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Research Article

Early-life adversity modulates growth trajectories and red blood cell mitochondrial metabolism in king penguin chicks

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In many avian species, variation in breeding phenology is known to affect reproductive success. In king penguins, the breeding cycle lasts more than a year, and the start of egg-laying extends over three months, with early-breeders laying eggs around December and late-breeders around February. Consequently, late-born chicks have less time to grow and build-up their energy reserves before the austral winter. Variation in metabolic rate is one important pathway affecting differences in growth patterns, as it is directly involved both in energy allocation processes and fitness. The conversion of resources into energy occurs in mitochondria, and the efficiency of this conversion is likely to play a fundamental role in explaining individual variation in growth and survival. The purposes of this study were to investigate the differences in red blood cell mitochondrial metabolism between early- and late-born king penguin chicks and to assess whether growth patterns could be explained by differences in mitochondrial metabolism. Late chicks expressed higher mitochondrial metabolism than early chicks at 100 days post-hatching, probably linked to the stress related to winter environmental conditions and a decrease in parental feeding rates. We did not find a clear association between chick mitochondrial metabolism and growth patterns, suggesting that environmental conditions mostly contributed in explaining different metabolic phenotypes. As king penguin populations in Crozet may face changing breeding conditions (changes in feeding area and foraging trips duration) in relation to global changes, studying the physiological traits and adaptations underlying the chick growth and survival may help in understanding the response of king penguins facing challenging conditions.

Keywords: *Aptenodytes patagonicus*, breeding success, cellular metabolism, growth, phenology

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Introduction

Two important aspects influence the success of the breeding season: the timing of breeding (i.e. phenology) and the investment of individuals in breeding – both influencing reproductive performance and fitness (Both et al. 2006, Verhulst and Nilsson 2008, Gilsenan et al. 2020, Shipley et al. 2020). On one hand, the timing of breeding depends on environmental cues, such as photoperiod and ambient temperature, both of which influence the peak availability of food resources in the environment (Visser et al. 1998, Nilsson and Källander 2006, Helm et al. 2013, Marrot et al. 2018, Bonamour et al. 2019). On the other hand, the timing of breeding is also influenced by intrinsic factors such as breeder experience (age) and body condition (e.g. physiological capacity for the female to produce eggs). In many species, experienced and older breeders tend to breed earlier in the season (e.g. blackbirds, kittiwakes; Coulson and White 1956, Snow 1958, Perrins 1970). Phenological variation during the breeding season has been extensively studied to understand the effect of such variation on reproductive success (reviewed by Dunn 2004).

According to the match–mismatch hypothesis the timing of breeding and subsequent offspring growth should be synchronized with the peak of resource availability to maximize breeding success (Stenseth et al. 2002, Both et al. 2006, Ramírez et al. 2017, Kharouba and Wolkovich 2020). Breeding early in the season (but not too early) can bring several advantages, including more chances to find a good breeding habitat (nest, cavity, territory in a colony), to match the maximal food availability period, but also more chance of finding a good partner (Arvidsson and Neergaard 1991, Coté 2000, Currie et al. 2000, Both et al. 2006, Sutton and Freeman 2023). For some species, early-born offspring experience lower predation rate compared to late-born ones (Coté 2000, Götmark 2002, Sutton and Freeman 2023). All these reasons contribute to explain why experienced breeders tend to breed early in the season in many species, and why selection pressures often favor early breeding (Van Noordwijk et al. 1995, Visser et al. 1998, Marrot et al. 2018, De Villemereuil et al. 2020).

Among avian species with a large variation in the timing of reproduction, king penguins *Aptenodytes patagonicus* have a laying onset distributed over several months during the austral summer (November to mid-April), with early-breeders (egg hatching around January) and late-breeders (egg hatching around March). As the single chick reaches independence about 12 months after hatching, a successful breeding season (including pre-breeding molt) takes more than one year to complete (Stonehouse 1956, 1960, Weimerskirch et al. 1992, Brodin and Olsson 1998, Viblanc et al. 2014). During winter, the king penguin chicks remain in the colony on land (gathered all together in crèches), while parental food provisioning decreases in relation to the changes in the Antarctic polar front position (main feeding area during summer) and marginal ice zone (main feeding area during winter) (Bost et al. 1997, 2004, Charassin and Bost 2001, Scheffer et al. 2010, Saraux et al. 2012). Therefore, chicks may experience prolonged fasting periods during winter, leading to significant body mass loss (usually ca 40%) and a high mortality, especially for late-born chicks (Cherel et al. 1987, VanHeezik et al. 1993, Verrier 2003, Stier et al. 2014, Bost et al. 2015, Fernandes 2023).

Successful breeders attempting to breed the following season will be delayed and start breeding later in the austral summer. For the late-born chicks (hereinafter late-born versus early-born chicks are referred to as late-chicks versus early-chicks), the growth period is drastically reduced, resulting in a limited amount of time to accumulate body lipids and protein before winter, and as consequence late-chicks rarely survive the winter (first winter survival probability: 0.64 versus 0.29 for early- and late-chicks, respectively; VanHeezik et al. 1993, Geiger et al. 2012, Stier et al. 2014, Fernandes 2023, Fernandes et al. 2025). Prior studies showed that late-chicks can have a higher body mass and faster growth rates than early-chicks at the beginning of the growth period (VanHeezik et al. 1993, Stier et al. 2014). However, this difference is not maintained over the entire growth period: late-chicks are smaller and lighter than early-chicks before winter, and remain smaller at fledging (i.e. departure at sea) (VanHeezik et al. 1993, Verrier 2003, Stier et al. 2014, Fernandes 2023). Beside different growth trajectories, early- and late-chicks also differ in their physiology: late-chicks have higher blood corticosterone levels, higher oxidative stress and DNA damage, but also have reduced blood cell telomere length compared to early-chicks at 10 days post-hatching (Stier et al. 2014). Despite short-term benefits, accelerated growth rates most likely come at a cost for the king penguin chicks (Geiger et al. 2012). Overall, a late breeding attempt in the season has consequences not only on offspring survival and breeding success, but also on offspring phenotype.

On one hand, variation in growth trajectories and survival between early- and late-chicks could be linked to environmental factors, including forecast variability, access to nutritional resources (parental feeding rates) or predation pressure (with small chicks more likely predated) (Descamps et al. 2005, Eichhorn et al. 2011). On the other hand, variation in growth and survival could also be linked to differences in energy use between the two groups of chicks. Among plastic phenotypic traits, variation in metabolic rate is one important factor affecting growth patterns, as it is directly involved in energy allocation processes (Brown et al. 2018, Burger et al. 2019, 2021, Norin and Metcalfe 2019). The conversion of resources into energy occurs through oxidative phosphorylation in mitochondria, where metabolic fuels are transformed into energy (ATP synthesis). The efficiency of this conversion, as well as the rate of production of pro-ageing compounds is likely to play a fundamental role in explaining individual heterogeneity in growth and survival (Salin et al. 2019, Heine and Hood 2020, Koch et al. 2021, Goodrich and Clark 2023). Previous research showed that whole-organism metabolic rate and skeletal muscle metabolism can be modulated by the fasting process (Duchamp et al. 1989, Teulier et al. 2013, Monternier et al. 2014, Bourguignon et al. 2017).

For instance, skeletal muscle metabolic activity of king penguin chicks is globally reduced during long-term fasting (4–5 months), but ATP synthesis efficiency increases at the final stage of fasting (Bourguignon et al. 2017). However, whether mitochondrial metabolism and energy used by offspring differs depending on the timing of breeding (early versus late in the season) remains to be determined. Such variation may be expected since studies in other species have shown that the regularity and amount of provisioning patterns, as well as the type of food resources provided, affect mitochondrial function (in fish: Salin et al. 2016; in birds: Cooper-Mullin et al. 2021; in mammals: Kyriazis et al. 2022).

The purposes of this study were to investigate 1) the differences in growth trajectories and 2) red blood cell (RBC) mitochondrial metabolism between early- and late-born king penguin chicks and 3) relate potential differences in mitochondrial performances to growth trajectories. As avian RBCs are nucleated, mitochondrial metabolism can be measured in these cells and used as a proxy of respiration in other tissues, enabling less-invasive and longitudinal studies (Koch et al. 2021, Casagrande et al. 2023, Cossin-Sevrin 2024, Thoral et al. 2024b; for a validation in king penguin, Stier et al. 2017). Although RBCs may not directly be involved in the chick growth (as other tissues and organs), their function of carrying oxygen to the whole organism is expected to affect the individual growth. Moreover, prior studies demonstrated RBC mitochondrial metabolism to correlate with the whole-animal metabolism (Koch et al. 2021, Casagrande et al. 2023, Thoral et al. 2024a). King penguin chicks experience a rapid body mass increase during their first summer (beginning of the growth period), and then (usually) a decrease in body mass during winter when parental feeding decreases (VanHeezik et al. 1993, Verrier 2003, Stier et al. 2014, Fernandes 2023). To monitor these different stages, we collected blood samples in early- and late-chicks at different time-points: at the beginning of the rapid growth period (35 days post-hatching), at the end of the rapid growth period (100 days post-hatching) and during winter (150 days post-hatching). As prior literature showed that late-chicks express higher markers of oxidative stress (Stier et al. 2014), we expected late-chicks to express higher RBC mitochondrial metabolism (potentially leading to a higher production of oxidative stress markers). For example, higher oxidative stress in late-chicks could be linked to differences in mitochondrial proton leak, or in the usage of mitochondrial complex I, which is known to contribute to the cellular production of reactive oxygen species (ROS) (Hirst 2013, Wirth et al. 2016). Higher mitochondrial metabolism and ATP production would also be expected to sustain faster growth rates in late-chicks. Because a decrease in blood cell mitochondrial content with age during the growth period has been reported in several species (e.g. great tits, Japanese quails; Cossin-Sevrin et al. 2022, Stier et al. 2022, Cossin-Sevrin et al. 2023) we expected RBC mitochondrial metabolism of king penguin chicks to decrease as the chicks aged.

Material and methods

Ecology of king penguins and study area

This study was conducted on Possession Island in the Crozet Archipelago (46°25′S; 51°52′E) during the breeding seasons 2020–2021 and 2021–2022 in the king penguin colony 'La Grande Manchotière, Baie du Marin', hosting ca 20 000 breeding pairs (Barbraud et al. 2020). Following courtship and egg-laying (November–January), the single chick is fed by both parents until the beginning of winter. During winter (May–September), the chick remains on land in crèches in the colony and experiences long periods with a few feeding events, or no feeding at all (Cherel et al. 1987, Weimerskirch et al. 1992, Saraux et al. 2012). Parents start by regularly feeding the chick again during spring until the chick reaches independence a few months later (the subsequent summer, November–January), having completed its juvenile molt and being able to feed itself more than ca 12 months after egg laying (Stonehouse 1960, Weimerskirch et al. 1992, Ancel et al. 2013). As a result, king penguin breeders that had a successful breeding season cannot complete their breeding molt and the start of the following breeding cycle on time. The onset of egg-laying is thereby distributed over several months, with early breeding attempts between November/December (hatching around January) and late breeding attempts between January/February (hatching around March) (Fig. 1).

Data were collected on both early-born ($n_{2020}=16$, $n_{2021}=16$ individuals) and late-born king penguin chicks ($n_{2020}=15$, $n_{2021}=8$ individuals). In 2020–2021, the average hatching date (counted as the number of days in the year ± SD [range]) was in 17.8 ± 3.7 days [12, 26] for early-chicks and 59.7 ± 4.7 days [54, 70] for the late-chicks. In 2021–2022, the average hatching date was 11.2 ± 4.3 days [4, 18] for early-chicks and 65.6 ± 5.3 days [59, 77] for the late-chicks. Breeding pairs were randomly selected on the border of the colony (to minimize disturbance) during courtship and marked with a non-permanent animal dye (Porcimark). Breeding pairs were monitored daily to record egg-laying and hatching dates (± 24 h). The day where the chick was found completely outside of the egg shell was counted as 'day 1'. As the parents alternate between foraging trips at sea and fasting periods on land in the colony to complete incubation and brooding tasks, we closely monitored the identity of the parent (female or male) present on land with the offspring. Females and males were visually sexed according to the order of parental alternation in the colony (males are the first to incubate), vocalization and morphometrics (Stonehouse 1960, Weimerskirch et al. 1992, Kriesell et al. 2018). Chicks included in this study were individually marked with colour-coded fish tags (Floy Tag and MFG, Inc.; placed at 20 days post-hatching), and a Darvic plastic flipper-band from 100 days post-hatching (semi-rigid PVC Darvic bands; 25.8 mm wide, 1.9 mm thick, 7.4 g). Darvic flipper-bands were removed before the completion of the juvenile molt and the first departure at sea (ca 325 days post-hatching).

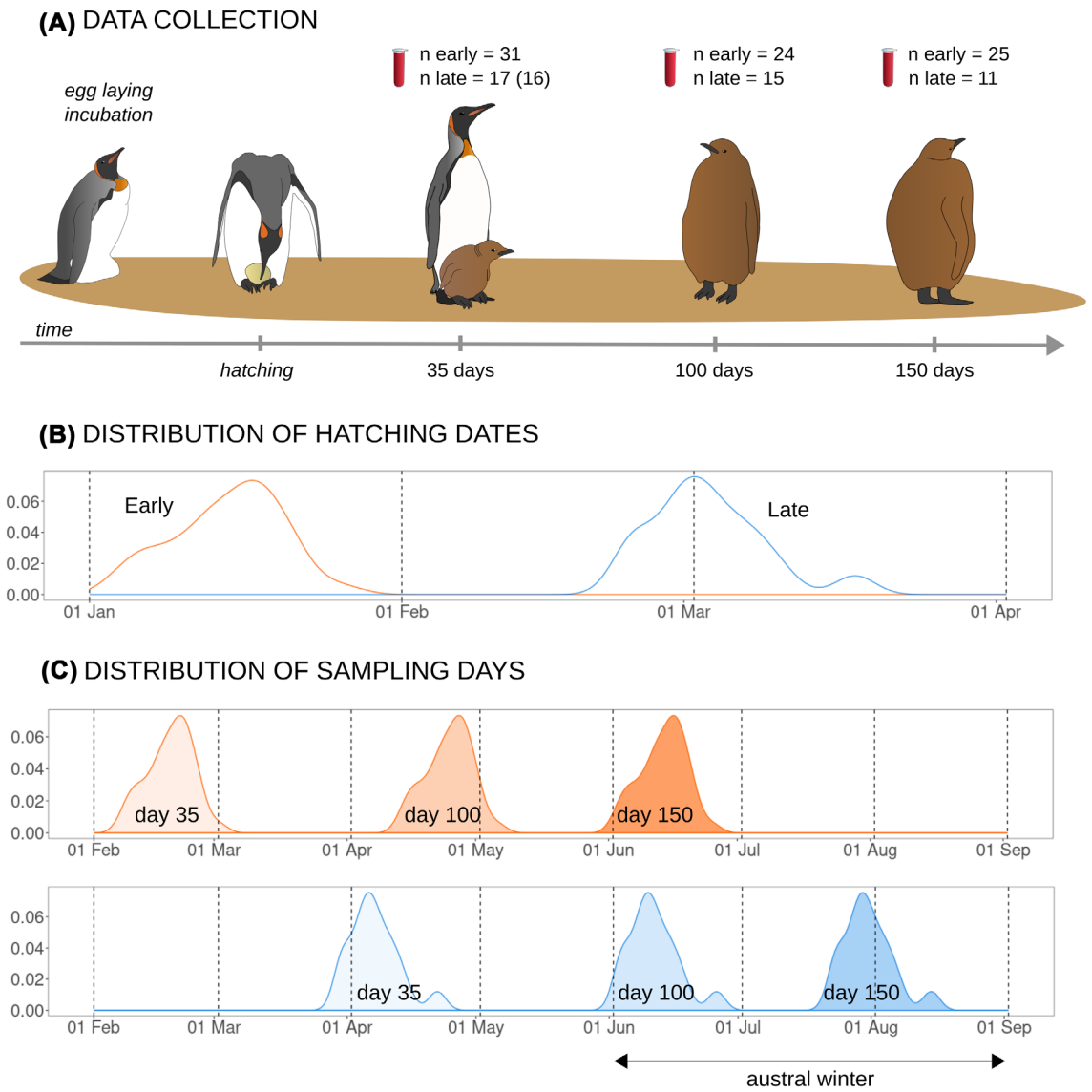

Figure 1. Diagram of the study design. Data collection of red blood cell (RBC) mitochondrial metabolic rates measurements (A), density of the distribution of hatching dates (B), density of the distribution of sampling days (C) across the breeding season for early- versus late-chicks. Note that the scale differs for (B) and (C). Sample sizes for RBC mitochondrial respiration measurements are indicated. A total of 55 individuals were included in this study: $n_{early} = 31$ and $n_{late} = 23$ individuals. For 35 days measurements, one value was missing for ROUTINE (sample size in brackets).

To investigate differences in growth trajectories, metabolism and survival between early- and late-chicks, king penguin chicks were captured in the colony for morphometric measurements and blood sampling (see below) at 35 days (mean ± SD = 35.2 ± 0.5 days), 100 days (99.9 ± 0.6 days) and 150 days (149.5 ± 1.2 days) post-hatching (Fig. 1). From 35 days to 100 days after hatching, the chick is experiencing a period of rapid body mass gain. At the beginning of winter (respectively ca 150 days for early-chicks and 100 days for the late-chicks in this study), the chick is experiencing a reduced number of feeding events by the parents (Cherel et al. 1987, Weimerskirch et al. 1992, Geiger et al. 2012, Saraux et al. 2012, Stier et al. 2014). According to the situation, the capture procedure was: 1) if the chick was incubated by the parent during the procedure, we covered the adult's eyes with a hood to reduce handling stress and provided a dummy egg to the adult while delicately removing the chick. The chick was carefully placed in a warm bag using a hot water bottle until the end of the procedure and then replaced under the parental brood patch. 2) If the chick was not incubated by the parent, the parent (if present) usually moved away over a few meters upon experimenter approach, leaving the chick behind. The chick was then captured and returned to the colony in the same area after the procedure. The return of the adult was then monitored from a distance, using binoculars, until the chick was reunited with its parent. Chick body mass was obtained using a spring scale (± 5 g) for 35 day-old chicks and using an electronic scale (± 2 g) for 100 and 150

days-old chicks. Flipper length (an index of structural size) was measured using a solid metal ruler (± 1 mm).

RBC mitochondrial aerobic metabolism

To measure RBC mitochondrial aerobic metabolism, blood samples were collected from the marginal flipper vein using a G25 needle fitted to a 1 ml heparinized syringe for the 35 days-old chicks (maximum 0.9 ml) and with a G23 needle fitted to a 2.5 ml heparinized syringe for the 100 and 150 days-old chicks (maximum 1.5 ml). Blood samples were transferred within 5–10 min to a laboratory facility located in the vicinity of the king penguin colony for processing. After refrigerated centrifugation (+4°C, 5 min, 800 g), RBCs were resuspended in PBS and kept at +4°C until high-resolution respirometry measurements could be performed at the research station located a couple of kilometers from the colony, a few hours later (median, 1st quartile–3rd quartile: 5.25 h, 3.8–6.6 based on 103 individuals), using two Oroboros instruments. Mitochondrial respiration has previously been shown to remain preserved for at least 24 h after sampling (Stier al. 2017); therefore, we did not include the time interval between sampling and measurement as a covariate in our analyses. RBCs were centrifuged a second time to remove PBS (+4°C, 5 min, 800 g) and 50 µl of packed RBCs (or 25 µl in cases of limited blood sample volume) were resuspended in MIR05 respiration buffer to measure mitochondrial metabolism on permeabilized RBC at 38°C using a similar protocol as described in Stier et al. (2019) and Cossin-Sevrin et al. (2023, 2025): digitonin (20 µg × ml^{-1}), pyruvate (5 mM), malate (2 mM), ADP (1.25 mM), succinate (10 mM), oligomycin (2.5 µM), antimycin A (2.5 µM).

Five distinct respiration rates were calculated and analysed: 1) the endogenous cellular respiration rate before permeabilization (ROUTINE), 2) the maximum respiration rate fuelled with exogenous substrates of complex I (pyruvate/malate), as well as ADP ($OXPHOS_{CI}$), 3) the maximum respiration rate fuelled with exogenous substrates of complexes I and II (succinate), as well as ADP ($OXPHOS_{CI+II}$), 4) the respiration rate contributing to the proton leak after adding oligomycin in the presence of exogenous substrates of complexes I and II ($LEAK_{CI+II}$), and 5) the respiration rate supporting ATP synthesis through oxidative phosphorylation, here referred to as mitochondrial aerobic scope (mtAS, calculated as $OXPHOS_{CI+II} - LEAK_{CI+II}$). Non-mitochondrial respiration was measured after the addition of antimycin A (2.5 µM) (raw data average ± SD: 0.90 ± 0.30 pmol × s^{-1}) and was subtracted from all respiration rates. We also calculated two mitochondrial flux ratios (FCR): 1) OXPHOS coupling efficiency (OxCE, calculated as [1 – ($LEAK_{CI+II}$/mtAS)], and 2) the proportion of maximal respiration capacity being used under endogenous cellular condition ($FCR_{R/CI+II}$) calculated as (ROUTINE/$OXPHOS_{CI+II}$). Respiration rates were standardized by the quantification of total protein measured in each sample, using a Pierce BCA protein assay kit. Preliminary analyses confirmed that total protein levels did not differ according to hatching group, and could thereby be used for standardisation (see below). Repeatability of the quantification of total protein (based on sample-duplicate) was high: R [CI 95%] = 0.980 [0.976, 0.984]. Technical repeatability of mitochondrial metabolic rates based on nine duplicates was: ROUTINE: R [CI 95%] = 0.712 [0.451, 0.905]; $OXPHOS_{CI}$: R = 0.937 [0.867, 0.98]; $OXPHOS_{CI+II}$: R = 0.878 [0.733, 0.963]; $LEAK_{CI+II}$: R = 0.841 [0.674, 0.949]; and mtAS: R = 0.915 [0.816, 0.975].

Statistical analysis

Statistical analyses were conducted using R ver. 4.0.2. software (www.r-project.org). For morphometrics, our final sample size included 137 measurements from 32 early-chicks and 23 late-chicks: n_{early} = 32, 27, 28 versus n_{late} = 22, 16, 12 at day 35, 100 and 150, respectively. For RBC mitochondrial metabolism measurements, the final sample size encompassed 123 measurements from 31 early-chicks and 17 late-chicks: n_{early} = 31, 24, 25 versus n_{late} = 17 (16 for ROUTINE), 15, 11 at day 35, 100 and 150, respectively. Our final sample size was lower than expected as a result of missing data due to field constraints, such as bad weather and success at collecting blood samples. Furthermore, king penguin chicks experience a natural mortality during the growth period, which is stronger in late- than early-chicks (Weimerskirch et al. 1992, VanHeezik et al. 1993, Stier et al. 2014, Fernandes 2023). Here, chicks' mortality before day 100, after day 100 and after day 150 respectively were: n_{early} = 0, 0, 5 versus n_{late} = 1, 4, 2 dead individuals for 2020–2021 and n_{early} = 0, 0, 1 versus n_{late} = 6, 0, 2 dead individuals for 2021–2022.

To investigate the differences in growth trajectories between early- and late-chicks, we used linear mixed models (LMMs, 'lme4' package in R) (Bates et al. 2015; www.r-project.org) with chick body mass, body size (flipper length) and body condition (scale mass index as calculated in Peig and Green 2009) as response variables and chick age (three levels: 35, 100 and 150 days post-hatching) in interaction with the hatching group (two levels: early versus late), and the year of sampling (two levels: 2020 versus 2021), as independent variables in the model. Bird ID was included as a random effect to account for the non-independence of measures collected on the same chick.

To test if RBC mitochondrial metabolic rates were different between early- and late-chicks, we performed similar LMMs as described above (except for $LEAK_{CI+II}$), using mitochondrial metabolic rates as response variables. $LEAK_{CI+II}$ was analysed using a generalized linear mixed model (GLMM, 'lme4' package in R) with a gamma error distribution. We preliminarily tested whether total protein levels differ between hatching group to ensure this measure could be used to standardised RBC mitochondrial metabolic traits, by using a linear mixed model with hatching group and age as fixed factors and bird ID as random intercept. Total protein levels did not differ according to hatching group (LMM, p = 0.51; Supporting information), but increased with age ($p < 0.001$). Here, an increase of red blood cell total protein levels with age may be related to different nutritional and dehydration status linked to the changes in food resources in king penguin chick ecology

(Campbell 2015). For mitochondrial traits, we preliminarily tested the interaction between the age and the group in the models, but removed it if non-significant in order to properly interpret the main effects without scaling variables. In case of significant interaction, multiple post hoc comparisons were conducted using the 'emmeans' package and adjusted for Tukey honest significant differences (HSD) correction (Lenth and Piaskowski 2025). To complete LMMs, we also conducted a linear discriminant analysis (LDA) using the 'MASS' package in R (Venables and Ripley 2002), where the hatching group of chicks (early versus late) was discriminated according to RBC mitochondrial metabolic rates. With this complementary analysis, we aimed to test if we could separate early- and late-chicks according to their RBC mitochondrial metabolic profile, including ROUTINE, $OXPHOS_{CI}$, $OXPHOS_{CI+II}$ and $LEAK_{CI+II}$. mtAS and FCR(s) could not be included in the LDA because of their non-independence with other metabolic rates (e.g. mtAS calculated as $OXPHOS_{CI+II} - LEAK_{CI+II}$). To test if RBC mitochondrial metabolism was associated with chick body mass, we performed separate LMMs using chick body mass as a continuous variable, and by including the age (three levels: day 35, day 100, day 150), and year of sampling (two levels: 2020 versus 2021) as fixed effects. Bird ID was included as random effect. Following our findings (see Results), we used complementary LMs to test if RBC mitochondrial metabolisms measured at 100 days were associated with the environmental context. To achieve this aim, we used each RBC mitochondrial metabolic rate as a response variable and included the date of sampling (in number of weeks in the year) and the year of sampling (two levels: 2020 versus 2021) as fixed factors.

We used LMs to test the association between growth trajectories (body mass gain) and RBC mitochondrial metabolism during the rapid growth period (from day 35 to day 100 post-hatching). Body mass gain (differences in body mass between 35 and 100 days) was specified as the response variable. For each RBC mitochondrial metabolic rate, measurements collected at day 35 and day 100 were both included as continuous variables in the models, while the hatching group (two levels: early versus late) and the year of sampling (two levels: 2020 versus 2021) were included as fixed factors. Finally, to test the association between body mass change during winter and RBC mitochondrial metabolism, we performed similar analyses as described above, using the body mass changes between 100 and 150 days as a response variable. Potential multicollinearity between mitochondrial predictors measured at different time points was assessed using variance inflation factors (VIFs, all < 3.6).

Fledging success of the chicks was monitored (individuals fledgling around ca 325 days post-hatching), and chick survival was modelled using GLMs with a logistic binary distribution of the response variable (survival: 0 = dead, 1 = alive), with the hatching group (two levels: early versus late) and year of sampling (two levels: 2020 versus 2021) included as fixed factors.

Results

Differences in growth trajectories and survival between early- and late-chicks

We found a significant interaction of the age and the hatching group for chick body mass (age × group: $F_{2,89.1} = 61.68$, $p < 0.001$), body size (age × group: $F_{2,78.8} = 86.12$, $p < 0.001$) and body condition (age × group: $F_{2,87.2} = 19.8$, $p < 0.001$). Body mass, size and condition did not differ between early- and late-chicks at day 35 (post hoc comparisons: early versus late, all $p > 0.10$). At 100 and 150 days post-hatching, however, late-chicks were lighter (post hoc comparisons: early versus late, day 100: −40.3% and day 150: −43.3% for late-chicks; all $p < 0.001$; see Table 1A, Fig. 2A), smaller (post hoc comparisons: early versus late, day 100: −22.3% and day 150: −19.3% for late-chicks, all $p < 0.001$; Table 1B, Fig. 2B) and with lower body condition than early-chicks (Tukey HSD post hoc comparisons: early versus late, day 100: −16.5% and day 150: −25.3% for late-chicks, all $p \leq 0.001$; Table 1). Chick body condition was significantly lower (−10%) during the 2021–2022 breeding season compared to 2020–2021 ($F_{1,51.41} = 11.49$, $p = 0.001$). Fledging success (i.e. chick departure at sea) was significantly lower for late-chicks (early: 23 chicks fledged out of 32; late: two chicks fledged out of 23) ($p < 0.001$), but was not affected by the year of sampling ($p = 0.06$).

RBC mitochondrial metabolism

The interaction between age and hatching group was statistically significant for ROUTINE (age × group: $F_{2,85.8} = 6.96$, $p = 0.002$) and $FCR_{R/CI+II}$ (age × group: $F_{2,84.7} = 7.42$, $p = 0.001$). For both ROUTINE and $FCR_{R/CI+II}$, differences between early- and late-chicks were only significant at 100 days post-hatching, with higher RBC metabolic rates for late-chicks (post hoc comparisons early versus late at day 100: all $p < 0.001$; Fig. 3).

$OXPHOS_{CI}$ was significantly higher in late-chicks than early-chicks (estimate ± SE = 0.63 ± 0.30, $F_{1,38.1} = 4.32$, $p = 0.04$; Table 2, Fig. 4). In contrast, $OXPHOS_{CI+II}$, $LEAK_{CI+II}$, mtAS and OXPHOS coupling efficiency were not different between early- and late-chicks (Table 2).

When conducting supplementary analyses to assess differences observed at day 100: ROUTINE, $OXPHOS_{CI}$ and $FCR_{R/CI+II}$ were not associated with chick body mass measured at day 100 days (all $F < 0.88$, all $p > 0.35$; Supporting information), but significantly increased with sampling date (number of weeks in the year) (all $F > 5.12$, all $p < 0.03$; Supporting information).

Except for $OXPHOS_{CI}$, all RBC metabolic rates were significantly impacted by sampling year, with higher ROUTINE, $FCR_{R/CI+II}$ and $LEAK_{CI+II}$ (all $F > 7.25$, all $p < 0.01$), but lower mtAS and OXPHOS coupling efficiency in the breeding season 2021–2022 (year 2021) than 2020–2021 (year 2020) (all $F > 6.77$, all $p < 0.01$).

All RBC mitochondrial metabolic rates decreased with age (all $F > 29.32$, all $p < 0.001$; Table 2, Fig. 4).

Table 1. Results of linear mixed models (LMMs) testing the effect of the interaction between the chick age (number of days post-hatching) and hatching group (early versus late) on (A) chick body mass (g) and (B) chick body size (flipper length, mm). Observations day 35: $n_{early}/n_{late}=32/22$; day 100: $n_{early}/n_{late}=27/16$, day 150: $n_{early}/n_{late}=28/12$; n = 55 individuals in total. Final sample sizes are lower than expected due to missing data (Material and methods). Estimated marginal means are reported with their SE. Post hoc comparisons with Tukey HSD correction are presented for the early and late groups. Bird ID was included as a random intercept in all models. σ^2, within-group variance; $\tau 00$, between-group variance. Sample size (n) along with marginal (fixed effects only) and conditional (fixed and random effects). Bold indicates significance ($p < 0.05$).

Predictors	(A) Body mass (g)			(B) Body size (flipper length, mm)		
	Estimate	SE	p	Estimate	SE	p
(Intercept)	3281.5	211.06	**< 0.001**	156.84	4.09	**< 0.001**
Age (day 100)	4918.4	191.58	**< 0.001**	142.30	2.80	**< 0.001**
Age (day 150)	4286.82	189.23	**< 0.001**	144.22	2.76	**< 0.001**
Hatching group (late)	−149.21	275.71	0.59	−8.84	5.24	0.10
Year (2021)	−370.82	234.97	0.12	1.94	4.78	0.69
Age (day 100) × group (late)	−3080.96	314.33	**< 0.001**	−57.99	4.70	**< 0.001**
Age (day 150) × group (late)	−3045.54	355.64	**< 0.001**	−49.46	5.04	**< 0.001**
Post hoc comparisons for:						
Day 35: early versus late	149	276	0.590	8.84	5.24	0.10
Day 100: early versus late	3230	311	**< 0.001**	66.82	5.66	**< 0.001**
Day 150: early versus late	3195	331	**< 0.001**	58.29	5.92	**< 0.001**
Random effects:						
σ^2	719.8			10.44		
$\tau 00$ bird	682.4			15.59		
n observations	137			136		
n individuals	55			55		
Marginal R^2 /conditional R^2	0.91/0.82			0.98/0.93		

We used a linear discriminant analysis (LDA) to test the differences in RBC mitochondrial metabolic profile (ROUTINE, $OXPHOS_{CI}$, $OXPHOS_{CI+II}$, $LEAK_{CI+II}$) between early- and late-chicks. Early and late-chicks were statistically different at day 100 (ANOVA: $F_{1,37}=15.49$, $p < 0.001$; Fig. 5), but not at 35 days or 150 days post-hatching (ANOVA: all $F < 2.19$, $p > 0.15$; Fig. 5). When comparing RBC mitochondrial metabolic profile measured in early- and late-chicks during a similar period during the season (early-chicks at day 150 and late-chicks at day 100; Fig. 1), LDA analysis revealed a significant difference between groups (ANOVA: $F_{1,38}=12.32$, $p=0.001$; Fig. 5).

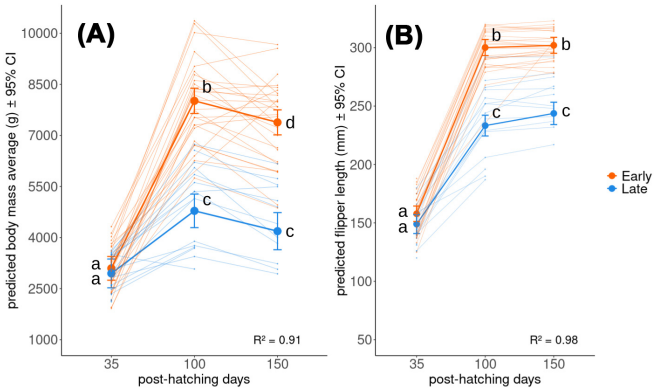

Figure 2. Predicted body mass (g) (A) and body size (flipper length, mm) (B) of king penguin chicks from 35 to 150 post-hatching days according to hatching group (early versus late). Predicted averages (dots) are shown with their 95% confidence interval (CI). Results from Tukey HSD post hoc tests are reported. The letters indicate significance of the post hoc comparisons: if groups share a letter, they are not significantly different. R^2 from LMMs are shown (see details and sample sizes in Table 1). Raw data are plotted behind predicted average (lines). Orange colour refers to the early-chicks, blue colour refers to the late-chicks.

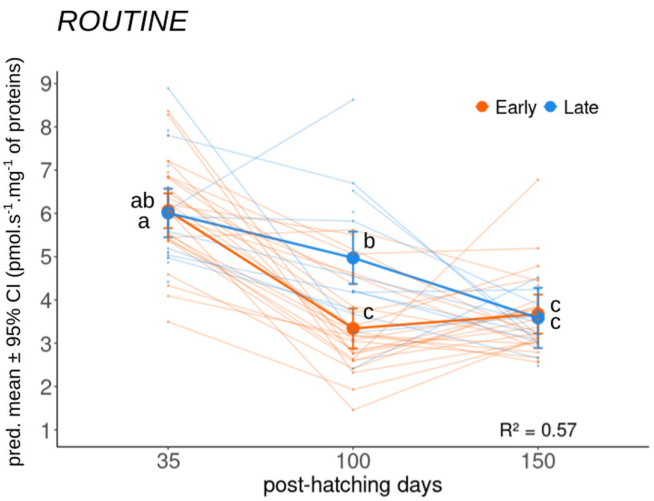

Figure 3. Predicted ROUTINE metabolic rate (pmol × s^{-1} × mg^{-1} of proteins) measured in king penguin chicks from 35 to 150 post-hatching days according to hatching group (early versus late). Predicted averages (dots) are shown with their 95% confidence interval (CI). Results from Tukey HSD post hoc tests are reported. The letters indicate significance of the post hoc comparisons: if groups share a letter, they are not significantly different. R^2 from LMM is presented. Raw data are plotted behind predicted average (lines). Orange colour refers to the early-chicks, blue colour refers to the late-chicks.

Table 2. Results of linear mixed models (and generalized linear mixed model for $LEAK_{CI+II}$) testing the effect of chick age (in number of days post-hatching), hatching group (early versus late) and year (2020 versus 2021) on chick RBC mitochondrial metabolic rates (pmol × s^{-1} × mg^{-1} of proteins) and FCRs. Observations day 35: n_{early}/n_{late} = 31/17; day 100: n_{early}/n_{late} = 24/15; day 150: n_{early}/n_{late} = 25/11; n = 55 individuals in total. Age × hatching group interaction was initially included in models, but was removed when found to be non-significant. OxCE refers to OXPHOS coupling efficiency. Estimates are reported with their SE. σ^2, within-group variance; τ_{00}, between-group variance. Sample size (n) along with marginal (fixed effects only) and conditional (fixed and random effects). Bold indicates significance ($p < 0.05$).

Predictors	$OXPHOS_{CI}$		$OXPHOS_{CI+II}$		$LEAK_{CI+II}$		mtAS		OxCE	
	Estimate ± SE	p	Estimate ± SE	p	Estimate ± SE	p	Estimate ± SE	p	Estimate ± SE	p
(Intercept)	5.96 ± 0.29	**< 0.001**	12.58 ± 0.40	**< 0.001**	0.59 ± 0.06	**< 0.001**	10.72 ± 0.37	**< 0.001**	201.66 ± 21.32	**< 0.001**
Age (day 100)	−1.84 ± 0.31	**< 0.001**	−4.09 ± 0.43	**< 0.001**	−0.32 ± 0.05	**< 0.001**	−3.53 ± 0.39	**< 0.001**	−0.02 ± 0.01	**0.03**
Age (day 150)	−2.23 ± 0.32	**< 0.001**	−5.10 ± 0.45	**< 0.001**	−0.41 ± 0.05	**< 0.001**	−4.38 ± 0.40	**< 0.001**	−0.03 ± 0.01	**0.01**
Hatching group (late)	0.62 ± 0.30	**0.04**	0.54 ± 0.41	0.19	0.03 ± 0.06	0.58	0.43 ± 0.39	0.27	-5×10^{-4} ± 0.01	0.96
Year (2021)	−0.29 ± 0.29	0.33	−0.16 ± 0.40	0.69	0.44 ± 0.06	**< 0.001**	−0.99 ± 0.38	**0.01**	−0.10 ± 0.01	**< 0.001**
Random effects										
σ^2	1.42		1.99		0.23		1.77		0.05	
τ00 bird	0.39		0.41		0.10		0.61		0.02	
n observations	123		123		123		123		123	
n individuals	55		55		55		55		55	
Marginal R^2/ conditional R^2	0.39/0.35		0.58/0.57		0.51/0.42		0.59/0.54		0.56/0.50	

Predictions of growth trajectories by RBC mitochondrial metabolism

Except for $FCR_{R/CI+II}$, differences in body mass gain from day 35 to day 100 were neither associated with RBC mitochondrial metabolic rates measured at day 35 nor at day 100 (all $p > 0.07$; Supporting information). We found a significant association between body mass gain and $FCR_{R/CI+II}$ measured at day 100 ($p = 0.04$); however, this association was driven by a single measure from a late-chick with a high $FCR_{R/CI+II}$ (> 1) at day 100 (Supporting information) and was not significant anymore if this value was excluded ($p = 0.08$). Body mass gain in the beginning of the growth period was strongly associated with the hatching group, with lower body mass gain for the late-chicks (all $p < 0.001$; Supporting information).

Again, except for $FCR_{R/CI+II}$, changes in body mass from day 100 to day 150 were not associated with RBC metabolic rates measured at day 100 or at day 150 (all $p > 0.07$; Supporting information). For $FCR_{R/CI+II}$, changes in body mass were significantly associated with $FCR_{R/CI+II}$ measured at day 150 ($p = 0.01$; Supporting information) and this effect remained significant even with the exclusion of the maximum value ($p = 0.03$; Supporting information).

Discussion

We found marked differences in the growth trajectories of king penguin chicks that hatched early or late in the breeding season. Early- and late-chicks had similar body masses and sizes at 35 days post-hatching. In line with previous studies, chick body mass rapidly increased from 35 to 100 days post-hatching, and then decreased (significantly for the early-chicks) from 100 to 150 days post-hatching (Geiger et al. 2012, Stier et al. 2014). This decrease in body mass is most likely linked to a reduction in the number of feeding events for both groups following the rapid growth period (Saraux et al. 2012). Parental chick feeding decreases in association with the Antarctic polar front position (abundant distribution of myctophid fishes), shifting southward towards marginal ice zone during winter, and therefore extending the foraging trips for the parents (Cherel and Ridoux 1992, Jouventin et al. 1994, Bost et al. 2004, Freeman et al. 2016). Whereas the accurate position of the Antarctic polar front varies between years, its withdrawal may even take place a few weeks earlier before the beginning of winter (starting in May) (Stonehouse 1956, 1960, Charrassin and Bost 2001, Freeman et al. 2016, Cristofari et al. 2018). For instance, Charrassin and Bost (2001) reported an average foraging trip distance of ca 340 km, ca 900 km and ca 1610 km during austral summer, autumn and winter, respectively, for the king penguin population in Crozet (data collected between 1994 and 1997, but similar to results presented in Bost et al. 2015). In our study sample, late-chicks were around 100 days-old when facing winter conditions, while early-chicks were around 150 days-old. This means that the beginning of the growth period (from day 35 to day 100) took place during favorable conditions for early-chicks (summer–autumn),

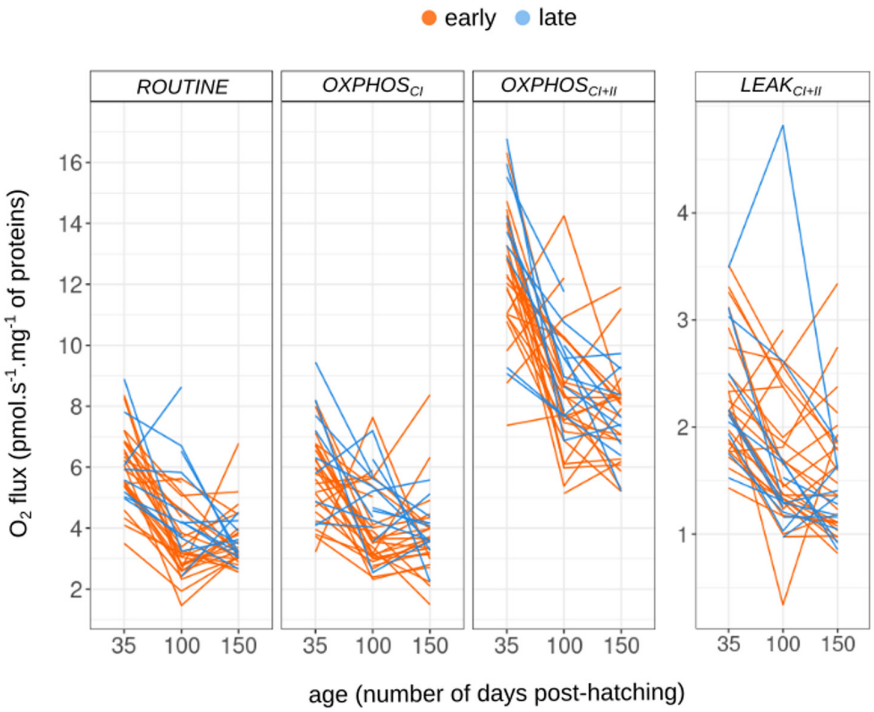

Figure 4. Changes in red blood cell (RBC) mitochondrial metabolic rates (pmol × s^{-1} × mg^{-1} of proteins) in king penguin chicks from 35 to 150 post-hatching days according to hatching group (early versus late). Raw data are presented. Orange colour refers to the early-chicks, blue colour refers to the late-chicks. Observations day 35: n_{early}/n_{late} = 31/17; day 100: n_{early}/n_{late} = 24/15; day 150: n_{early}/n_{late} = 25/11; n = 55 individuals in total. See details of the models in the Supporting information.

while for late-chicks this period coincided with the end of autumn–beginning of winter. When comparing similar ages, late-chicks were significantly lighter and smaller than the early-chicks from 100 days post-hatching and onwards as reported in the literature (VanHeezik et al. 1993, Stier et al. 2014, Fernandes 2023). Prior studies showed that late-chicks can experience faster growth rates, and as a result express different markers of physiological stress (higher ROS levels and telomere loss) (Geiger et al. 2012, Stier et al. 2014). While we did not strictly compare the body masses of early and late-chicks a few days after hatching in our study, and therefore cannot assess if late-chicks experienced a faster growth rate at the beginning of the rearing period, we show that late-chicks did not manage to catch up to an equivalent body mass and size of early-chicks before winter.

As expected, we found a significantly lower fledging success for late-chicks, probably because of their small body mass and size, which prevented them from surviving during winter (Weimerskirch et al. 1992, VanHeezik et al. 1993, Stier et al. 2014, Fernandes 2023). In our case, it is important to consider that we selected individuals that survived at least until 35 days to include them in this study – constituting a biased sample size with selective disappearance (mortality before 35 days post-hatching not taken into account). However, similar patterns have been found on larger sample sizes (Fernandes 2023) and are well documented in the literature (Weimerskirch et al. 1992, VanHeezik et al. 1993, Stier et al. 2014).

The main objective of this study was to test if early- and late-chicks expressed different red blood cell (RBCs) mitochondrial metabolic rates during the rapid growth period (from day 35 to day 100), but also during the winter period. We expected late-chicks to have higher RBC mitochondrial metabolism and a higher coupling efficiency to meet the energy requirement linked to faster or compensatory growth. As late-chicks have been found to exhibit higher oxidative stress levels (Stier et al. 2014), we expected to find higher respiration of complex I ($OXPHOS_{CI}$) and proton leak ($LEAK_{CI+II}$) in these individuals. Both the oxygen consumption before RBC permeabilization (endogenous respiration, ROUTINE) and the ratio between the endogenous respiration and the maximum respiration of complexes I and II ($FCR_{R/CI+II}$) were higher for late-chicks at 100 days post-hatching. In other words, at similar ages, late-chicks consumed more oxygen and had a higher usage of the complexes I and II respiration maximum capacity at the end of the rapid growth period (day 100). Additionally, the maximal respiration capacity of the complex I ($OXPHOS_{CI}$) was significantly higher in late-chicks at all ages. Yet, the respiration linked to oxidative phosphorylation (mitochondrial aerobic scope, mtAS) did not differ between early- and late-chicks, meaning that late-chicks had higher, but not more efficient RBC metabolism than the early-chicks when comparing same ages (i.e. no difference observed in OXPHOS coupling efficiency).

These results were also confirmed by linear discriminant analysis (LDA), which was able to well separate the

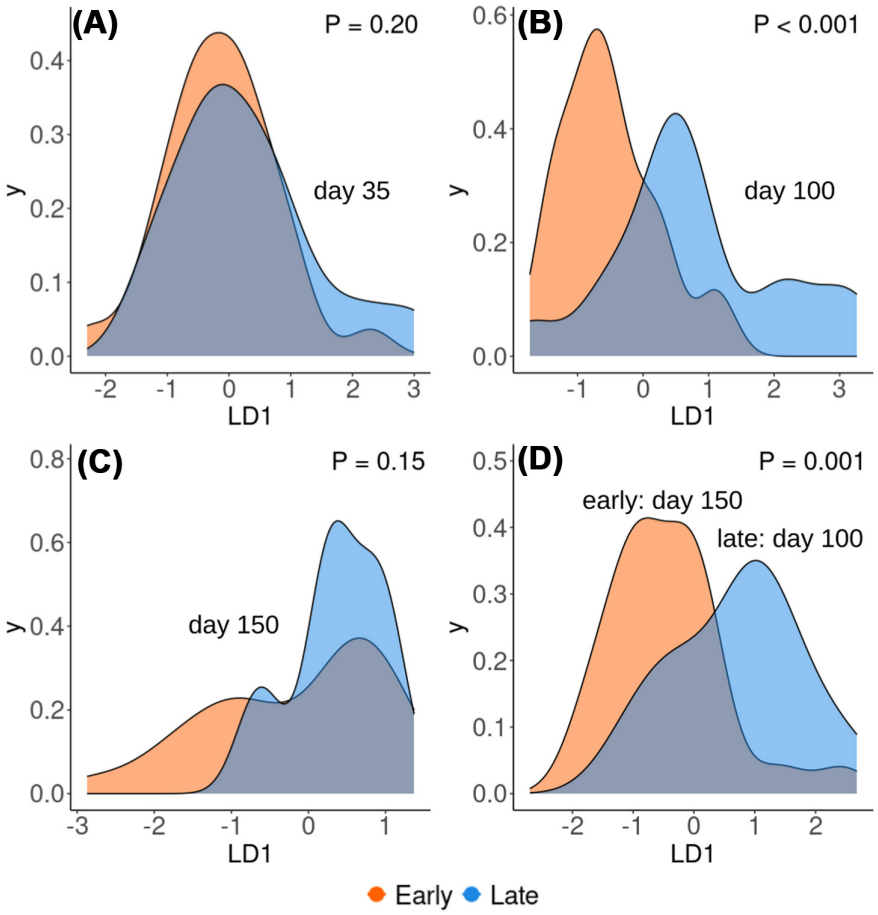

Figure 5. Results of the linear discriminant analysis (LDA) testing the differences between early- versus late-chicks according to red blood cell (RBC) mitochondrial metabolic rates measured at 35 days (A), 100 days (B) and 150 days (C) post-hatching. Comparisons between chicks facing similar environmental conditions (early-chicks day 150 and late-chicks day 100) are also presented (D). The separation between early- and late-chicks was tested as a function of their RBC mitochondrial metabolic profile, including ROUTINE, $OXPHOS_{CI}$, $OXPHOS_{CI+II}$ and $LEAK_{CI+II}$. p-values from ANOVA are shown. The x-axis represents the first discriminant function (LD1), which is a linear combination of four variables: ROUTINE, $OXPHOS_{CI}$, $OXPHOS_{CI+II}$, $LEAK_{CI+II}$. The *y*-axis shows the density distribution of both early- and late-chicks on this axis. In (A) and (C), the distributions of both groups overlap: as a result early- and late-chicks cannot be separated according to RBC mitochondrial metabolic rates tested. However, in (B) and (D), the overlap of the distributions is reduced and both groups are significantly distinct when taking into account their RBC mitochondrial metabolic profile. Orange colour refers to the early-chicks, blue colour refers to the late-chicks.

early- and late-group when taking into account ROUTINE, $OXPHOS_{CI}$, $OXPHOS_{CI+II}$ and $LEAK_{CI+II}$. Interestingly, these metabolic differences at day 100 were not associated with the discrepancy in chick body mass between early- and late-chicks, but did correlate with the date of sampling: a proxy of the environmental conditions. Our results seem reasonable in light with the fact early-chicks reached 100 days-old during austral autumn (ca end of April) and the late-chicks during winter (ca beginning of June). Different RBC metabolic profiles can be linked to different environmental conditions, but also with the stress associated with growing in adverse conditions and experiencing food restriction during growth (Blas 2015). This hypothesis would be supported by the fact that late-chicks have been shown to express higher corticosterone levels – a hormone involved in stress response (Breuner et al. 2013, Cockrem 2013, Stier et al. 2013, 2014, 2017, Blas 2015). Although increased glucocorticoid levels are expected to modulate RBC mitochondrial metabolism in birds (Manoli et al. 2007, Psarra and Sekeris 2009, Cossin-Sevrin et al. 2022, Casagrande et al. 2023), the direction of the effect (increase or decrease in mitochondrial respiration) seems to be context-dependent. As early- and late-born king penguin chicks face different growth patterns and most likely fasting stage (see below), quantifying chronic stress in the late-chicks is challenging and would require complementary measurements, such as corticosteroid-binding globulin levels or glucocorticoids receptors between early- and late-chicks (Breuner et al. 2013, Lin et al. 2021, Lemonnier 2024).

In our study, late-chicks ended up smaller and lighter than early-chicks during winter. However, prior studies demonstrated that small king penguin chicks can exhibit compensatory growth after winter, a few months before fledging,

which may partially offset their initial growth disadvantage (Geiger et al. 2012). As suggested above, late-chicks may express a higher RBC mitochondrial metabolism (higher endogenous respiration, higher respiration of the complex I and higher respiration of the complexes I and II) to ensure a higher production of ATP to sustain the energy requirements linked to a faster growth rate. This hypothesis would be supported by recent research reporting that late-born king penguin chicks may have some adaptations linked to a faster growth, by the over-expression of genes potentially involved in food uptake efficiency, body mass increase and accumulation of abdominal fat (Fernandes 2023).

An alternative hypothesis is the differences in fasting stages between chicks. Indeed, since late-chicks reached 100 days at the beginning of winter (versus autumn for early-chicks), they may have experienced more frequent and longer fasting events than early-chicks at the same age. Previous studies carried out in king penguin chicks reported a variation in skeletal muscle mitochondrial metabolism according to fasting stages (increase in metabolism efficiency between phase II and phase III of fasting) (Teulier et al. 2013, Monternier et al. 2014, Bourguignon et al. 2017). Here, a higher complex I respiration in the late-chicks could be linked to a variation in the usage of metabolic substrates compared to early-chicks and could reflect energetic adjustments linked to nutritional status (last feeding event), substrates availability and body composition (lipid and protein stores), but also fasting stage (glycolysis with NADH generation versus β-oxidation with production of $FADH_2$-derived electrons and electron-transferring flavoprotein pathway) that could affect the relative contribution of the complex I respiration in the electron transport system (Bernard et al. 2003, Liesa and Shirihai 2013, Secor and Carey 2016, Gnaiger 2020, 2024, Bochkova et al. 2025). For instance, RBC mitochondrial metabolism has been shown to vary (to some extent) according to fasting stages in adult king penguins (Cossin-Sevrin et al. 2025). Thus, differences in RBC mitochondrial metabolic rates could be also associated with different fasting stages between early- and late-chicks.

All RBC mitochondrial metabolic rates decreased with age, as reported in other species (Cossin-Sevrin et al. 2022, 2023, Stier et al. 2022) and this decrease was independent from the variation in total protein levels with age (Supporting information). In the studies mentioned above, the decrease in RBC mitochondrial metabolism was closely linked to a reduction in RBC mitochondrial content (mitochondrial density) across the growth period and we may expect similar patterns during the beginning of the growth period of king penguin chicks (day 35 to day 100). A large reduction in mitochondrial content per cell would lead to a decrease in RBC mitochondrial metabolism, providing an explanation for our results. In our case, as body masses and sizes differed between early- and late-chicks at 100 days, one possibility is that early- and late-chicks were not at similar developmental stages when drawing comparisons at similar ages. Distinct developmental stages might explain different RBC mitochondrial metabolic rates, for instance through a variation in mitochondrial content as explained above. Additionally, rapid growth has been shown to increase mitochondrial content per cell (in japanese quails), which may also explain higher metabolic rates for late-chicks (Jimenez et al. 2014). Yet, if the late-chicks possessed a higher number of mitochondria per cell, we could expect all RBC mitochondrial metabolic rates to increase (including mtAS and $OXPHOS_{CI+II}$), but we did not find such patterns in this study.

Most of the RBC mitochondrial metabolic rates were affected by sampling year. This effect is most likely due to a bias in our sample size, with very few late-chicks monitored during the season 2021–2022, 2022 being an overall poor-quality year with lower chick-survival at the level of the colony (only two late-chicks monitored from 100 post-hatching in 2022 versus 10 individuals in 2021). However, mitochondrial metabolism in endotherms has been shown to vary according to environmental conditions: including ambient temperature and food restrictions (Gyllenhammer et al. 2020, Mahalingam et al. 2020, Le Roy et al. 2021, Zitkovsky et al. 2021). Here, we cannot totally exclude the impact of different environmental conditions between years and further studies would be needed to comprehensively assess the effect of the environment on king penguin chick RBC mitochondrial metabolism.

One of our aims was to assess if differences in growth trajectories between early- and late-chicks could be predicted by differences in RBC mitochondrial metabolism. We only found a positive association between changes in body mass after the rapid growth period (day 150–day 100) and $FCR_{R/CI+II}$ measured at day 150. This would suggest that individuals expressed a lower usage of their maximal respiration of complexes I and II when experiencing a body mass drop between day 100 and day 150. However, $R^2$for this set of analysis was low ($R^2 = 0.3$), meaning that differences in RBC mitochondrial metabolism were not the main effect contributing to differences in growth. As suggested above, differences in environmental conditions and in parental food provisioning (and associated nutritional stress) were likely primary causes for differences in RBC metabolic profiles at day 100 between early- and late-chicks. Furthermore, this hypothesis is strengthened by the fact that RBC mitochondrial metabolic profiles were similar between early- and late-chicks at day 150, when both groups of chicks were facing winter conditions. It is worth noting that when comparing chicks at different ages during a similar period (early-chicks at day 150 and late-chicks at day 100; Fig. 1), RBC metabolic profiles remained different between the hatching groups (early versus late), suggesting that other factors influenced differences in chick metabolism (as suggested above: stress, faster growth rate, mitochondrial content per cell).

In conclusion, phenotypic differences between early- and late-born king penguin chicks were visible from 100 days post-hatching and onwards. Late-chicks had lower body mass and size, most likely explaining low survival chances (fledging success) compared to early-chicks. When comparing similar ages, late-chicks had higher endogenous RBC mitochondrial metabolism (ROUTINE) and higher usage of the complex I respiration ($OXPHOS_{CI}$), but also higher usage of the

complexes I and II respiration ($OXPHOS_{CI+II}$). These differences were more pronounced 100 days post-hatching, most likely due to differences in environmental conditions: number of feeding events and/or the stress associated with facing adverse conditions at younger age for the late-chicks. For instance, adverse weather events have been shown to impact king penguin chick metabolic budget and heat production (heterothermy), most probably impacting the animal metabolic rate (Eichhorn et al. 2011). Due to global changes, the breeding conditions are expected to change for king penguin population in Crozet Archipelago as the polar front contracts around the Antarctic continent moving further from the breeding colony, and as sea surface temperatures modify the growth and size of king penguin prey (Péron et al. 2012, Saunders et al. 2020, Brisson-Curadeau et al. 2023). These scenarios are expected to influence the king penguins' breeding success, although king penguins appear to exhibit some resilience through adjustments in their breeding phenology (Bardon et al. 2026). Understanding how nutritional changes (quantity and quality) may impact the king penguin chicks' metabolism, growth and survival (both early- and late-born chicks) would ultimately help in predicting the response of king penguin populations facing changing conditions.

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Permits – All the procedures were approved by the French Ethical Committee (APAFIS#31268-2021042117037897 v3 and APAFIS#16465-2018080111195526 v4) and the Terres Australes et Antarctiques Françaises (Arrêtés TAAF A-2020-82 and A-2021-49).

Author contributions

Nina Cossin-Sevrin: Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Katja Anttila**: Investigation (equal); Supervision (equal); Validation (equal); Writing – review and editing (equal). **Mathilde Lejeune**: Data curation (equal); Writing – review and editing (equal). **Céline Bocquet**: Data curation (equal); Writing – review and editing (equal). **Maëlle Fusillier**: Data curation (equal); Writing – review and editing (equal). **Thomas Faulmann**: Data curation (equal); Writing – review and editing (equal). **Camille Lemonnier**: Data curation (equal); Writing – review and editing (equal). **Natacha Garcin**: Data curation (equal); Writing – review and editing (equal)**. Anne Cillard**: Data curation (equal); Writing – review and editing (equal). **Pierre Bize**: Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Validation (equal); Writing – review and editing (equal)**. Jean-Patrice Robin**: Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Validation (equal); Writing – review and editing (equal). **Suvi Ruuskanen**: Investigation (equal); Supervision (lead); Validation (equal); Writing – review and editing (equal). **Vincent A. Viblanc**: Conceptualization (equal); Funding acquisition (equal); Methodology (equal); Project administration (lead); Validation (equal); Writing – review and editing (equal).

Transparent peer review

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Data availability statement

Data are available from the InDoRES public repository: https://doi.org/10.48579/PRO/KC44HE (Cossin-Sevrin et al. 2026).

Supporting information

The Supporting information associated with this article is available with the online version.

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