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## Biological Sciences

# Physiological response of *Pygoscelis* penguins in a rapidly changing region

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**Abstract**

We evaluated the physiological condition of the *Pygoscelis* penguins at Isla 25 de Mayo/King George Island (Antarctica Peninsula). Samples were collected from adults and chicks of Adélie (*Pygoscelis adeliae*, *n* = 20 each), gentoo (*Pygoscelis papua*, *n* = 20 chicks and *n* = 24 adults) and chinstrap penguins (*Pygoscelis antarcticus*, *n* = 18 each). We analysed haematological and biochemical parameters as indicators of health, immune response and nutrition. Gentoo penguin chicks exhibited higher haematocrits, indicating development linked to erythropoiesis and reticulocyte release from bone marrow or signalling dehydration related to fasting periods in chicks. Adélie penguins had increased total leukocyte counts, basophils and eosinophils, whereas gentoo penguins showed elevated heterophils and decreased lymphocytes, resulting in a higher heterophil/lymphocyte ratio stress index, possibly due to the impact of human activities. Chinstrap penguins from a remote area exhibited the lowest heterophil/lymphocyte ratio values. Adélie penguins showed more erythrocytic nuclear abnormalities, indicating sensitivity to environmental deterioration due to human impacts. The biochemical results were less consistent; Adélie penguins had higher cholesterol, whereas gentoo penguins had elevated triglycerides. Gentoo penguins showed dietary adaptability based on prey availability in this area. Our findings highlight the vulnerability of Adélie penguins and contribute to a 20 year physiological monitoring dataset for Antarctic penguins, which will aid future comparative studies.

**Key words:** Antarctic penguins; cell immune parameters; genotoxic parameters; nutritional parameters; physiology

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## Introduction

Three sympatric *Pygoscelis* penguin species inhabit the northern part of the western Antarctic Peninsula and are exhibiting different responses in their population distributions and abundances to rapid environmental change (Ducklow *et al.* 2007). The population of gentoo penguins (*Pygoscelis papua*), which avoids ice, has increased and expanded southwards, whereas ice-dependent Adélie penguins (*Pygoscelis adeliae*) have decreased and shifted polewards (Juáres *et al.* 2024). Chinstrap penguins (*Pygoscelis antarcticus*) exhibited a southwards shift in their nesting range (Ducklow *et al.* 2007).

The South Shetland Islands provide breeding sites for the three species of *Pygoscelis* penguins. For example, at Stranger Point on Isla 25 de Mayo/King George Island, Adélie and gentoo penguins breed sympatrically (D'Amico *et al.* 2016a, Juáres *et al.* 2020, 2024), and chinstrap penguins breed in a very close colony at Narebski Point (Peninsula Barton) ~10–15 km from Stranger Point. The population change of the three penguin species in these colonies aligned with the regional pattern (Ducklow *et al.* 2007). The Adélie penguin breeding pairs decreased by 89.8% in the last 27 years (-8.4% per annum; Juáres *et al.* 2024), whereas gentoo penguins increased by 74.6% (+3.1% per annum; Juáres *et al.* 2020), and the chinstrap penguin breeding population seems to have stabilized at ~3000 pairs since 2006/2007 (Kim 2002). However, as with Adélie penguins, chinstrap penguin populations in the western Antarctic Peninsula have notably declined (Juáres *et al.* 2024 and references therein). In this region, Antarctic krill (*Euphausia superba*) is a crucial food source for penguins, comprising 99.9% of the diets of Adélie and chinstrap penguins (Panasiuk *et al.* 2020). Gentoo penguins consume krill as well, but their diet also includes fish (Ainley & Blight 2009, Panasiuk *et al.* 2020). Moreover, human activities such as commercial fishing and tourism adversely affect these species, as penguins are sensitive to anthropogenic influences (Barbosa *et al.* 2012, Bertellotti *et al.* 2013). Previous research indicates that, in Antarctic regions with significant human activity, baseline levels of physiological parameters in *Pygoscelis* species have been altered (Barbosa *et al.* 2007, 2013, Carlini *et al.* 2007, Cebuhar *et al.* 2017).

We hypothesized that natural and anthropogenic environmental changes differentially affect the *Pygoscelis* penguin species based on observed distribution and abundance shifts. To assess this, we evaluated the physiological condition of the three species by examining haematological (haematocrit, erythrocyte abnormalities, leukocytes) and biochemical (lipids, carbohydrates, proteins) metrics indicative of nutritional condition, immune function and genotoxicity. We examined whether the selected physiological parameters indicate the vulnerability to human

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impacts (disturbance and/or pollution resulting from tourism and scientific bases) of Adélie penguins relative to gentoo and chinstrap penguins, as reported previously in the area (D'Amico *et al.* 2016a, Di Fonzo 2019).

## Materials and methods

The study was conducted during the breeding season of the three closely related penguin species: the chinstrap, the gentoo and the Adélie penguins. In our research, chicks and adults were sampled at Isla 25 de Mayo/King George Island (Punta Stranger Point, 62°15′S, 58°37′W, for gentoo and Adélie penguins; and Narebski Point, 62°14′S, 58°46′W, for chinstrap penguins) during January and February 2012. The sampling occurred during the guard phase of chicks (10–15 days after birth) to avoid potential variation related to the breeding period. Penguins were captured and immediately sampled at the nest. We sampled 120 individuals of the three penguin species (58 adults and 62 chicks). Sampled penguins showed no external signs of illness or injuries. Blood samples (1.0 ml) were extracted from the foot vein. All birds were sampled within 5 min after capture to avoid leukocyte production due to the stress of handling (Davis *et al.* 2008). Blood was placed into heparinized microcapillary tubes for the haematocrit measurements. Thin blood smears were prepared with a drop of fresh blood from each individual, placed on slides, air-dried, fixed with ethanol for 3 min and stained with Tinción 15 (Biopur). Finally, the rest of the blood was stored in an Eppendorf tube for biochemical analyses. In total, we measured 13 physiological parameters, including haematological and biochemical parameters.

### *Haematological analysis*

Haematocrit was obtained by measuring red cells and the total sample with a microhematocrit ruler (J. P. Selecta, Abrera, Spain) calibrated in percentages after centrifuging the microcapillary tubes in a haematocrit centrifuge for 12 min at 12 000 *g*. Haematocrit in birds can be considered to be an index of condition when evaluated with other haematological parameters (Fair *et al.* 2007).

Erythrocytic nuclear abnormalities (ENAs) were obtained by analysing blood smears with a light microscope (1000× oil immersion). The frequency of ENAs was scored in each blood smear to 2000 mature erythrocytes (D'Amico *et al.* 2016a). ENAs measure genotoxic effects derived from pollution (Van Ngan *et al.* 2007). Most contaminants are known to be genotoxic, and thus they can affect DNA, causing genetic alterations and leading to mutations. Differences in the level of contamination due to human activity can therefore be monitored through the level of ENAs present in species such as penguins. Nuclear abnormalities were recorded following Kursa & Bezrukov (2008), classifying the abnormalities as micronucleus, segmented nucleus or two-lobe nucleus, and as tailed and buddings nucleus. The sum of all ENAs per individual was used for the statistical analysis.

Blood smears were also examined with a light microscope (1000× oil immersion) to assess the counting of leukocytes (Campbell 1995). Total leukocyte count was estimated by counting all white blood cells in 10 consecutive 40× monolayer fields (D'Amico *et al.* 2016b). Leukocyte proportions were obtained as the part of each leukocyte type (basophils, heterophils, eosinophils, lymphocytes and monocytes) in a sample of 100 leukocytes (Campbell 1995). Leukocyte counts, as a measure of the cellular immune response, can objectively assess health status and support the diagnosis of various pathological states of individuals (Campbell 1995). Among birds, heterophils and lymphocytes are the most abundant leukocyte types (Campbell 1995). Heterophils are the primary phagocytic leukocytes, and they proliferate in the circulation in response to infections, inflammation, stress or malnutrition (Campbell 1995). Lymphocytes are involved in various immunological functions such as immunoglobulin production and modulation of immune defence (Campbell 1995). The remaining phagocytic leukocytes correspond to a combination of eosinophils, which play a role in the inflammation process and are associated with defence against parasites, monocytes (which are associated with defence against bacterial infections) and basophils that are related to inflammatory processes (Campbell 1995).

The heterophil/lymphocyte (H/L) ratio has been described as a good measure of stress in birds (Davis *et al.* 2008). During chronic stress, such as poor feeding conditions, plasma baseline corticosterone levels become elevated, leading to adaptive changes in physiology and behaviour, including increased H/L ratios (Davis *et al.* 2008).

### *Biochemical analysis*

Blood samples were centrifuged to separate plasma in order to obtain the biochemical parameter concentrations. Plasma for biochemical analyses was processed on a spectrophotometer (Automatic Biochemistry Analyzer CM250, Wiener Lab) to determine total proteins (TPs; g/dl), cholesterol (CHOL; mg/dl), triglycerides (TGLs; mg/dl) and glucose (GLU; mg/dl). Plasma biochemical parameters contribute to knowledge regarding body condition and health status (Brown 1996).

TPs are associated with food intake energy reserves and immunity, and they have been proposed as good indicators of overall fitness in birds (Brown 1996).

Lipids are directly related to the fat reserves of the animals (Brown 1996). CHOL is a lipid fraction of the blood that usually increases in level after feeding in most animals. The differences in this parameter can indicate the quality of food consumed (Brown 1996). TGLs were positively correlated with body fat, constituting the energy storage of the birds (Brown 1996).

GLU is the carbohydrate that represents the source of cellular energy, and it reflects the nutritional status and blood values depending on the type and quality of food consumed (Brown 1996).

### *Statistical analysis*

The haematological and biochemical parameters were described statistically and compared separately by age for the three penguin species using the *SPSS* statistical package. When possible, we used a two-way analysis of variance (ANOVA) to compare the differences in each parameter between the groups, which were divided by two independent factors - species and sex - and their interaction. Regarding the data distribution, normality and homoscedasticity were tested previously with parametric (ANOVA) and non-parametric (Kruskal-Wallis and Mann-Whitney) tests.

## Results

We obtained physiological parameters from 20 Adélie penguin adults (11 males and 9 females) and 20 chicks (11 males and 9 females), 20 gentoo penguin adults (12 males and 8 females) and 24 chicks (11 males and 13 females) and 18 chinstrap penguin adults

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**Table I.** Physiological parameters of chicks and adult penguins of the genus *Pygoscelis*. Values are expressed as mean ± standard error and ranges are given in parentheses.

| Physiological parameter | Adélie penguin | | Gentoo penguin | | Chinstrap penguin | |
|---|---|---|---|---|---|---|
| | Adult ($n$ = 20) | Chick ($n$ = 20) | Adult ($n$ = 20) | Chick ($n$ = 24) | Adult ($n$ = 18) | Chick ($n$ = 18) |
| HTO (%) | 55.6 ± 0.7 | 48.1 ± 0.7 | 52.2 ± 1.2 | 55.9 ± 0.7 | 54.0 ± 0.9 | 41.4 ± 0.9 |
| | (51.7–61.3) | (41.8–53.0) | (35.7–60.0) | (50.0–63.6) | (45.9–62.2) | (35.8–49.3) |
| WBC ($n$) | 49.2 ± 3.7 | 75.5 ± 3.7 | 76 ± 4.0 | 64.4 ± 3.9 | 68.9 ± 4.0 | 65 ± 6.1 |
| | (27–91) | (42–112) | (53–124) | (43–109) | (42–99) | (0.4–1.4) |
| B (%) | 3.7 ± 0.6 | 1.2 ± 0.2 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.9 ± 0.3 | 0.6 ± 0.2 |
| | (0–9) | (0–2.8) | (0–1.9) | (0–1) | (0–3.6) | (0–2.7) |
| E (%) | 7.7 ± 1.1 | 6.1 ± 1.4 | 4.5 ± 0.5 | 4.4 ± 0.5 | 1.7 ± 0.4 | 2.8 ± 0.6 |
| | (1.9–17.6) | (1.0–27.5) | (0.9–10.7) | (0.9–9.8) | (0–6.0) | (0–7.2) |
| H (%) | 40.1 ± 2.7 | 35.5 ± 2.8 | 52.7 ± 2.4 | 49.7 ± 1.4 | 35.1 ± 2.3 | 39.9 ± 2.2 |
| | (18.6–69.0) | (15.9–69.7) | (31.7–75.2) | (36.2–61.2) | (18.9–52.9) | (24.8–57.3) |
| L (%) | 44.8 ± 2.4 | 57.9 ± 2.6 | 37.8 ± 2.1 | 41.9 ± 1.3 | 58.8 ± 2.4 | 54.1 ± 2.3 |
| | (17.2–60.6) | (37.7–78.8) | (15.4–54.2) | (30.6–51.9) | (40.0–75.5) | (39.6–71.7) |
| M (%) | 3.7 ± 0.6 | 2.6 ± 0.6 | 4.8 ± 0.7 | 3.8 ± 0.4 | 3.6 ± 0.5 | 2.6 ± 0.4 |
| | (0–9.9) | (0–11.4) | (0–12.2) | (0.9–7.8) | (0–9.1) | (0–6.6) |
| H/L | 1.1 ± 0.2 | 0.6 ± 0.1 | 1.6 ± 0.2 | 1.2 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.1 |
| | (0.3–4.0) | (0.2–1.4) | (0.6–4.9) | (0.7–2.0) | (0.3–1.3) | (0.4–1.4) |
| ENAS ($n$) | 26.2 ± 3.2 | 31.3 ± 4.6 | 14.6 ± 2.0 | 15.3 ± 1.8 | 8.2 ± 1.3 | 5.0 ± 0.8 |
| | (5–62) | (1–95) | (4–32) | (3–36) | (1–22) | (0–13) |
| CHOL (mg/dl) | 289.0 ± 9.7 | 223.1 ± 6.9 | 169.4 ± 15.9 | 215.0 ± 7.6 | 220.9 ± 12.0 | 185.3 ± 10.1 |
| | (156–363) | (171–300) | (57–308) | (156–282) | (114–302) | (110–260) |
| TGL (mg/dl) | 134.3 ± 15.8 | 126.6 ± 10.3 | 84.1 ± 7.9 | 131.5 ± 9.7 | 196.1 ± 42.3 | 152.2 ± 18.4 |
| | (63–382) | (75–267) | (42–147) | (86–336) | (51–660) | (66–323) |
| GLU (mg/dl) | 211.6 ± 8.4 | 199.1 ± 6.9 | 203.4 ± 5.7 | 189.9 ± 5.4 | 179.6 ± 4.2 | 217.8 ± 3.0 |
| | (166–301) | (117–265) | (168–254) | (102–243) | (147–214) | (200–247) |
| TP (g/dl) | 7.0 ± 0.1 | 5.9 ± 0.1 | 7.0 ± 0.1 | 5.9 ± 0.04 | 6.7 ± 0.2 | 5.5 ± 0.1 |
| | (6.1–8.1) | (5.2–7.0) | (6.1–7.7) | (5.5–6.5) | (5.9–8.5) | (5.1–5.9) |

B = basophils; CHOL = cholesterol; E = eosinophils; ENA = erythrocytic nucleus abnormality; GLU = glucose; H = heterophils; H/L = heterophil/lymphocyte ratio; HTO = haematocrit; L = lymphocytes; M = monocytes; TGL = triglycerides; TP = total proteins; WBC = white blood cell total.

(9 males and 9 females) and 18 chicks (7 males and 11 females). Descriptive values of the physiological parameters considered for the three pygoscelid penguins are shown in Table I. No significant differences were found between the sexes or the species × sex interaction in any of the parameters analysed, nor in the adults nor the chicks of the three species (all $P > 0.05$; see Table S1).

### *Haematological parameters*

Haematological parameters varied significantly by species and age. Haematocrit values were similar among adult species ($F = 2.65$, $P = 0.80$). However, haematocrit was significantly higher in the gentoo penguin chicks than in the Adélie penguin chicks and again were higher in the Adélie penguin chicks than in the chinstrap penguin chicks ($F = 79.58$, $P < 0.001$).

Total adult leukocyte counts differed among species ($F = 12.18$, $P < 0.001$). Adélie penguins had significantly lower counts than gentoo and chinstrap penguins (Tukey test, $P < 0.03$), whereas chinstrap and gentoo penguin counts were similar (Tukey test, $P = 0.45$). In chicks, no differences were observed ($F = 1.65$, $P = 0.20$). Lymphocyte counts were higher in chinstrap penguin adults compared to Adélie penguin adults, which in turn were higher than in gentoo penguin adults ($F = 22.24$, $P < 0.0001$; all Tukey tests $P < 0.05$). Chicks also displayed significant differences ($\chi^2 = 24.34$, $P < 0.0001$), with gentoo penguins being lower than Adélie penguins ($U = 49$, $P < 0.0001$) and chinstrap penguins ($U = 70$, $P < 0.0001$); Adélie and chinstrap penguins were similar ($U = 150$, $P = 0.38$). Gentoo penguin adults exhibited significantly higher heterophil counts ($F = 13.23$, $P < 0.000$; Tukey test, $P < 0.001$), as did gentoo penguin chicks ($F = 11.90$, $P < 0.0001$; all Tukey tests $P < 0.007$). Basophils varied by species, being significantly higher in Adélie penguin adults ($\chi^2 = 28.25$, $P < 0.0001$, all contrasts $P < 0.05$) and their chicks ($\chi^2 = 18.63$, $P < 0.0001$). Eosinophils were also elevated in Adélie penguin adults ($\chi^2 = 24.17$,

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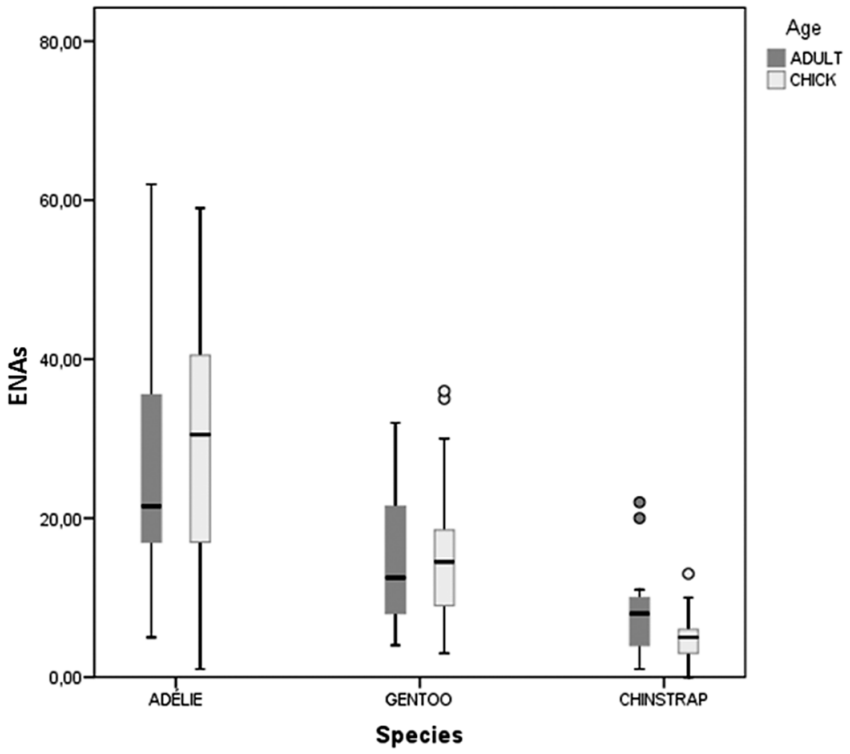


**Figure 1.** Box plot (medians, quartiles and 95% confidence intervals) showing the frequency of erythrocytic nucleus abnormalities (ENAs) among adults and chicks of *Pygoscelis* species.

$P < 0.0001$, all contrasts $P < 0.05$), but no differences were observed in chicks ($F = 2.87$, $P = 0.06$). No significant differences in monocyte counts were found among adults ($\chi^2 = 1.86$, $P < 0.39$) or chicks ($\chi^2 = 4.86$, $P = 0.089$).

The H/L index varied among adult penguin species ($F = 7.43$, $P = 0.001$), being significantly lower in chinstrap than in gentoo penguins (Tukey test, $P = 0.001$), whereas other comparisons were not significant (all Tukey tests $P > 0.05$). Among chicks, the H/L index also differed ($F = 18.44$, $P < 0.0001$), with chinstrap and Adélie penguin chicks showing lower values than gentoo penguin chicks (Tukey test, $P = 0.0001$) but being similar to each other (Tukey test, $P = 0.3230$).

Finally, ENAs were significantly higher in the Adélie penguin adults ($F = 17.42$, gl = 2, $P < 0.0001$) and chicks ($F = 28.91$, gl = 2, $P < 0.0001$) compared to the adults and chicks of the other two species (Fig. 1).

### *Biochemical parameters*

Biochemical parameters varied significantly by species and age. Chinstrap penguin adults had lower GLU ($F = 6.30$, $P = 0.004$), whereas gentoo and Adélie penguin adults showed similar levels (*post hoc* test, $P = 0.66$). In chicks, GLU was significantly higher in chinstrap penguins ($F = 7.71$, $P = 0.001$). CHOL was significantly higher in Adélie penguin adults ($F = 22.49$, $P < 0.001$; all *post hoc* tests $P < 0.02$). In chicks, CHOL also differed by species ($F = 5.54$, $P = 0.006$), particularly between chinstrap and Adélie penguins ($P = 0.006$) and between chinstrap and gentoo penguin ($P = 0.03$). TGLs were lower in gentoo penguin adults ($\chi^2 = 8.94$, $P = 0.011$), whereas no differences were noted for chicks ($\chi^2 = 1.15$, $P = 0.56$). No significant differences in TPs were found among adults of the species ($F = 1.72$, $P = 0.190$). However, TPs in chicks varied by species ($F = 17.69$, $P < 0.001$), being significantly lower in chinstrap penguins compared to Adélie and gentoo penguins (all *post hoc* tests $P < 0.0001$), with there being no significant differences between Adélie and gentoo penguins (all *post hoc* tests $P = 0.99$).

## Discussion

Our results offer a comprehensive set of physiological parameters for adults and chicks of three penguin species that breed together on Isla 25 de Mayo/King George Island, substantially improving the baseline database collected in the region.

### *Haematological parameters*

The haematological values were in the general range of previously reported values for the region provided by other authors (D'Amico *et al.* 2016a,b, Di Fonzo 2019). For instance, haematocrit levels naturally vary among endothermic species (Stark & Schuster 2012), with our study showing ranges of 35.8–63.6% for chicks and 35.7–62.2% for adults. Our results showed that haematocrit varied only among chicks of the species, with gentoo penguin chicks showing the highest values. This aligns with the understanding that haematocrit changes at different developmental stages, because elevated haematocrit results from erythropoiesis (the production of new erythrocytes) and/or the release of reticulocytes (immature erythrocytes) from the bone marrow, triggered by hypothalamus-pituitary-adrenal-mediated stress (Voorhees *et al.* 2013). Increases and decreases in oxygen-carrying capacity, along with related factors such as haematocrit, can indicate various life-history events and trade-offs (see Fair *et al.* 2007 for a review). On the other hand, haematocrit variation may also reflect dehydration

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processes associated with food intake, especially in chicks, which may undergo prolonged periods of time without eating (Ibañez *et al.* 2015).

Leukocyte profiles and indicators of health, immune status and stress in birds did not present a clear pattern. In this study, Adélie penguin adults showed higher values for the total leukocyte count in comparison to the adults of the other two species, but this difference was not translated to their chicks, which did not show differences among them. Generally, a decreased total leukocyte count could be an indicator of poor condition and reduced immune system health, and *vice versa* (Salvante 2006).

Lymphocyte levels were lower in both the adults and chicks of the gentoo penguins, whereas heterophil proportions were higher, resulting in an elevated H/L stress index. The increased heterophil levels may indicate a robust innate immune response to gastrointestinal parasites obtained through diet (Shutler & Marcogliese 2011). At Stranger Point, gentoo penguins host a wide variety of gastrointestinal helminth parasites (Diaz *et al.* 2013), probably due to their diverse diet. Similarly, Barbosa *et al.* (2007) found that gentoo penguins in this area had the highest levels of immunoglobulin (IgY), probably due to greater exposure to parasites or pathogens. Human activities significantly affect physiological parameters in in penguins, including H/L ratios (Barbosa *et al.* 2013). Our findings revealed that chinstrap penguin adults and chicks had the lowest H/L ratio values at this more isolated location, unlike the gentoo and Adélie penguins breeding at Stranger Point, where human activity is notably higher.

Adélie penguin adults and chicks also displayed the highest values of basophils. Previous studies found similar trends, indicating that Adélie penguins had the highest average basophil counts compared to chinstrap and gentoo penguins along the Antarctic Peninsula (D'Amico *et al.* 2016a). Olmastroni *et al.* (2024) also reported elevated basophil levels in three clustered Adélie penguin colonies in the Ross Sea. Basophils and eosinophils, which were higher in Adélie penguin adults in this study, are implicated in the acute inflammatory response in birds. However, this may not always manifest as basophilia or eosinophilia in the leukogram (Campbell 1995).

Adélie penguins showed significantly higher levels of ENAs in both chicks and adults than gentoo and chinstrap penguins. ENAs represent a key indicator of genomic damage related to environmental pollution (Van Ngan *et al.* 2007, Frixione *et al.* 2024). Therefore, their increased values in Adélie penguins probably reflect the heightened sensitivity of this species to environmental deterioration, such as the release of harmful contaminants into the environment.

The literature on Antarctic wild animals remains limited. However, some previous studies suggest that Adélie penguins are vulnerable to environmental stressors such as contamination, as indicated by increasing numbers of ENAs (De Mas *et al.* 2015, D'Amico *et al.* 2016a,b, Olmastroni 2024). One study noted erythrocytic abnormalities in gentoo penguins, linking nuclear abnormalities to environmental contaminants (Barbosa *et al.* 2013), but it also found ENAs in only 4 out of 2000 erythrocytes, which is far less than that observed in Adélie penguins in this study (Fig. 1). Previous studies have indicated that penguins at the Stranger Point colony exhibit elevated levels of heavy metal contamination (Jerez *et al.* 2011). Adélie penguins there have the highest concentrations of chromium (Cr) and selenium (Se) in their feathers, whereas gentoo penguins show the highest levels of zinc (Zn; Jerez *et al.* 2011). Both species present similar percentages of lead (Pb), marking the highest levels reported among Adélie and gentoo penguins in the Antarctic Peninsula (Jerez *et al.* 2011). Pb and Cr are heavy metals associated with human activities, and feather analysis reveals that these elements are most prevalent in regions with significant human activity, such as Isla 25 de Mayo/King George Island (Jerez *et al.* 2011). Furthermore, ice melt from global warming may be releasing pollutants that have accumulated over decades, exacerbating their impact on nearby penguin populations (Cabrerizo *et al.* 2013).

Additionally, Adélie penguins exhibit high ENAs in the analysis of their erythrocyte. Breeding penguins from three locations in central Victoria Land, Ross Sea, showed ENA values ranging from 77.05 ± 46.93 to 110.62 ± 70.48 (Olmastroni *et al.* 2024). Penguins from Torgensen and Avian islands had ENA values of between 41.20 ± 40.10 and 46.90 ± 46.50 (De Mas *et al.* 2015). ENA values reported in Adélie penguins from the Yalour Islands and Isla 25 de Mayo/King George Island were 109.90 ± 80.00 and 72.00 ± 35.30, respectively (De Mas *et al.* 2015). Higher ENA values in Adélie penguins breeding in Antarctica may further support the hypothesis of low genome instability in this species (Olmastroni *et al.* 2019, 2024).

### *Biochemical parameters*

Biochemical metrics related to nutritional status showed no clear pattern. In our studied area, our findings suggest that gentoo penguins exhibit dietary plasticity, although the diet of Adélie penguins during the crèche period also contained small portions of fish (Juáres *et al.* 2018). Previous research indicates that, during the breeding period, all three penguin species on Isla 25 de Mayo/King George Island consume krill of similar sizes, implying that changes in prey availability (e.g. fish) may increase dietary overlap (Wawrzynek-Borejko *et al.* 2022).

Consistent with previous findings at Stranger Point indicating no differences in GLU levels between sympatrically breeding Adélie and gentoo penguins (D'Amico *et al.* 2016a), our results also revealed no differences in GLU levels between Adélie and gentoo penguin adults and chicks. In contrast, chinstrap penguin adults exhibited the lowest GLU levels, whereas their chicks exhibited the highest levels, but they were within the normal range reported by other authors (D'Amico *et al.* 2016a, Di Fonzo 2019). However, other studies indicated that gentoo penguins had higher GLU concentrations than Adélie and chinstrap penguins, which was attributed to their glucogenic mechanisms arising from their feeding strategies, which involve consuming larger average prey sizes and body masses (Ibañez *et al.* 2015).

Lipid concentrations in free-living birds primarily reflect nutritional status (Jenni-Eiermann & Jenni 1994). The CHOL concentration was within the range reported for these species in the area, being higher for adult Adélie penguins, as in previous reports (D'Amico *et al.* 2016a,b). The TGL levels observed in this study were also consistent with previously reported values for these species, showing a similar pattern whereby gentoo penguins exhibited the lowest levels (Di Fonzo 2019). The penguins in this area have comparable diets, predominantly consisting of krill and high-fat fish (Juáres *et al.* 2016). Indeed, the diets of Adélie and gentoo penguins at the Stranger Point colony, analysed through stable isotopes and stomach contents, revealed a predominance of Antarctic krill. However, a dietary shift occurred in gentoo penguins, transitioning from a krill-dominant diet to one that included krill, fish and squids (Juáres *et al.* 2016). Adults of the three species exhibited similar values of TPs, whereas chinstrap penguin chicks showed the lowest values. Some previous studies indicated higher TP levels in

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chicks, whereas others reported the opposite (Dawson & Bortolotti 1997). This discrepancy suggests a potential area for future research to explore the specific proteins contributing to these differences and their ecological and fitness implications related to age-based variations in protein concentrations.

### *Integrative physiological approach*

The haematological and biochemical values observed in this study may fluctuate due to specific nutritional, pathological or environmental factors. The breeding colonies on Isla 25 de Mayo/King George Island are located very close to areas where there is high human activity (Carlini *et al.* 2007), which can further affect their physiological conditions. Adélie penguins have shown sensitivity to anthropogenic impacts, as was also reflected by the increased ENA values observed in previous studies (De Mas *et al.* 2015, D'Amico *et al.* 2016a, Olmastroni *et al.* 2019, 2024). In this study, this could be reflected in the higher ENA values in Adélie penguins compared to gentoo and chinstrap penguins, suggesting that Adélie penguins are more vulnerable to environmental contaminants at this site, a vulnerability that is probably intensified by climate change (e.g. ice melting; Cabrerizo *et al.* 2013). In addition, previous researchers found that Adélie penguins breeding in Esperanza/Hope Bay, a site with year-round human activity, exhibited reduced body condition. Indicators such as body mass, haematocrit and plasma metabolites were significantly lower in individuals from high-human-disturbance zones, reflecting poorer body condition (Graña Grilli *et al.* 2018, Ibañez *et al.* 2018). Furthermore, proteins related to antioxidant processes, immune functions, vitamin transport, metabolism and stress responses were overexpressed in Adélie penguins breeding in a high-human-disturbance area (Ibañez *et al.* 2021).

## Conclusion

We evaluated the physiological condition of pygoscelid penguin populations in the Antarctic Peninsula using haematological and biochemical parameters. Although research on the effects of global change, mainly related to human activities and increasing temperatures, on penguin species has mainly focused on distribution, abundance, diet and phenology (areas with extensive historical data), it is essential also to understand their physiological responses. However, collecting physiological data is complex, rendering long-term comparisons challenging due to the various analytical processes and measurement methodologies involved. Aside from the crucial role of ENAs, other physiological parameters generally do not show clear patterns among penguin species, especially their chicks. It is also essential to systematically investigate the impacts of climate-related diseases by gathering relevant data. Future research on the health of penguins and other wildlife near areas with increasing human activity should be prioritized to prepare for changes in the Antarctic ecosystem. Nonetheless, our results confirm previous findings that Adélie penguins are particularly sensitive to such impacts. This research contributes to a comprehensive database of ongoing physiological monitoring of Antarctic penguins over the past 20 years, which is highly valuable for future comparative studies.

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