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Environmental Drivers of Growth and Oxidative Status during Early Life in a Long-Lived Antarctic Seabird, the Adélie Penguin

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ABSTRACT

In vertebrates, developmental conditions can have long-term effects on individual performance. It is increasingly recognized that oxidative stress could be one physiological mechanism connecting early-life experience to adult phenotype. Accordingly, markers of oxidative status could be useful for assessing the developmental constraints encountered by offspring. Although some studies have demonstrated that developmental constraints are associated with high levels of oxidative stress in offspring, it remains unclear how growth, parental behavior, and brood competition may altogether affect oxidative stress in long-lived species in the wild. Here, we investigated this question in a long-lived Antarctic bird species by testing the impact of brood competition (e.g., brood size and hatching order) on body mass and on two markers of oxidative damage in Adélie penguin chicks. We also examined the influence of parental effort (i.e., foraging trip duration) and parental body condition on chick body mass and oxidative damage. First, we found that brood competition and parental traits had significant impacts on chick body mass. Second, we found that chick age and, to a lesser extent, chick body mass were two strong determinants of the levels of oxidative damage in Adélie penguin chicks. Finally, and importantly, we also found that brood competition significantly increased the levels of one marker of oxidative damage and was associated with a lower survival probability. However, parental effort and parental condition were not significantly linked to chick levels of oxidative damage. Overall, our study demonstrates that sibling competition can generate an oxidative cost even for this long-lived Antarctic species with a limited brood size (maximum of two chicks).

Keywords: oxidative stress, Adélie penguin, brood competition, early life, hatching order, brood size, reactive oxygen metabolites, protein carbonyls.

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Introduction

Early life is a critical life history stage because events during this period affect the ontogeny of multiple organ systems, with immediate and potentially long-term consequences for animal performance (Lindström 1999; Metcalfe and Monaghan 2001; Monaghan 2008; Costantini and Marasco 2022). During development, an organism faces important energetic demands (e.g., ontogeny of behavioral and physiological systems) that may raise life history trade-offs because the limited available resources must be allocated to competing traits. Nutritional conditions are therefore crucial because they determine the amount of energy and nutrients that can be allocated to the growth and development of body traits. For example, reduced food availability is usually associated with reduced mass at independence and with increased risk of mortality before emancipation (Gebhardt-Henrich and Richner 1998; Rodríguez et al. 2016). Poor nutrition can also lead to long-lasting phenotypic alterations and to lower longevity or reproductive success (Van De Pol et al. 2006).

In addition to food availability, postnatal nutritional conditions are also determined by two biotic factors. First, parental investment determines the amount and quality of food that will be delivered to the offspring (Clutton-Brock 1991). In birds, the ability of parents to acquire food resources and to return quickly to the nest affects the amount of energy that can be dedicated to growth and to the ontogeny of organismal systems. Second, sibling competition can also affect how energy is transferred to and used

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by offspring. In many bird species, parents raise multiple chicks simultaneously, and within-brood competition is one of the main factors that influence the nutritional status of offspring (Dijkstra et al. 1990; Royle et al. 1999). In addition, meteorological conditions and nest predation risk also affect offspring conditions (Coslovsky and Richner 2011; Maness and Anderson 2013; de Zwaan et al. 2019) because thermoregulation and antipredator behavior have energetic costs. In colonial species, such effects of nest predation and meteorological conditions can be modulated by the size of the colony, and individuals breeding in large colonies generally perform better (Götmark and Andersson 1984; Coulson 2002; Schmidt et al. 2021).

There is growing interest in using physiological markers to better understand the effect of such external variables on the fitness of developing individuals (e.g., Losdat et al. 2010; Bourgeon et al. 2011; Reichert et al. 2015; Gil et al. 2019). It is increasingly recognized that markers of oxidative stress provide valuable tools to test the link between physiological status and individual fitness traits (Beaulieu et al. 2009*a*; Colominas-Ciuró et al. 2017; Wada and Heidinger 2019). Depending on the focus of the study, oxidative stress can be defined differently (Costantini 2019). Classically, the biochemical definition of oxidative stress often refers to a disturbance of prooxidant-antioxidant balance in favor of the former, leading to potential damage (Sies 1991). Recently, Costantini (2019, p. 2) emphasized the importance of defining oxidative stress in a relevant biological context, proposing that biological oxidative stress be defined as "any change in one of the molecular components of the redox system that has an effect on any metric of the Darwinian fitness." Here, we rely on the biological definition because we specifically examine the ecological determinants of specific markers of oxidative damage and their link with fitness components. The complex mechanisms regulating oxidative status and reactive oxygen species (ROS) production are not fully understood yet, but oxidative stress has clear consequences for developing organisms (López-Arrabé et al. 2016; Wada and Coutts 2021). ROS are continuously created (mainly through metabolism, mitochondrial activity, and immune processes; Andreyev et al. 2005; Chen et al. 2018), but they are usually high under (i) increased metabolic activities (but see Salin et al. 2015; Colominas-Ciuró et al. 2022), (ii) physiological stress (i.e., high circulating levels of blood glucocorticoids; Costantini et al. 2011; Haussmann et al. 2012), and (iii) environmental stress (i.e., pollution [Koivula and Eeva 2010], temperatures [Costantini et al. 2012; Paital et al. 2016]). ROS can harm cell constituents (e.g., proteins, membranes, DNA) and lead to cell senescence, impaired fertility, and other significant damage. These detrimental impacts of ROS can be countered by antioxidants, structural tissue resistance, and repair mechanisms, for example (Davies 2000).

Oxidative stress is also intrinsically linked with metabolism, growth, and cell replication (Andreyev et al. 2005; Metcalfe and Alonso-Alvarez 2010; Smith et al. 2016), and it is therefore linked to nutritional conditions during the demanding developmental phase (Alonso-Alvarez et al. 2007). Quantifying the oxidative status of developing chicks may therefore also help us understand the effect of nutritional conditions on offspring during this demanding stage. Standard growth metrics, for body size or condition at independence, might not capture the impacts of temporarily poor nutritional conditions due to compensatory growth (Mangel and Much 2005; Criscuolo et al. 2008). For example, great tit (*Parus major*) nestlings maintain their growth under poor nutritional conditions at the cost of increased oxidative stress (Giordano et al. 2015). Late hatching and temporarily poor nutritional conditions have been associated with compensatory growth and higher oxidative damage in king penguins (*Aptenodytes patagonicus*; Geiger et al. 2012). Ultimately, the occurrence of oxidative stress in early life detrimentally affects subsequent fitness (e.g., Alonso-Alvarez et al. 2004; Bourgeon et al. 2011; Noguera et al. 2012; Losdat et al. 2014; López-Arrabé et al. 2016).

Although parental care is expected to play an important role in offspring development, it remains unclear whether and how it may affect the oxidative status of the offspring. Indeed, only a few studies have investigated the proximate drivers of oxidative stress (here, referred to as oxidative damage), especially during early life, for long-lived and wild bird species (Costantini et al. 2006; Rubolini et al. 2006). In particular, it has not been established yet whether monitoring the oxidative status of chicks could be a useful tool for assessing offspring quality.

The circumpolar Adélie penguin (*Pygoscelis adeliae*) is considered a sentinel species of the Antarctic marine ecosystem (Ainley 2002), and it shows contrasted populations trends: declining in the Antarctic Peninsula and stable in East Antarctica. Such variable trajectories are prompting the development of multiple indicators to monitor Adélie penguin populations and to attempt to provide robust predictions of future population trends in response to ongoing environmental changes (Reid et al. 2005).

Here, we focus on oxidative damage in and growth and survival of Adélie penguin chicks to investigate to what extent these metrics can help us monitor the nutritional constraints that occur in Antarctic populations of Adélie penguins during the breeding period. We thus quantified the two markers of oxidative damage in and the growth of Adélie penguin chicks from hatching to fledging. These two markers (reactive oxygen metabolites [ROMs] and protein carbonyls) have been shown to give a representative overview of oxidative damage in free-living birds (Beaulieu and Costantini 2014). Our specific aims were to (i) relate conditions at the nest (brood size and hatching order as proxies of brood competition), parental condition, and foraging behavior to chick growth; (ii) relate chick growth and conditions at the nest to chick oxidative damage to better understand whether they provide complementary information about a chick's health status; and (iii) test whether chick growth and/or oxidative damage are linked to fledging success. First, we predicted that large brood size (i.e., increased brood competition), delayed hatching relative to siblings, poor parental condition (i.e., low parental body condition), and poor parental foraging effort (i.e., excessive foraging trip duration) will be associated with poor chick nutritional conditions and therefore with slower growth. Second, and according to existing literature (Smith et al. 2016), we predicted that chick growth and chick body mass will be associated with increased levels of oxidative damage. After controlling for this effect of growth/age/body mass on oxidative damage, we predicted that poor nutritional conditions (i.e., large brood size, late-hatched nestling, poor parental condition,

poor parental foraging effort) at the nest will be associated with higher levels of oxidative damage. Finally, we predicted that slow growth, poor body condition, delayed hatching relative to siblings, and elevated levels of oxidative damage will be associated with a higher risk of mortality during the developmental period.

Methods

Fieldwork: Study Site and Season

We conducted the study at the French research station Dumont d'Urville on Petrel Island, Terre Adélie, East Antarctica (66°40′S, 140°00′E) during the austral summer of 2018–2019. Adélie penguins are endemic Antarctic seabirds that stay at the edge of the pack ice during their winter migration (Thiebot et al. 2019). Adélie penguins feed mainly on ice krill (*Euphausia crystallorophias*), Antarctic krill (*Euphausia superba*), and small Antarctic silverfish (*Pleuragramma antarctica*; Ainley 2002). When the chicks hatch, the guard stage lasts for approximately 20 d. During that period, adults perform shorter (1-d) trips at sea to feed themselves and their chicks. In mid-January, chicks gather in creches. At this stage, both adults feed the chicks at the same time, and nests are no longer occupied. In February, the chicks moult, and adults stop feeding them, stimulating chicks to fledge at approximately 50 d (Ainley 2002). Petrel Island hosts ca. 18,000 breeding pairs across many different subcolonies, ranging from just a few nests to several hundred nests (Barbraud et al. 2020). We conducted the monitoring of the studied colony from the beginning of the season (mating) to the end of the season (fledging; Ropert-Coudert et al. 2018). We randomly selected 118 nests during courtship, captured both parents of each nest, and then weighed the parents, measured their flippers, and individually marked them with tape glued onto the back feathers. We monitored all nests by observing them from a distance of approximately 10 m every 2 h during the chick-rearing period to record the presence/absence of both parents and the foraging trip duration (FTD). We determined the sex of the adults by using a combination of morphometric measurements, observations of mating behavior, and incubation patterns (the female takes the first trip at sea after egg laying). We accurately determined laying and hatching dates by visually observing the nest content once per day during the expected periods of laying/hatching.

Chick Monitoring

All chicks from monitored nests were captured twice (at 12 and 32 d), representing a total of 156 chicks (156 chicks at 12 d and 143 chicks at 32 d) from 101 nests (17 nests failed during incubation or early chick rearing, i.e., before the chicks reached 12 d old). While 12 d correspond to the beginning of intense growth, 32 d occur toward the end of growth and close to the plateau when growth stabilizes (Ainley and Schlatter 1972). At 12 d, the chicks were marked with tags (T-bar colored tags, Hallprint; 105 mm long; inserted into the skin on the back) to identify them at 32 d old, which is when they leave the nest to form creches (fig. S1). At 12 d old, the biggest chick was identified as the first-hatched chick, following a pilot study conducted the year before. In this pilot study, 24 chicks from 12 nests were tagged with a piece of loose Tesa tape around the leg as soon as they hatched, and they were weighed at 12 d old. In all 12 nests, the first-hatched chick was always the biggest. In this study, chicks hatched on the same day in 14 of the 101 nests (13.9%). Survival of each chick was monitored by recording their presence/absence in the colony at the age of 32 d; at this time, all tags were removed. During each capture, weight, head-bill length, and flipper lengths were measured, and blood was collected from the tarsus vein using 25-G needles and 1-mL heparinized syringes; 200 μL and 1 mL of blood were collected at 12 and 32 d, respectively. Blood was immediately centrifuged, and plasma was separated from the red blood cells. All samples were then kept frozen at −20°C until laboratory analyses. Survival of each chick was monitored by recording its presence/absence in the colony at the age of 32 d.

Laboratory Analyses

We performed molecular sexing to determine the sex of the chicks at the Service d'Analyses Biologiques of the Centre d'Etudes Biologiques de Chizé. We carried out a DNA extraction with 2 μL of pellets (red blood cells) and using a chelex resin (chelex 100 molecular biology grade resin, Bio-Rad; 10%) associated with proteinase K as written in the manufacturer's instructions. We then performed a polymerase chain reaction with amplification of the CHD gene by following standard procedures validated on penguins in Lee et al. (2010).

We measured two markers of oxidative damage at the laboratory of the Muséum National d'Histoire Naturelle (Unité Physiologie Moléculaire et Adaptation). We used the d-ROMs test (Diacron International, Grosseto, Italy) to measure ROMs following the manufacturer's instructions. ROMs absorbance was read at 505 nm. The d-ROMs test has been used effectively in penguins (Beaulieu et al. 2009*a*; Stier et al. 2019); it measures early oxidation compounds (e.g., including hydroperoxides and endoperoxides; Costantini 2016). ROMs are expressed as millimolars of H_2O_2 equivalents. We analyzed each plasma sample in duplicate; the intra- and interplate coefficients of variation were 9.69% and 12.31%, respectively.

We quantified the carbonyl content of plasma samples by using the protein carbonyl colorimetric assay (Cayman Chemical, Ann Arbor, MI). This was performed according to the protocol described in Levine et al. (1990) and applied to penguins in Stier et al. (2019). Protein carbonyls were first purified using streptomycin to remove carbonyls derived from nucleic acids. Then, absorbance of the sample was read at 370 nm. Mean absorbance of control tubes was subtracted. The extinction coefficient of 2,4-dinitrophenylhydrazine (0.022 μmol L^{-1} cm^{-1}) was used to calculate protein carbonyl content, which was expressed as nanomoles per milligram of protein. We analyzed each plasma sample in duplicate; the intra- and interplate coefficients of variation were 10.92% and 13.03%, respectively.

Statistical Analysis

All analyses were performed using R (ver. 4.0.5; R Core Team 2022). ROM variables were log transformed to satisfy normality. For

all tested variables (i.e., ROMs, protein carbonyls, and chick body mass), normality and heterogeneity were assessed by visual inspection of qq plots, residual plots, and histograms of residuals. We performed linear mixed effects models (LMEMs) using the lme4 package for the models that were testing the first two hypotheses.

To test our predictions (see the introduction), we built different sets of models to examine the influence of explanatory variables on our response variables (i.e., body mass, ROMs, protein carbonyls). First, we tested the influence of the age (12 vs. 32 d) and sex of a chick on the response variables by using LMEMs with chick identity and nest identity as random effects. Plate number was also included as a random effect when oxidative marker (i.e., ROMs, protein carbonyls) was a dependent variable. The similarity in oxidative damage levels (i.e., ROMs, protein carbonyls) between siblings was then measured with the rptR package for testing repeatability.

Second, we tested the influence of nest condition (hatching order and brood size as proxies of brood competition), mass (when ROMs and protein carbonyls were the response variables), and sex on the response variables (body mass, ROMs, protein carbonyls) by performing LMEMs with nest identity and plate number as random effects. When analyzing ROMs and protein carbonyls, we had to separate the analysis by age (12- or 32-d-old chicks) because of the strong correlation between age and chick body mass (body mass is obviously much higher in 32-d-old chicks than in 12-d-old chicks). Indeed, running models with two highly correlated variables can lead to spurious results. For consistency, we also separated the statistical analyses by age when analyzing the chick body mass. In all these analyses, hatching order and brood size were merged into one factor variable called brood size and hatching order (BSHO) with the following three levels: (i) single chick (i.e., alone in the nest), (ii) first-hatched chick from nests with two siblings, and (iii) second-hatched chick from nests with two siblings. The BSHO category of a given chick was defined as the brood size/hatching order of the chick when it was 12 d old. We defined the BSHO category at 12 d old (i.e., the day of blood sampling) to be the most representative of the potential effect of the brood size on the condition of the chicks. Eighteen nests had two chicks at hatching and only one chick left at the time of measurement (12 d after hatching). The exact day of death was unknown for these 18 nests because the nests were not monitored between hatching and 12 d after hatching. This category was kept unchanged for the analysis of the 32-d-old chicks, even if the sibling of the nest died between 12 and 32 d old, because it was not possible to know the exact day of the sibling death. Such mortality occurred very rarely (13 of 165 chicks) in our study and is unlikely to bias our results. At 12 d old, the BSHO variable includes 46 single chicks, 55 first-hatched chicks from nests with two chicks, and 55 second-hatched chicks from nests with two chicks.

Third, we tested whether (i) parental condition (i.e., body condition calculated as the residuals between prebreeding body mass and flipper lengths; male: $F_{1,97} = 16.39$, $P < 0.001$; female: $F_{1,97} = 7.45$, $P = 0.007$) and (ii) parental foraging effort (i.e., FTD) would be associated with chick growth and levels of oxidative damage. To test whether parental condition was associated with chick growth and levels of oxidative damage, we built one model with chick body mass as a response variable; age, sex, and parental body condition as explanatory variables; and chick identity and nest identity as random effects; we also built two models for ROMs and protein carbonyls in 12- and 32-d-old chicks, with mass, sex, and parental body condition as explanatory variables (i.e., five models in total) and with nest identity and plate number as random effects. In these models, we separated the analysis by age (12- or 32-d-old chicks) because of the strong correlation between age and chick body mass. To test whether parental FTD was associated with chick growth and levels of oxidative damage, we built similar models including maternal and paternal FTDs as explanatory variables. Sex differences in adult FTD were tested using a mixed model with FTD as the dependent variable, sex as an explanatory variable, and nest as a random factor.

Finally, for testing the influence of the levels of oxidative damage, chick body mass, and brood competition on mortality, we used a generalized linear model for binomial data with death (i.e., yes/no) as a response variable and with mass, BSHO, ROMs, or protein carbonyls at 12 d as explanatory variables (separately). Level of significance was set at $P = 0.05$. When interactions were significant, contrast tests were used to compare groups by using the emmeans package (with degrees of freedom estimated with the Kenward-Roger method).

Results

Influence of Age and Sex Effect on Body Mass and Markers of Oxidative Damage

Chicks weighed on average 959 ± 207 g (±SD) and 3,005 ± 638 g when they were 12 and 32 d old, respectively. Chick body mass was affected by sex, age, and the sex × age interaction (table 1, pt. A), demonstrating that the influence of sex on body mass differed with age. In addition, male chicks were heavier than female chicks, but this was true only when they were 32 d old (contrast: $t = -4.60$, $P < 0.001$) and not when they were 12 d old (contrast: $t = -1.39$, $P = 0.166$). Levels of ROMs and protein carbonyls were affected only by the age of the chick: 12-d-old chicks had higher levels of ROMs and protein carbonyls than 32-d-old chicks (table 1, pts. B, C; fig. 1). Finally, using rptR analysis, we showed that protein carbonyl levels for chicks from the same nest were associated when the chicks were 12 d old ($P = 0.002$, $r^2 = 0.37$) but not when they were 32 d old ($P = 0.080$, $r^2 = 0.180$). However, the levels of ROMs were not similar among siblings at any age (12-d-old chicks: $P = 0.179$, $r^2 = 0.11$; 32-d-old chicks: $P = 0.121$, $r^2 = 0.14$).

Influence of Brood Size and Hatching Order on Body Mass and Markers of Oxidative Damage

Chick body mass was affected by BSHO at 12 and 32 d old (table 2, pt. A). For 12-d-old chicks, the body masses of single chicks and first-hatched chicks from nests with two siblings were

Table 1: Influence of age, sex, and their interactions on chick body mass (pt. A), levels of reactive oxygen metabolites (ROMs; pt. B), and levels of protein carbonyls (pt. C)

	df	*F*	*P*
	A. Body mass (299)		
Age	1, 152.08	2,102.46	**<.001**
Sex	1, 155.22	14.66	**<.001**
Age × sex	1, 152.22	7.74	**.006**
	B. Levels of ROMs (295)		
Age	1, 86.51	80.03	**<.001**
Sex	1, 151.42	.24	.628
Age × sex	1, 150.29	.54	.465
	C. Levels of protein carbonyls (294)		
Age	1, 202.44	4.24	**.041**
Sex	1, 189.45	.56	.455
Age × sex	1, 200.90	2.18	.141

Note. Chick identity and chick nest were included as random factors in all models. Sample sizes are indicated in parentheses for each dependent variable. Significant results (i.e., $P < 0.05$) are in bold.

similar (contrast: $t = 0.08$, $P = 0.93$; fig. 2), and first-hatched chicks were larger than second-hatched chicks (contrast: $t = 5.40$, $P < 0.001$). For 32-d-old chicks, single chicks were heavier than first-hatched chicks (contrast: $t = 3.17$, $P = 0.002$; fig. 2), and both were heavier than second-hatched chicks (contrast, single chicks: $t = 6.92$, $P < 0.001$; first-hatched chicks: $t = 5.02$, $P < 0.001$).

The ROMs analysis showed an effect of mass and BSHO only on 32-d-old chicks (table 2, pt. B; fig. 3). Specifically, heavier chicks had higher levels of ROMs ($F_{1, 136.54} = 12.80$, $P < 0.001$), and second-hatched chicks from nests with two siblings had higher levels of ROMs than single chicks and first-hatched chicks from nests with two siblings (contrast, single chicks: $t = -2.19$, $P = 0.030$; first-hatched chicks: $t = -2.28$, $P = 0.024$); the levels of ROMS did not differ between single chicks and first-hatched chicks from nests with two siblings (contrast: $t = -0.22$, $P = 0.83$). The level of protein carbonyls was not significantly affected by body mass, brood size, or hatching order in 12- and 32-d-old chicks (table 2, pt. C).

Influence of Parental Quality on Body Mass and Markers of Oxidative Damage

Chick body mass was affected by the maternal body condition × sex interaction (table 3), with female chicks being heavier when their mothers were in better condition ($F_{1, 42.82} = 5.07$, $P = 0.029$; fig. 4*A*); this was not the case for male chicks ($F_{1, 55.08} = 1.18$, $P = 0.282$). In contrast, chick body mass was not affected by paternal body condition (table 3).

Chick body mass was affected by the average paternal FTD × age interaction (table 4). Specifically, 32-d-old chicks were heavier when their fathers performed shorter trips (fig. 4*B*), but this effect was not significant at 12 d old (32-d-old chicks: $F_{1, 89.82} = 8.01$, $P = 0.006$; 12-d-old chicks: $F_{1, 93.90} = 1.40$, $P = 0.240$; table 4). In contrast, chick body mass was not affected by average maternal FTD (table 4).

The levels of ROMs and protein carbonyls were differently affected by the maternal and paternal body conditions, depending on the age of the chicks. At 12 d old, the levels of ROMs were affected by the maternal body condition × sex interaction (table 5). Specifically, maternal body condition was significantly and negatively correlated with the levels of ROMs in male chicks ($F_{1, 57.07} = 4.43$, $P = 0.040$), but it was not significantly correlated with the levels of ROMs in female chicks ($F_{1, 69.77} = 0.58$, $P = 0.450$). The levels of protein carbonyls at 12 d old were affected by the paternal body condition × sex interaction (table 5). Paternal body condition showed a weak, nonsignificant negative correlation with the levels of protein carbonyls in female chicks ($F_{1, 56.65} = 3.57$, $P = 0.064$), but it

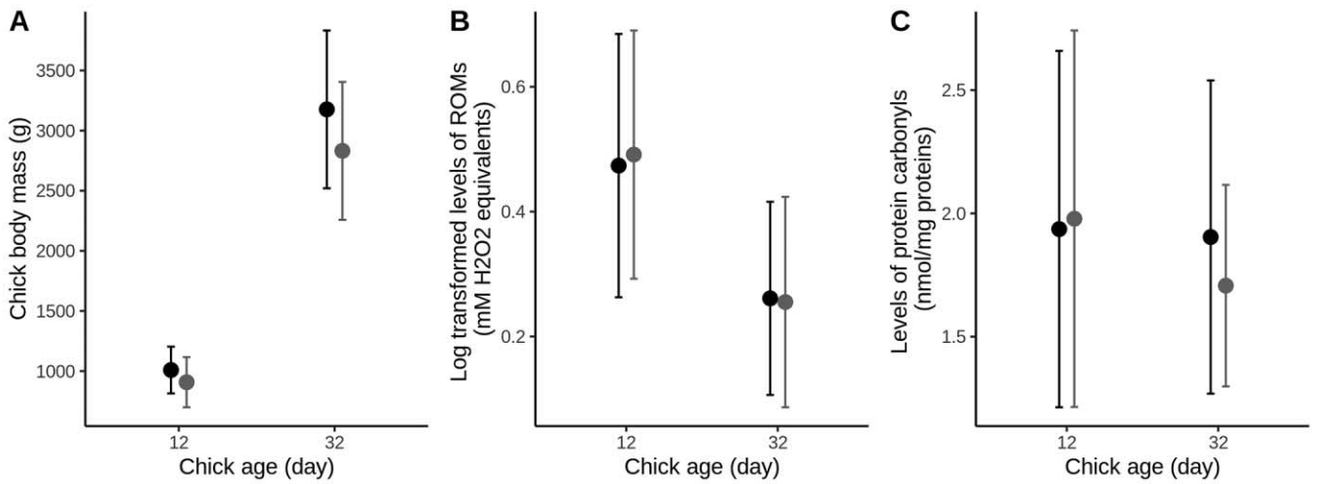

Figure 1. Influence of the age and sex of a chick on body mass (*A*), levels of reactive oxygen metabolites (ROMs; *B*), and levels of protein carbonyls (*C*). Males are represented by black dots, and females are represented by gray dots. Mean ± SD calculated from raw data is represented.

Table 2: Influence of sex, brood size and hatching order (BSHO), and their interactions on body mass (pt. A), levels of reactive oxygen metabolites (ROMs; pt. B), and levels of protein carbonyls (pt. C) in Adélie penguin chicks

	df	*F*	*P*	df	*F*	*P*
	12-d-old chicks (156)			32-d-old chicks (143)		
A. Body mass:						
Sex	1, 147.99	9.89	**<.001**	1, 136.65	16.8	**<.001**
BSHO	2, 72.31	39.51	**<.001**	2, 66.05	26.82	**<.001**
Sex × BSHO	2, 97.14	.65	.530	2, 96.5	2.82	.070
	12-d-old chicks (152)			32-d-old chicks (143)		
B. Levels of ROMs:						
Sex	1, 137.52	1.64	.202	1, 128.56	.30	*.582*
BSHO	2, 137.22	.85	.428	2, 136.22	3.16	**.045**
Mass	1, 138.70	2.32	.130	1, 136.54	12.80	**<.001**
Sex × BSHO	2, 137.35	.03	.973	2, 128.52	1.40	*.251*
Sex × mass	1, 137.84	2.36	.127	1, 128.51	.19	*.666*
BSHO × mass	2, 137.19	.99	.376	2, 128.62	.93	*.396*
Sex × BSHO × mass	2, 137.29	.10	.903	2, 128.50	1.31	*.273*
	12-d-old chicks (151)			32-d-old chicks (143)		
C. Levels of protein carbonyls:						
Sex	1, 133.10	.00	.947	1, 128.19	1.32	*.252*
BSHO	2, 89.27	1.29	.281	2, 115.51	1.28	*.283*
Mass	1, 132.64	.01	.935	1, 126.87	.62	*.433*
Sex × BSHO	2, 132.42	.16	.854	2, 119.34	1.42	*.247*
Sex × mass	1, 129.23	.01	.938	1, 126.88	2.14	*.146*
BSHO × mass	2, 89.01	1.54	.219	2, 117.60	.93	*.399*
Sex × BSHO × mass	2, 133.68	.25	.780	2, 117.68	.90	*.408*

Note. Chick nest was included as a random factor in all models. Model selection was performed by using a stepwise backward approach and by eliminating the nonsignificant terms shown in italics. Sample sizes are indicated in parentheses for each dependent variable. Significant results (i.e., $P < 0.05$) are in bold.

was not significantly correlated with the levels of ROMs in male chicks ($F_{1, 62.06} = 0.60$, $P = 0.439$).

At 32 d old, the levels of ROMs and protein carbonyls in chicks were not affected by maternal or paternal body condition or by average maternal or average paternal FTD (tables S1, S2). Finally, fathers performed, on average, significantly shorter trips than mothers during the chick-rearing period ($F_{1, 95.42} = 51.59$, $P < 0.001$).

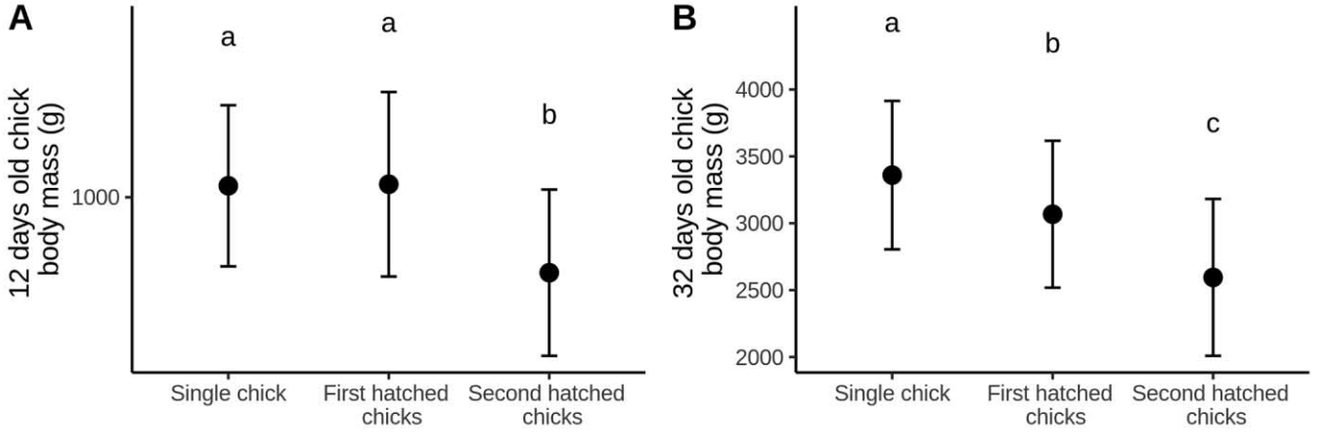

Figure 2. Influence of brood size and hatching order on the body mass of 12-d-old Adélie penguin chicks (*A*) and 32-d-old Adélie penguin chicks (*B*). First- and second-hatched chicks are from nests with two siblings. Different lowercase letters indicate a statistical difference between groups. Mean ± SD calculated from raw data is represented.

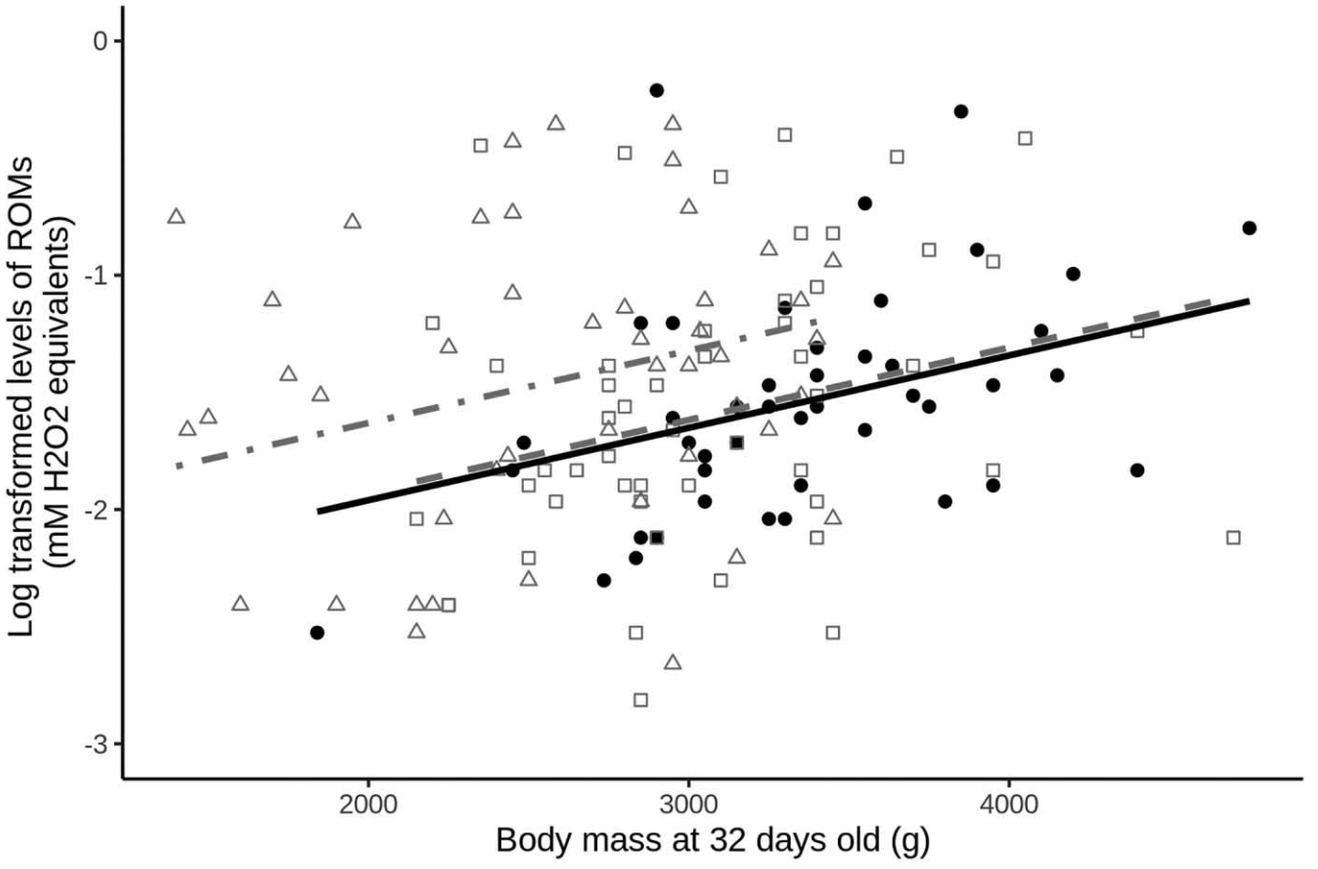

Figure 3. Influence of body mass and brood size and hatching order on the levels of reactive oxygen metabolites (ROMs) in 32-d-old Adélie penguin chicks. Single chicks, first-hatched chicks from nests with two siblings, and second-hatched chicks from nests with two siblings are represented by black dots, white squares, and white triangles, respectively. The solid line, the dashed line, and the dot-dashed line represent the predicted values of ROMs in single chicks, first-hatched chicks from nests with two siblings, and second-hatched chicks from nests with two siblings, respectively.

Table 3: Influence of age, sex, maternal body condition, and paternal body condition on chick body mass

	df	*F*	*P*
Age	1, 145.36	2,220.71	**<.001**
Sex	1, 149.54	13.59	**<.001**
Maternal body condition	1, 97.93	.55	.459
Paternal body condition	*1, 107.21*	*.06*	*.809*
Age × sex	1, 145.51	9.04	**.003**
Age × maternal body condition	1, 146.30	.00	.991
Sex × maternal body condition	1, 148.17	5.71	**.018**
Age × paternal body condition	*1, 143.51*	*.00*	*.970*
Sex × paternal body condition	*1, 147.59*	*.00*	*.984*
Age × sex × maternal body condition	1, 146.34	3.97	**.048**
Age × sex × paternal body condition	*1, 143.83*	*.08*	*.781*

Note. Chick identity was included as a random factor. Model selection was performed by using a backward stepwise approach and by eliminating nonsignificant terms shown in italics; $n = 293$ for all models. Significant results (i.e., $P < 0.05$) are in bold.

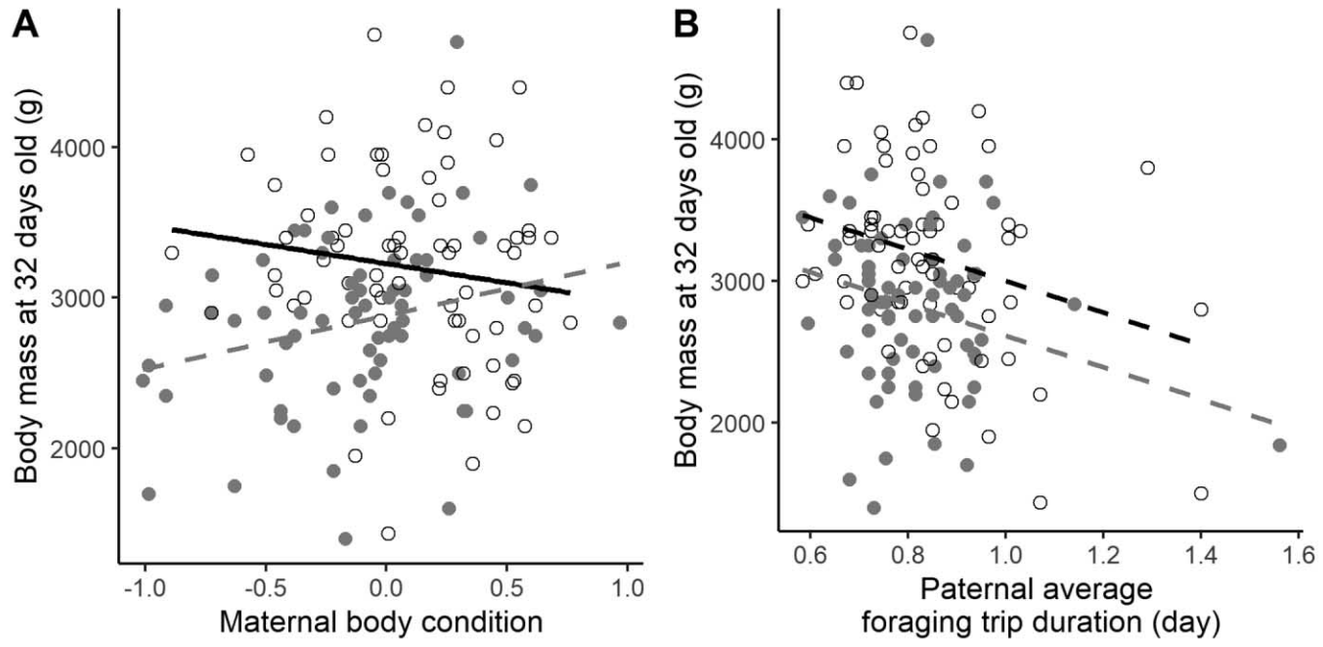

Figure 4. Influence of maternal body condition (*A*) and average paternal foraging trip duration during the chick-rearing period (*B*) on the body masses of 32-d-old Adélie penguin chicks. Males and females are represented by white dots and gray dots, respectively. The black and gray dashed lines represent the statistically significant relationship between our variables of interest for male and female chicks, respectively; the solid line represents the nonsignificant relationship between male chick body mass at 32 d and maternal body condition. The lines represent the predicted values from the model.

Chick Survival

The probability to survive from 12 to 32 d old was significantly affected by body mass, with smaller chicks having a lower survival probability ($Z = -2.47$, $P = 0.013$; fig. 5). In addition, chicks presenting lower levels of ROMs at 12 d old had a lower survival probability ($Z = -2.28$, $P = 0.023$); however, no association was found with their protein carbonyl levels at 12 d old ($Z = 1.53$, $P = 0.13$). Finally, chick survival was associated with brood competition, with second-hatched chicks having a higher probability to die than first-hatched and single chicks (contrast analysis, single chicks compared to first-hatched chicks from nests with two siblings: $Z = -0.78$, $P = 0.436$; single chicks compared to second-hatched chicks from nests with two siblings: $Z = -2.13$, $P = 0.033$; first-hatched chicks from nests with two siblings compared to second-hatched chicks from nests with two siblings: $Z = -1.36$, $P = 0.047$).

Discussion

In this study, we found that brood size, hatching order, and to a lesser extent parental condition and foraging behavior affected the growth of and oxidative damage in the chicks of a long-lived Antarctic species.

Growth, Age, and Levels of Oxidative Damage

We found that the levels of ROMs and protein carbonyls decrease with the age of Adélie penguin chicks and that this is consistent with our predictions. This sharp decrease in the levels of molecular oxidative damage with age is consistent with findings across multiple vertebrate species (e.g., Costantini et al. 2006, 2007; Noguera et al. 2011; Burraco et al. 2017). The relatively high levels of ROMs in 12-d-old Adélie penguin chicks probably result from the rapid growth rate, intense cell divisions, and mitochondrial activity at that age, all of which generate high levels of ROS (Lenaz 2001; Harper et al. 2004; Costantini et al. 2007), but measures of mitochondrial activity would be needed to validate this hypothesis. The age of a chick had a stronger effect on the levels of ROMs than

Table 4: Influence of age, sex, and average foraging trip duration (FTD) during the chick-rearing period on chick body mass

	df	*F*	*P*
Age	1, 148.86	46.13	**<.001**
Sex	1, 152.37	.07	.792
Maternal FTD	1, 139.96	.16	.693
Paternal FTD	1, 163.76	10.00	**.002**
Age × sex	1, 148.86	.03	.859
Age × maternal FTD	1, 137.52	.31	.581
Sex × maternal FTD	1, 139.96	1.55	.216
Age × paternal FTD	1, 158.24	9.63	**.002**
Sex × paternal FTD	1, 163.76	.98	.325
Age × sex × maternal FTD	1, 137.52	2.96	.088
Age × sex × paternal FTD	1, 158.24	2.15	.144

Note. Chick identity was included as a random factor; $n = 291$ for all models. Significant results (i.e., $P < 0.05$) are in bold.

Table 5: Influence of sex, chick body mass, and maternal and paternal body conditions on the levels of reactive oxygen metabolites (ROMs; pt. A) and protein carbonyls (pt. B) in 12-d-old Adélie penguin chicks

	df	*F*	*P*
	A. Levels of ROMs		
Mass	1, 140.50	2.09	**<.001**
Sex	1, 140.17	1.14	.288
Maternal body condition	1, 93.82	1.04	.310
Paternal body condition	*1, 99.56*	*.20*	*.655*
Mass × sex	*1, 122.24*	*1.41*	*.237*
Maternal body condition × sex	1, 134.29	7.49	**.007**
Paternal body condition × sex	*1, 138.07*	*.05*	*.825*
	B. Levels of protein carbonyls		
Mass	1, 138.81	.02	.889
Sex	1, 108.66	.00	.980
Maternal body condition	*1, 92.48*	*.79*	*.376*
Paternal body condition	1, 100.84	1.32	.253
Mass × sex	1, 104.86	.01	.937
Maternal body condition × sex	*1, 139.97*	*.49*	*.486*
Paternal body condition × sex	1, 141.92	5.42	**.021**

Note. Nest identity was included as a random factor. Model selection was performed by using a backward stepwise approach and by eliminating nonsignificant terms shown in italics; $n = 149$ for all models. Significant results (i.e., $P < 0.05$) are in bold.

on the levels of protein carbonyls, which is also consistent with previous studies showing that all markers of oxidative stress do not necessarily follow the same pattern (Sepp et al. 2012). Because ROMs and protein carbonyls are produced by the oxidation of different molecules (i.e., fatty acids, proteins, etc.; Beaulieu and Costantini 2014), our results may reflect differences in the composition of the plasma content between chicks (i.e., more variability in fat content than in protein content between chicks), although this hypothesis should be formally tested. In addition, ROMs and protein carbonyls follow different metabolic ways (i.e., ROMs are

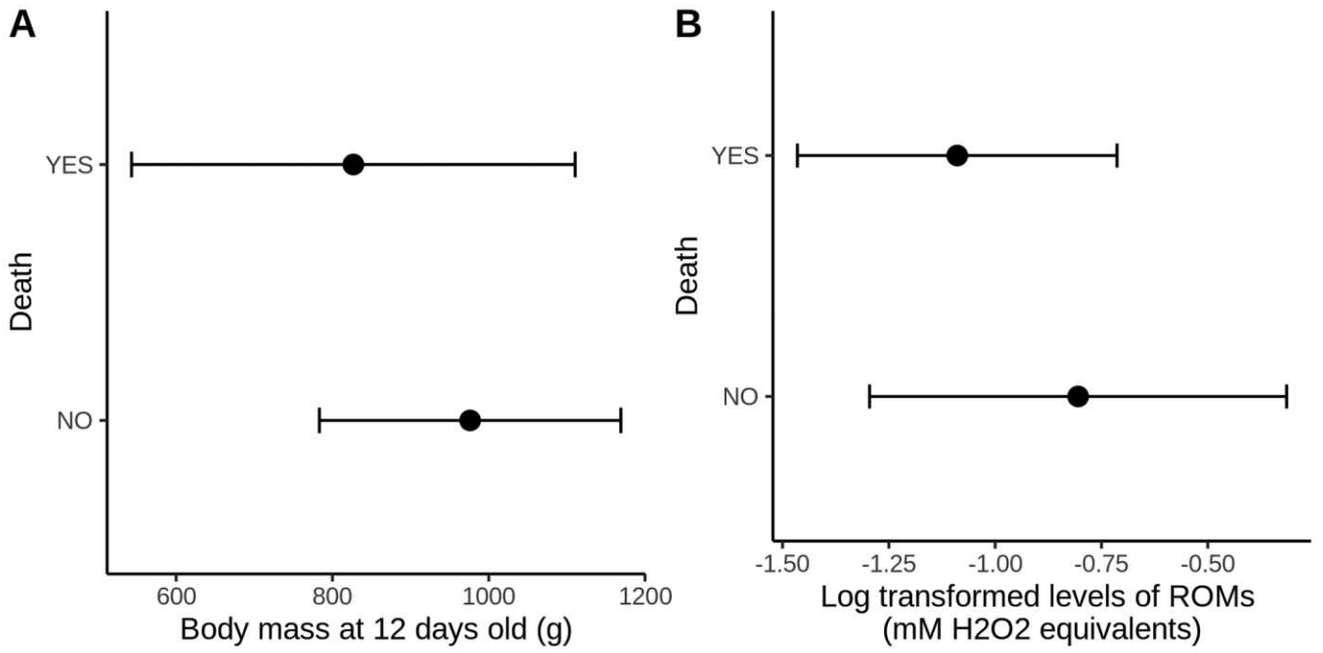

Figure 5. Influence of the body mass of 12-d-old chicks (*A*) and the levels of reactive oxygen metabolites (ROMs) in 12-d-old chicks (*B*) on the probability of Adélie penguin chicks to survive from 12 to 32 d; $n = 140$ for chicks that were alive at 32 d, and $n = 14$ for chicks that died. Mean ± SD calculated from raw data is represented.

expected to be generated and eliminated faster than protein carbonyls), and if proteins are more important for growth and development, protecting these might be prioritized by the organism. Furthermore, even though most usual antioxidants are not specific to a given tissue, some of them can have a preferred substrate (e.g., hydrogen peroxide for the catalase; Halliwell and Gutteridge 2015), and this could potentially explain why the effect of age on oxidative damage was more apparent for ROMs than for protein carbonyls. Additional analysis measuring antioxidant levels would help evaluate the underlying mechanisms leading to such an oxidative status and apprehend more precisely the interdifferences in oxidative damage between chicks.

Heavier chicks also had higher ROMs levels at 32 d old. This could be explained by higher metabolism in heavier chicks (e.g., Culik et al. 1990). Furthermore, smaller chicks may be fed less often, and fasting is known to reduce oxidative stress (Ensminger et al. 2021), except in cases of severe and prolonged fasting when oxidative stress is then increased (Schull et al. 2016). In contrast, this effect was not apparent in 12-d-old chicks, probably because there was much less interindividual variation in body mass (12-d-old chicks: SD = ±207 g; 32-d-old chicks: SD = ±638 g). Also, the levels of ROMs at this age might be very high and less variable because of fast growth and high cellular activity in 12-d-old chicks compared to 32-d-old chicks. The influence of mass on ROM levels supports the idea that fast growth entails physiological costs (Christensen et al. 2016; see Smith et al. 2016 for a meta-analysis; Burraco et al. 2017) and that developing chicks may face a trade-off between growth and physiological stress.

Brood Competition, Growth, and Oxidative Damage

Brood competition had a strong influence on the growth of Adélie penguin chicks. Comparing the growth of and the level of oxidative damage in three categories of chicks (single chick, first-hatched chick, and second-hatched chick), we found that hatching order had a very strong effect on body mass in nests with two siblings. Second-hatched chicks had a significantly lower body mass than the first-hatched chicks, a finding evident at both 12 and 32 d old. This effect was significant, although some single chicks might have had a sibling during an unknown period before 12 d (when their sibling died between hatching and 12 d after hatching). This effect is unlikely related to egg size, which is similar between first- and second-laid eggs in Adélie penguins (Crossin and Williams 2016). Hatching asynchrony is a major determinant of brood competition in birds (Magrath 1990), and it often results from incubation asynchrony because the first-laid egg is incubated earlier than the second-laid egg (~1–2-d difference in penguins; Williams and Croxall 1991). In Adélie penguins, the larger chick has a clear advantage over its sibling when parents deliver the meal because it has a better competitive ability. Therefore, the second-hatched chick is usually fed only when the first-hatched and dominant chick reaches a state of satiety. Accordingly, hatching order has been shown to convincingly explain differences in growth trajectories between siblings in other species (e.g., Ploger and Medeiros 2004; Merkling et al. 2014; Hildebrandt and Schaub 2018).

Brood size also had a significant influence on the body mass of Adélie penguin chicks. At 32 d, single chicks were always heavier than chicks from a nest with two chicks, demonstrating that competition had a detrimental effect on chick development. Brood competition is certainly exacerbated as chicks grow and require larger amounts of food, and this may be why this effect is particularly obvious in 32-d-old chicks. We also found a more subtle effect of brood competition on ROMs. Although the levels of ROMs are mainly explained by the age of the chick, they were also affected to a lesser extent by BSHO in 32-d-old chicks. After controlling for body mass, the levels of ROMs in second-hatched chicks were higher than those in first-hatched or single chicks, which is a novel finding. Previous studies investigating the influence of brood competition on the oxidative status of developing chicks had mixed results. Despite the fact that the use of many different markers of oxidative status can complicate comparisons, in the majority of these studies brood size and hatching order had no effect on the oxidative status of chicks (Bourgeon et al. 2011; Losdat et al. 2014; Nettle et al. 2015; López-Arrabé et al. 2016; Gil et al. 2019; Espín et al. 2020). For example, Young et al. (2017) found no effect of brood size and hatching order on the levels of protein carbonyls, uric acid, and total antioxidant status in a long-lived seabird, the black-legged kittiwake (*Rissa tridactyla*). In contrast, enlarged brood size was associated with higher levels of oxidative stress in the zebra finch (*Taeniopygia guttata*; Reichert et al. 2015). In our study, brood size and hatching order influenced the levels of ROMs in 32-d-old chicks, but importantly, this effect was apparent only if body mass was taken into account, suggesting that growth and chick body mass might be important factors when investigating the role of other abiotic and biotic factors in the levels of oxidative damage. These effects could be linked to the nutritional and social stresses of brood competition, which are known to increase the levels of glucocorticoids, important mediators of oxidative stress (Losdat et al. 2010; Costantini et al. 2011; Reichert et al. 2015; Stier et al. 2015). The survival consequences of oxidative damage and their persistence later in life now need to be investigated to better understand the fitness consequences of developmental conditions for individuals (reviewed in Wada and Coutts 2021). We did not find any influence of brood competition or body mass on the levels of protein carbonyls at any age. Again, protein carbonyls might occur only when individuals suffer from intense stressors, and this was unlikely to occur in our study because this breeding season was characterized by a high average breeding success (average breeding success: 1.23 fledglings per pair). In comparison, other years since 1995 variably ranged between 0.6 and 1.2 fledglings per pair—except for two specific years that were characterized by massive breeding failure, with zero fledglings per pair (see Ropert-Coudert et al. 2018).

Parental Traits, Growth, and Oxidative Damage

Maternal condition was positively related to chick mass, although the effect was apparent only in female chicks. The origin of this sex-dependent effect is not clear, but it may be due to a higher sensitivity of female chicks to environmental constraints or due to a differential investment of mothers in their daughters

and sons (e.g., maternal effects through egg composition). Indeed, female Adélie penguins modulate their foraging efforts depending on the sex of their offspring (Beaulieu et al. 2009*b*). Male and female Adélie penguin chicks are not fed with the same diet (Jennings et al. 2016), further suggesting that parents adjust their investment according to the sex of their offspring.

In contrast, we did not find any influence of paternal body condition on chick body mass. This suggests that paternal body condition may be a less reliable predictor of breeding success than maternal body condition. This may be explained by the effects of maternal body condition, mass, and food availability on egg composition, which can influence chick size and development (Steiger 2013; Ruffino et al. 2014). Maternal body condition may therefore be a reliable proxy of maternal quality, with females in better condition being better at providing parental care to their chicks.

Although maternal FTD was unrelated to the body mass of the chicks, shorter paternal foraging trips were associated with a larger chick mass at 32 d. In penguins, shorter trips during the chick-rearing period are associated with greater parental effort: parents increase their foraging effort to feed their chicks as frequently as possible to sustain their growth (e.g., Weimerskirch et al. 2003; Angelier et al. 2008), and such increased parental effort is usually associated with increased oxidative stress for parents (Colominas-Ciuró et al. 2017). In Adélie penguins, the father may have a strong influence on chick growth because it performs shorter trips and feeds the chicks with a higher-quality diet than the mother (Clarke et al. 1998; Beaulieu et al. 2010; Jennings et al. 2016). Accordingly, fathers were performing shorter trips on average than mothers in our study, which is consistent with the literature showing that males forage in more coastal areas and more on fish (Widmann et al. 2015; Colominas-Ciuró et al. 2018; Michelot et al. 2020).

We found only weak evidence that the levels of oxidative damage in chicks were related to parental body condition. In 12-d-old chicks, the levels of ROMs were slightly correlated with maternal body condition, but this was only in male chicks ($P = 0.040$). In addition, in 12-d-old chicks the levels of protein carbonyls were slightly and almost significantly correlated with paternal body condition, but this was only in female chicks ($P = 0.064$). These trends were not apparent in 32-d-old chicks, suggesting that the influence of parental body condition on the levels of oxidative damage in the parents' chicks weaken as the chicks grow. Supporting this idea, the levels of protein carbonyls were similar between siblings at 12 d old but not at 32 d old.

Adult quality may therefore affect the levels of oxidative damage in chicks, most especially during their first days of life. Such effects could be mediated by several non–mutually exclusive mechanisms, such as the maternal transfer of antioxidant molecules into the eggs (e.g., Rubolini et al. 2006; Watson et al. 2018), through potential heritability of the mechanisms leading to oxidative stress (López-Arrabé et al. 2016) or through the expression of parental care (e.g., poor incubation or brooding behavior leading to oxidative stress; Berntsen and Bech 2021). Adult foraging ability should be linked to the oxidative status of the chicks not only because food limitation can induce oxidative stress in growing chicks (Costantini 2008) but also because the parents could select specific antioxidant-rich prey to limit oxidative stress in their progeny (e.g., Beaulieu et al. 2015; Colominas-Ciuró et al. 2021). Nonetheless, we found no evidence that parental foraging ability affected the levels of oxidative damage in the chicks. It is important to note that breeding success and food availability were high during the year of the study and that our results therefore suggest that inter-individual variability in parental foraging behavior (at least in trip duration) does not influence the levels of oxidative damage in the chicks when food availability is high.

Survival

As expected, and previously reported in numerous bird species (reviewed in Maness and Anderson 2013) including penguins (e.g., Williams and Croxall 1991; Ainley et al. 2018), we found that smaller chicks had a lower probability of surviving to 32 d and, accordingly, that second-hatched chicks tended to survive less than single chicks and first-hatched chicks from nests with two siblings. Our study suggests that brood competition affects chick survival, although this result should be carefully interpreted given the high breeding success this year and the low number of chicks that died in our study ($n = 13$).

We found that the levels of ROMs in 12-d-old chicks were positively associated with the probability of surviving until 32 d. Higher levels of ROMs might reflect a higher pace of growth or may counterintuitively be a sign of a better nutritional state; for example, on one side, a large intake of fatty acids provides energetic stores, but on the other side, it provides substrates for ROS-caused oxidation. Indeed, we found that the levels of ROMs were positively correlated with body mass, although this relationship was significant only in 32-d-old chicks. It is likely more beneficial to grow rapidly than to avoid oxidative stress in the short term, especially considering that ROMs can be rapidly eliminated (e.g., through the activity of peroxidases; Halliwell and Gutteridge 2015). In addition, fasting is associated with reduced oxidative stress, probably because of a lower metabolic activity (reviewed in Ensminger et al. 2021). Therefore, lower levels of ROMs could be associated with a lower feeding frequency and an overall poorer nutritional status. However, because of the strong positive correlation between ROMs and body mass, we must remain cautious in regard to our interpretations. Indeed, the link between ROMs and survival cannot be distinguished from the link between body mass and survival. In addition, the survival costs of bearing elevated ROMs may become apparent later in life when cell and tissue damage accumulate (Wada and Heidinger 2019), as high oxidative damage is often related to lower survival both in adults and during the first years of life (Noguera et al. 2012; Herborn et al. 2016). In our study year, the environmental conditions were particularly good for penguins. Thus, we cannot exclude that the relationship between oxidative damage and survival could be different under harsher conditions that would make trade-offs more severe.

Conclusions

Our study provides rare evidence that oxidative damage might be a physiological cost of sibling competition, even in a bird species

that generates a maximum of two chicks per reproductive event. Further experimental studies are now needed to better evaluate the determinants of interindividual variations in oxidative stress in Adélie penguin chicks (e.g., cross-fostering experiments manipulating brood size and hatching order). The results of our work also suggest that growing large has short-term survival benefits for chicks, even if this strategy would incur increased production of oxidative damage. As emphasized recently by Wada and Coutts (2021), future studies should relate oxidative status at fledging to recruitment probability to explore further the consequences of early-life oxidative stress and, more generally, developmental stress for mid- to long-term survival. In addition, measuring antioxidant levels would help obtain a wider picture to fully evaluate the oxidative status in young penguins. Finally, our work shows that using physiological markers may be a promising tool for monitoring the effects of environmental challenge on wildlife populations, especially in a context of global change.

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