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Living in a challenging environment: Monitoring stress ecology by non-destructive methods in an Antarctic seabird

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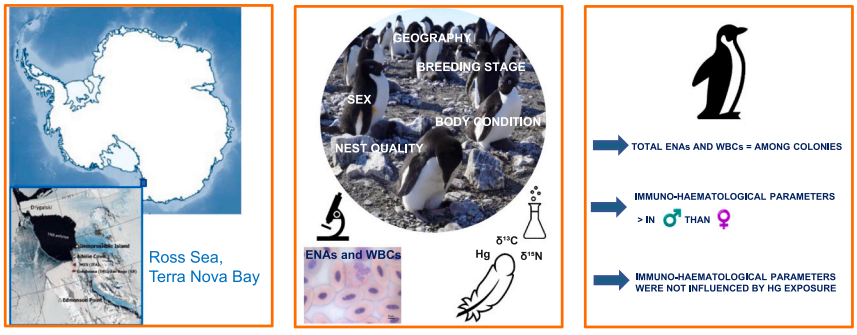
HIGHLIGHTS

- Immuno-haematological parameters can detect stressors acting on wildlife.
- Adélie penguins are indicators of environmental changes in Antarctica.
- Individual life-history traits (sex, nest position, body condition) influenced penguin's biomarkers.
- Immuno-haematological parameters were not influenced by Hg exposure.
- The study sets a baseline on penguin health status across years and various conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

How Antarctic species are facing historical and new stressors remains under-surveyed and risks to wildlife are still largely unknown. Adélie penguins *Pygoscelis adeliae* are well-known bioindicators and sentinels of Antarctic ecosystem changes, a true canary in the coal mine. Immuno-haematological parameters have been proved to detect stress in wild animals, given their rapid physiological response that allows them tracking environmental changes and thus inferring habitat quality. Here, we investigated variation in Erythrocyte Nuclear Abnormalities (ENAs) and White Blood Cells (WBCs) in penguins from three clustered colonies in the Ross Sea, evaluating immuno-haematological parameters according to geography, breeding stage, and individual penguin characteristics such as sex, body condition and nest quality. Concentrations of mercury (Hg) and stable isotopes of carbon and nitrogen (as proxies of the penguin's trophic ecology) were analysed in feathers to investigate the association between stress biomarkers and Hg contamination in Adélie penguins. Colony and breeding stage were not supported as predictors of immuno-haematological parameters. ENAs and WBCs were respectively ~30 % and ~20 % higher in male than in female penguins. Body condition influenced WBCs, with penguins in the best condition having a ~22 % higher level of WBCs than those in the worst condition. Nest position affected the

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proportion of micronuclei (MNs), with inner-nesting penguins having more than three times the proportion of MNs than penguins nesting in peripheral positions. Heterophils:Lymphocytes (H:L) ratio was not affected by any of the above predictors. Multiple factors acting as stressors are expected to increase prominently in Antarctic wildlife in the near future, therefore extensive monitoring aimed to assess the health status of penguin populations is mandatory.

1. Introduction

Human activities, pollution, and biological invasions summed up to climate changes represent an increasing pressure on Antarctic ecosystems and their biodiversity (Gutt et al., 2021). Although a reliable amount of scientific literature now undermines the description of the Antarctic continent as a remote and unchanged place, the human footprint is expanding and new actions devoted to protecting, restoring, and managing Antarctic biodiversity are mandatory.

The Antarctic Treaty System devoted to protecting living resources (Berkman, 2011) is facing conservation challenges associated with rapid environmental changes and increasing human interests in the region (Chown et al., 2012; Meyer and Kawaguchi, 2022). Antarctic Specially Protected Areas (ASPAs) and Marine Protected Areas (MPAs) provided that national research programs comply with Antarctic guidelines and codes of conduct for their participants and logistics (Environment Protocol Annex V, https://www.ats.aq/e/protocol.html 27/11/2023). Creation of ASPAs and MPAs may all contribute to protecting biodiversity (Wauchope et al., 2019) and promote conservation of ecosystems (Hughes et al., 2018). Since the establishment of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) in 1982, several pressures have been exacerbated in the Southern Ocean and Antarctica's living marine resources are under increasing threat (Brooks et al., 2022). Overfishing of Antarctic krill (*Euphausia superba*) associated with future climate impacts is expected to pose the greatest risk to populations of krill-feeders, such as penguins (Watters et al., 2020). In such a context, the establishment of MPA in the Ross Sea (Brooks et al., 2021), acknowledged as the largest of the pristine marine ecosystems in the world, has provided significant action, not only toward the protection and conservation of marine living resources but also assuring the entire resilience of the Southern Ocean (Capurro et al., 2021). Increasing human activities such as commercial fishing, tourism, and point-source discharges, together with long-range transport of contaminants, represent significant sources of pollution in the Antarctic atmosphere, plateau, and coastal marine and terrestrial habitats (Bargagli, 2016; Corsolini and Ademollo, 2022; Perfetti-Bolaño et al., 2022). Scientific research stations and their activities have been recognized as a source of disturbance to bird populations due to logistics (vehicular traffic noise) and discharges/waste disposal (Giese and Riddle, 1999; Caccavo et al., 2021) and plants (Tejedo and O'Neill, 2018). Also, recreational activities and touristic routes (Tejedo et al., 2022) have brought more and more humans to interact with local fauna (Coetzee and Chown, 2016). Although the assessment of the extent of pollution from various sources relies on established international initiatives, knowledge of the biological effects on wildlife is still insufficient and may vary among organisms, depending upon their abilities to respond to pressures and changes in their specific habitats (Scientific Committee On Antarctic Research, 2023).

Adélie penguins *Pygoscelis adeliae* are now considered sentinels of Antarctic ecosystem changes, a true canary in the coal mine (Ainley, 2002; Boersma, 2008). They are able to track back the effects of climate changes on the Antarctic marine ecosystem, either in habitat or in resource availability over time (Emmerson and Southwell, 2008; Dugger et al., 2014; Hinke et al., 2014). About four million breeding pairs of Adélie penguins were estimated to occur across the Antarctic continent in 2020 (Naveen et al., 2020). The Ross Sea is home to ~1,460,000 breeding pairs, which is ~36 % of breeders' world population. Adélies' colonies are distributed in the rare ice-free areas around Antarctica and are spatially distributed or clustered according to population size, food resources, presence of polynyas, and nesting habitat availability (Santora et al., 2019). In particular, ice-free areas are mostly located along the continent's coasts and have become extremely impacted by humans (Tin et al., 2009; Southwell et al., 2017; Brooks et al., 2019).

Along with human disturbance, potential environmental stressors for Antarctic wildlife include pathogens and diseases (Kerry and Riddle, 2009; Grimaldi et al., 2015; Smeele et al., 2018; Barbosa et al., 2021), plastic, chemical and noise pollution, competition with fisheries, acidification, and climate change (Trathan et al., 2014; Ropert-Coudert et al., 2019; Xavier and Trathan, 2020). All those factors can act synergistically on individuals and differently in distinct Antarctic regions. Among the emerging threats affecting seabirds' population worldwide, attention has returned to mercury (Hg) contamination and its effects on physiology, life traits, and ecology (Bargagli, 2008; Kessler, 2013; Ackerman et al., 2016; Mills et al., 2022). An extensive literature review on Hg toxicity data on seabirds translated into a blood-equivalent single toxicity benchmark revealed that various degrees of impairments in the behaviour, general health status, up to severe effects on physiology and reproduction (*e.g.*, reduced breeding success and reproductive failure), are associated with Hg exposure (Ackerman et al., 2016). Polar areas are considered to be a sink of global Hg from both natural geophysical processes or anthropogenic sources due to their peculiar environmental condition and associated rapid depletion events (Pfaffhuber et al., 2012). Bioaccumulation and biomagnification of methyl-Hg has received considerable attention in Arctic seabirds (*e.g.*, Provencher et al., 2014; Braune et al., 2015; Albert et al., 2021; Chastel et al., 2022) as well as in Antarctic ones (*e.g.*, Anderson et al., 2009; Carravieri et al., 2016; Becker et al., 2016). For Antarctic seabirds, latitudinal, age and sex differences in Hg contamination have been reported for several species such as wandering albatross (*Diomedea exulans*), emperor penguin (*Aptenodytes forsteri*) and Adélie penguin (*e.g.*, Carravieri et al., 2014a; Pilcher et al., 2020; Cusset et al., 2023). The overall consequences on Antarctic seabird populations are that Hg can negatively affect population dynamics (Goutte et al., 2014a; Goutte et al., 2014b).

Despite the sensitivity to Hg exposure that can differ among species, early warning responses to environmental perturbations as proxies for stress ecology can represent suitable tools for monitoring and assessing impact on seabird populations. Haematological parameters and blood smears examinations are considered to be a rapid tool to assess early cellular damages from genotoxic exposure (Van Ngan et al., 2007; Luzhna et al., 2013; Jara-Carrasco et al., 2015), and may indicate ongoing inflammatory or parasitic processes (Maceda-Veiga et al., 2015). Blood parameters such as erythrocyte nuclear abnormalities (ENAs), white blood cells (WBCs) and heterophil-to-lymphocyte ratio (H:L) have been demonstrated as reliable biomarkers of health status in Adélie penguins (Vleck et al., 2000; D'Amico et al., 2016a; Minias, 2019). The presence of ENAs (*e.g.,* micronuclei, MNs) is considered an index of cellular reaction to natural and anthropogenic environmental stressors, while an efficient method to assess the response of an organism to environmental factors consist also in the assessment of its leukocytes profile and in particular heterophils to lymphocytes ratio (H:L ratio). The latter is a suitable indicator of stress associated to reproductive cycle, seasonal changes, and injury. It can reflect food and water deprivation, extremes temperature, constant light, long-distance migration and social disruption too. All these stressors result in an increased level of heterophils (innate system), decreased number of lymphocytes (acquired immune system) and a high H:L ratio (Vleck

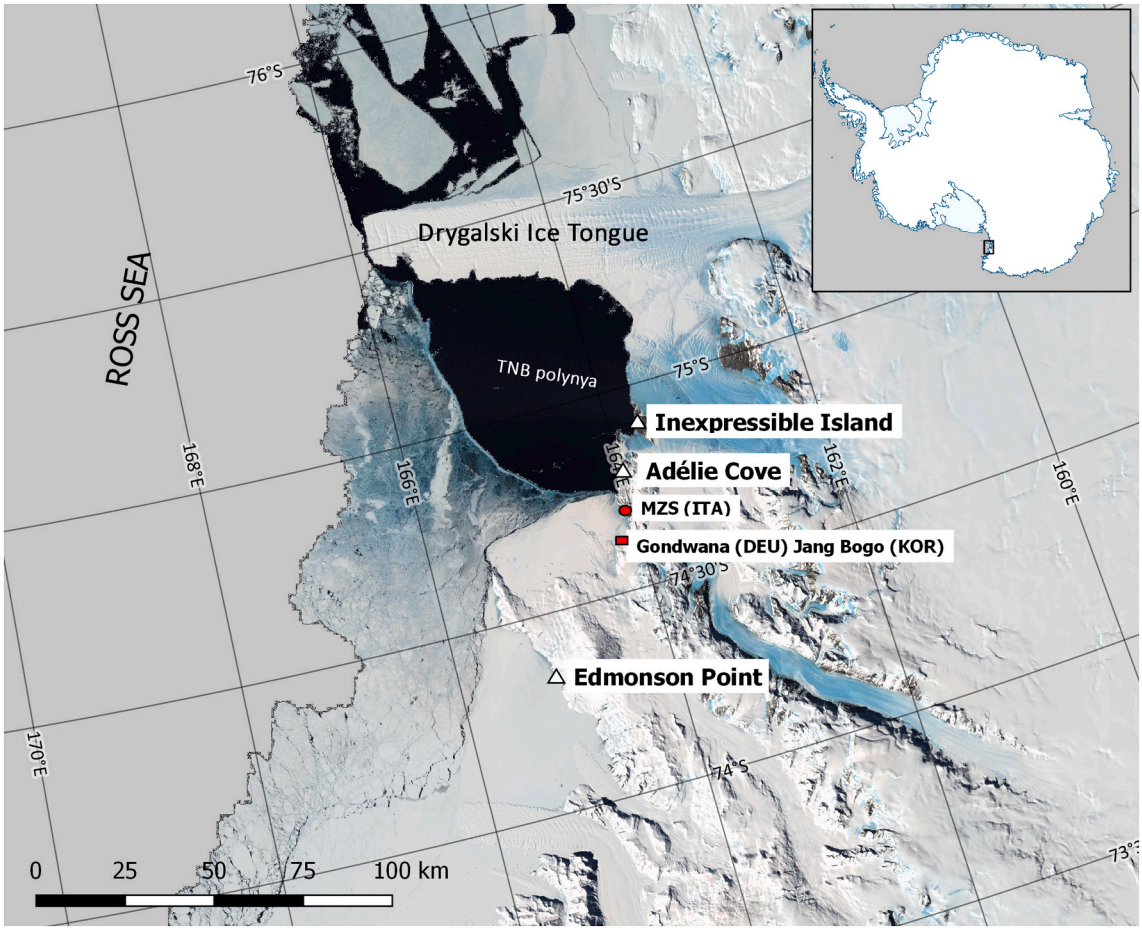

Fig. 1. Map of the study area, showing ice characteristics during the breeding season of Adélie penguins in Terra Nova Bay and Wood Bay. Triangles show the three Adélie penguin colonies (AdCo, EdPo, InIs). Research stations nearby are also shown with red symbols. Landsat image from Qantarctica3 (https://www.npolar.no/quantarctica 27/11/2023), LIMA Landsat image mosaic of Antarctica (15/240 m) USGS/Remote Sensing of Environment, 2008. MZS = Mario Zucchelli Station; TNB = Terra Nova Bay.

et al., 2000). A previous study provided a first baseline of haematological parameters obtained by a non-destructive method in an Adélie penguin colony in the Ross Sea as a rapid response to detect changes in their habitat and health status (Olmastroni et al., 2019). By expanding sampling sites from the previous Ross Sea investigation, the purpose of this study was to integrate the population monitoring program of this species with the measurement of a series of physiological parameters to: i) identify proxies of penguin's health by analysing small amounts of non-destructive samples, and ii) integrate these proxies with penguin bio-ecological responses to establish a baseline and detect potential changes at an early stage.

Here, we investigated variations in ENAs and WBCs in penguins from three clustered colonies in the Ross Sea, evaluating immuno-haematological parameters according to geography, breeding stage, and individual penguin characteristics including sex, body condition, and nest quality. Mercury levels and trophic ecology using penguin's feathers were also analysed in a subsample to investigate the association between stress biomarkers and Hg contamination in Adélie penguins. To our best knowledge, this is the first study simultaneously incorporating the relative contribution of different environmental and individual predictors into the assessment of blood parameters. Life-history traits may in fact modulate responses to stressors differently (Colominas-Ciuró et al., 2017). More extensive monitoring studies to assess the health status of penguin populations are mandatory in Antarctica. Multiple factors acting as stressors on wildlife are expected to increase more prominently in the study area in the near future. Our results represent a unique reference to detect wildlife risk assessment in future stress ecology studies.

2. Materials and methods

2.1. Study areas

Our study was conducted in three Adélie penguin colonies located between Wood Bay and Terra Nova Bay (TNB), in central Victoria Land, Ross Sea (Antarctica): Inexpressible Island (InIs; 74°54′S, 163°39′E), Adélie Cove (AdCo; 74°46′S, 164°00′E) and Edmonson Point (EdPo; 74°20′S, 165°08′E). The closest Adélie penguin colonies from our study areas are ~200 km away (South-East: Franklin Island; north: Coulman Island) (Fig. 1).

InIs, the southernmost colony, is located in front of the TNB polynya and occupies ice-free ground on the eastern shore, where the terrain is relatively flat, with a ridge of ~110 m above sea level elevation along the western flank. AdCo is located on a steep slope (80 m) in a small bay. EdPo is the northernmost colony and is in a small ice-free area within Wood Bay. While InIs and AdCo are affected by katabatic winds, EdPo is generally not affected and is characterised by several kilometres of fast ice that remain in place until late February. The three colonies markedly differ in relation to the number of breeding penguins, being greater at InIs, intermediate at AdCo, and smaller at EdPo (~29,000, 13,400, and ~2700 breeding pairs, respectively; www.penguinmap.com 27/11/2023; Olmastroni et al., 2022). Marine (leopard seal *Hydrurga leptonyx*,

killer whales *Orcinus orca*) and terrestrial (south polar skua *Stercorarius maccormicki*) predators of penguins occur in all colonies, but skua density per penguin nest differs substantially between colonies, being higher at EdPo, intermediate at AdCo and lower at InIs (Olmastroni et al., 2022). For further details on marine and terrestrial habitat characteristics in the three study areas, see Mori et al. (2021).

2.2. Data collection

2.2.1. Penguin blood collection

We sampled penguins according to our standardized protocol previously reported in Olmastroni et al. (2019). Blood samples were collected from adult breeders (>4 years; Ballerini et al., 2009) at the nest, in the period from mid-November 2017 to early-January 2018. Samples from AdCo and EdPo were collected throughout the breeding season, from late incubation to crèche stages, on previously selected nests, where we also recorded individual life-history traits and monitored breeding activities regularly (see Section 2.2.2). In these colonies, we attempted to balance blood samples collection according to sex (AdCo: $n = 14$ females, $n = 12$ males; EdPo: $n = 19$ females, $n = 23$ males) and breeding stage (mean sampling date, AC: 10th December; EP: 3rd December), to allow robust comparison among colonies. Conversely, harsh weather at InIs limited the opportunities to visit the colony to take samples and monitor penguins on a regular basis. Hence, samples from InIs were collected on two sampling events but, unfortunately, sex balance could not be achieved (2nd and 10th January; $n = 5$ females, $n = 14$ males). In all colonies, we were also able to balance sampling of penguins spatially, by (i) considering different subgroups within each colony, to limit differences in nesting habitat quality and sub-colony growth, (ii) maximizing distance among individuals, to avoid penguin inbreeding and relatedness (Cristofari et al., 2015). In particular, we sought to balance samples from AdCo and EdPo as to nest position (AdCo: $n = 11$ inner, $n = 15$ peripheral; EdPo: $n = 21$ inner, $n = 21$ peripheral).

Blood was collected from individuals showing no sign of disease or injury, including no ectoparasites, feather or skin changes, or emaciation. One drop of blood was obtained from the brachial vein using a heparinized syringe with a sterilized needle (22 gauge) and placed on a clean slide (15 min 10 % HCl and rinsed with Milli-Q water and oven-drying at 100 °C) according to the method of Owen (2011) and Olmastroni et al. (2019). Slides were then dried at ambient temperature and stored at +4 °C in a plastic box until analysis was performed at the University of Siena laboratory in Italy.

2.2.2. Penguin captures and individual traits assessment

The collection of blood samples occurred along with different research activities conducted in the framework of the PenguinERA project (Olmastroni et al., 2022). To reduce the stress of capture and manipulation, penguins were gently lifted from their nests and covered with a hood, while eggs and/or chicks were protected and kept warm during handling. Penguins were weighted to the nearest 50 g through a Salter scale, and bill depth, bill length, and flipper length were measured to the nearest mm. Up to six body feathers per individual were plucked from the chest area for Hg and stable isotope analyses. We marked penguins with passive transponders (TIRIS™ Texas Instruments Registration and Identification System) and externally with a temporary dye, to avoid recapturing the same individuals. We released each penguin in front of its nest after a 10 min maximum holding time and checked that individuals returned to their regular breeding activity. During field activities, we sexed penguins using morphometric equations specifically calibrated in one of our study colonies (Fattorini and Olmastroni, 2021). The sex of sampled penguins was later confirmed through feather-based molecular sexing (Mori et al., 2021). Individual body condition was measured using the body mass relevant to individual body size as an indicator of good health status. Following comparable studies on Adélie penguins (D'Amico et al., 2016a), individual body condition was calculated as the residual of the linear regression between body mass and bill depth. We took bill depth rather than bill length as an index of body size, as the former shows a greater sexual dimorphism in our study population (Fattorini and Olmastroni, 2021), thus providing a better proxy for the structural size of penguins compared to the latter.

2.3. Immuno-haematological analyses

Blood smears were processed for the analysis of erythrocyte nuclear abnormalities (ENAs) and complete leukocyte profiles (WBCs). A new combined protocol for erythrocyte and leukocyte staining was developed here based on Venier et al. (1997) and D'Amico et al. (2014) for ENAs and WBCs analysis, respectively. Slides of blood smears stored at +4 °C after sampling were fixed using cold methanol (100 % *v*/v) for 3 min, left to dry at room temperature (20 °C), and stained with a solution of 6 % Giemsa at pH 7.26 in Milli-Q (pure Giemsa) were previously filtered with folded filter, grade 3 hw (Sartorius) and solution with cellulose-acetate membrane syringe filters, 0.22 μm (Sartorius). Smears were then rinsed in Milli-Q water, covered with a coverslip, and observed under a light microscope (Olympus BX51) equipped with a Camera.

The following steps were used for ENAs and WBCs analysis. Firstly, slides were screened at 40× magnification, progressively moving from the *x* and *y* axes, to identify monolayer fields with similar density of well-stained erythrocytes. Then, nuclear abnormality counting was carried out over 10,000 erythrocytes under 100× (oil immersion) magnification according to Zúñiga-González et al. (2000). ENAs were identified according to the method by De Mas et al. (2015a) as follows: (a) micronucleus (MN), (b) lobed nucleus (LN), (c) tailed nucleus (TN), (d) two-lobed nucleus (TL), (e) budding nucleus (BN), (f) nucleus with cavity (WC), (g) kidney-shaped nucleus (KN), (h) unknown nuclear malformation (UN). The sum of total ENAs was also calculated for each individual. Finally, the percentage of WBCs was calculated as the number of leukocytes per class over 100 immune cells detected among 10,000 erythrocytes at 100× (oil immersion) magnification. Leukocytes were classified according to Samour (2006, Table 22.10, p. 597) as follows: (a) heterophils (H), (b) eosinophils (E), (c) lymphocytes (L), (d) basophils (B), (e) monocytes (M). H:L ratio was evaluated from the WBCs profile according to Davis et al. (2008).

2.4. Feather Hg concentrations and isotopic values

For a subsample of penguins captured within each colony ($n = 15$ per colony), total Hg concentrations were determined in feathers as well as carbon and nitrogen stable isotopes, which are established proxies of the feeding habitat and trophic position, respectively (Kelly, 2000). Since methyl-Hg accounts for >90 % of total Hg in seabird feathers (Renedo et al., 2017), total Hg was used as a proxy for methyl-Hg burden in Adélie penguin feathers. In penguins, the chemical composition of body feathers is homogenous (Brasso et al., 2013; Carravieri et al., 2014b). Therefore, measurements of Hg concentrations and isotopic values were made on a single randomly-selected feather for each bird. As described in Cusset et al. (2023), feathers were first cleaned with a chloroform: methanol mixture (2:1), sonicated for 3 min, rinsed twice in methanol and dried at 45 °C for 48 h. They were then cut with stainless steel precision scissors to obtain a homogenous powder. Second, Hg analyses were performed in duplicate or triplicate (*i.e.*, until the relative standard deviation between measurements reached <10 %) on feather homogenates (0.5–1.1 mg), using an Advanced Mercury Analyser (AMA 254, Altech). The AMA limit of quantification was 0.1 ng. Certified reference material (TORT-3, Lobster hepatopancreas, NRC, Canada) and blanks were analysed during each analytical session. Certified and measured values of TORT-3 were 0.292 ± 0.022 $\mu g \cdot g^{-1}$ and 0.293 ± 0.001 $\mu g \cdot g^{-1}$ ($n = 6$), respectively, showing a reliable recovery of the Hg (*i.e.*, 100.2 ± 0.5 %). Mercury concentrations are expressed as $\mu g \cdot g^{-1}$ dry weight (dw).

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analyses were

performed on feather homogenates (0.2–0.8 mg), loaded into tin cups (8 mm × 5 mm, Elemental Microanalysis Ltd., UK) using a microbalance (XPRUD5, Mettler Toledo, Switzerland). Values of $\delta^{13}C$ and $\delta^{15}N$ were determined with a continuous flow isotope ratio mass spectrometer (Delta V Plus with a Conflo IV Interface, Thermo Scientific, Germany), coupled to an elemental analyser (Flash 2000, Thermo Scientific, Italy). Results are expressed in the usual δ unit notation in per thousand (‰) relative to Vienna PeeDee Belemnite and atmospheric N_2 (air) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of reference materials (USGS-61 and USGS-63, US Geological Survey) indicated measurement uncertainties <0.10 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. For further methodological information, please refer to Cusset et al. (2023).

2.5. Statistical analysis

2.5.1. Data comparison among colonies

As a first step, we compared immuno-haematological parameters among our three study colonies. Due to their non-normal distributions, we used the Kruskal-Wallis test to compare individual biological responses among colonies. We also assessed multivariate differences in biological responses among colonies using Fisher's discriminant function analysis (DFA; Rencher, 1995). DFA reduces a multivariate dataset to two discriminant functions, which are linear combinations of the original variables and explain 100 % of variance, so that the variability among colonies is maximized and that within colonies is minimized. We performed DFA separately for haematological and immunological parameters. Hence, DFA conducted on the haematological dataset aimed at separating colonies according to levels of erythrocyte nuclear abnormality (ENAs/10,000 red blood cells, RBCs) and their composition (expressed as % of each ENA category); while DFA performed on the immunological dataset aimed at discriminating colonies following leukocyte levels (WBCs/10,000 RBCs) and their composition (expressed as % of each WBC category). Because DFA assumes no multicollinearity between explanatory variables, we excluded the H:L ratio in the dataset of immunological responses due to its substantial correlation ($|r| > 0.55$) to other parameters. We checked colony assignment by re-classification of data to each colony, providing the 'leave-one-out' (jackknifed) cross-validated percentage of correct re-classified individuals. Eventually, using the scores of the two discriminant functions obtained for each DFA, we depicted the separation of multivariate responses in two dimensions (Lovari et al., 2015).

To avoid the normality and homoscedasticity assumptions, for each of the two datasets, the overall difference in biological responses among the three colonies was tested formally through a non-parametric adaptation of the multivariate analysis of variances (PERMANOVA; Anderson, 2001). PERMANOVA was based on Euclidean distances between original variables. We computed significance by permutation of colony membership, with 99,999 replicates. We ran pairwise comparisons among colonies as *post-hoc* Mann-Whitney tests with Bonferroni correction. We performed DFA and PERMANOVA using the software Past (Hammer et al., 2001).

2.5.2. Life-history determinants of immuno-haematological parameters

In a second step, we focused on the two colonies (AdCo and EdPo) where we collected individual life-history traits of penguins at their nests. We used generalized linear mixed models (GLMMs) to investigate variations in Adélie penguin ENAs and WBCs according to individual characters. We fitted separate models for the total number of ENAs, the total number of WBCs, the proportion of MNs, and the H:L ratio determined in each penguin. Following statistical recommendations to model count data, continuous proportions, and real numbers, ENAs, and WBCs were modelled through Poisson errors (link: log), the proportion of MNs was modelled through beta errors (link: logit), while the H:L ratio was ln-transformed to meet model assumptions and modelled through Gaussian errors (link: identity). For the proportion of MNs, we converted 0 into 10^{-10} without altering the biological meaning of this parameter, to enable the use of beta errors.

For ~55 % of sampled individuals, we also sampled its mating partner, meaning that most penguins in our sample shared the same nest. Thus, we treated the nest identity as a random intercept. This precaution allowed us to account for potential sources of within-pair variability reflecting ecological constraints that could have acted on both members of the same pairs, potentially generating non-independent levels of ENAs and WBCs between individuals sharing the same nest. Such ecological constraints could be linked, for example, to the neighbouring penguins' behaviour, or related to the pair-specific effort and/or synchrony in parental care. In fact, members of the same pairs are expected to share similar energetic trade-offs, especially during chick-rearing (*e.g.*, Ballard et al., 2010; Cresswell et al., 2012).

We included five fixed effects in our models. (1) Colony (categorical; reference level: AdCo), to test differences between the two colonies; (2) nest position (categorical; reference level: inner nests), to examine the effect of nest location, as inner penguins are expected to experience a higher level of social stress due to a higher density of neighbouring penguins (Spurr, 1974; Viblanc et al., 2014); (3) sex (categorical; reference level: females), to assess intersexual differences in immuno-haematological levels, as shown by Olmastroni et al. (2019) in one of our study colonies; (4) days elapsed from 1st November (continuous, in days), to investigate the linear variation of the breeding progression reflecting the transition from late incubation through the guard and early crèche period on immuno-haematological parameters (Vleck et al., 2000); (5) body condition index (continuous), to account for differences in body status/health, ultimately reflecting the energetic allocation of breeding individuals. There was no collinearity ($|r| < 0.50$) among explanatory variables. Covariates were scaled to improve model convergence and to allow assessment of the relative importance of each predictor. It was not possible to measure the body mass of one individual at EP. Therefore, the present analysis included a sample size of 67 individuals. Additionally, we could not classify WBCs for one individual sampled at AC. Consequently, the model concerning H:L ratio had a sample size of 66 individuals.

For each response variable, we carried out an all-subset model selection according to the information-theoretic approach (Harrison et al., 2018). We started from the full model including all fixed effects, and we ranked and weighted all possible competing models, each one having a specific combination of predictors and therefore representing a different *a priori* hypothesis (Harrison et al., 2018). The null model (*i.e.*, the random intercept model) was also evaluated to compare model performance against a fixed baseline (Mac Nally et al., 2018). We considered the AIC_c value of each model (*i.e.*, the Akaike Information Criterion corrected for small sample size) and its difference (ΔAIC_c) with respect to the best model (*i.e.*, the model with the lowest AIC_c value). Following the nesting rule (Harrison et al., 2018), we did not select models having $\Delta AIC_c \geq 2$, as well as models with an AIC_c value greater than that of any simpler alternative, achieving either a set of top-ranked models or a single best model for each response variable. Model weight was standardized within the subset of selected models. Selected models are reported in Table S1 (Supplementary Material). For each response variable, we estimated coefficients of predictors, 95 % confidence intervals, and variance of random effects from the best model. The effects of predictors were assessed by checking whether 95 % confidence intervals of coefficients overlapped 0. Best models were validated by checking residual patterns (Zuur et al., 2009). GLMMs and model selection were conducted through the R packages *glmmTMB* (Brooks et al., 2017) and *MuMIn* (Bartòn, 2012).

2.5.3. Sex and colony effects on Hg concentration and isotopic niche

Bifactorial analyses were performed to test for differences in feather Hg concentrations, and $\delta^{13}C$ and $\delta^{15}N$ values between study colonies and sexes (two-way ANOVA). Normality and homoscedasticity of residuals were checked prior to interpretation using visual diagnostics, as well as Shapiro-Wilk and Breusch-Pagan tests (*lmtest* package in R,

Table 1
Erythrocyte nuclear abnormalities (ENAs) as total and detailed ENAs observed in mature blood erythrocytes of Adélie penguins from the three study colonies (n = sample size), Adélie Cove (AdCo), Edmonson Point (EdPo) and Inexpressible Island (Inls). Reported values are mean ± standard deviation and [range]. H: test statistic. *$p < 0.05$.

	Adélie Cove (AdCo) (n = 26)	Edmonson Point (EdPo) (n = 42)	Inexpressible Island (Inls) (n = 19)	H (p-value)
Total ENAs/ 10,000 RBCs	109.12 ± 57.40 [16–258]	110.62 ± 70.48 [22–356]	77.05 ± 46.93 [16–181]	H = 3.98 (p = 0.14)
% Micronucleus (MN)	4.21 ± 7.44 [0–39.29]	4.88 ± 5.43 [0–31.82]	5.15 ± 4.94 [0–18.52]	H = 2.49 (p = 0.29)
% Lobed nucleus (LN)	17.58 ± 8.49 [2.74–37.50]	17.74 ± 7.97 [0–36.11]	18.24 ± 7.12 [6.60–35.91]	H = 0.197 (p = 0.91)
% Tailed nucleus (TN)	21.18 ± 9.00 [5.36–39.53]	21.72 ± 8.70 [5.56–43.53]	26.12 ± 8.51 [6.25–38.78]	H = 5.22 (p = 0.073)
% Two-lobed nucleus (TLN)	4.26 ± 3.80 [0–14.62]	2.71 ± 2.82 [0–11.39]	4.50 ± 3.53 [0–12.50]	H = 5.76 (p = 0.056)
% Budding nucleus (BN)	13.32 ± 7.43 [0–34.09]	13.00 ± 6.20 [3.08–28.95]	11.21 ± 5.77 [0–20.16]	H = 0.802 (p = 0.67)
% Nucleus with cavity (NWC)	20.98 ± 12.24 [3.57–46. 03]	23.59 ± 10.30 [4.55–41.44]	14.76 ± 5.69 [0–25.00]	H = 7.81 (p = 0.02) *
% Kidney-shaped nucleus (KSN)	7.77 ± 5.02 [1.90–19.42]	6.17 ± 4.96 [0.90–21.88]	7.33 ± 3.57 [2.94–18.75]	H = 4.09 (p = 0.13)
% Unknown nuclear malformation (UNM)	10.71 ± 8.29 [0–34.51]	10.18 ± 6.90 [1.33–40]	12.70 ± 9.48 [3.23–39.62]	H = 0.85 (p = 0.65)

Version 4.2.2; R Core Team, 2022; Zeileis and Hothorn, 2002), respectively.

Finally, we evaluated whether the total number of ENAs, total number of WBCs, percentage of MNs, and H:L ratio assessed in each individual were related to relevant Hg concentrations by using both Pearson and Spearman correlation tests.

3. Results and discussion

3.1. Immuno-haematological parameters did not vary geographically

Total ENAs and single abnormalities found in Adélie penguins from the three colonies are listed in Table 1, and WBCs and the H:L ratio are reported in Table 2. No differences were found in either total ENAs, WBCs, and H:L ratio among individuals belonging to the three colonies, thus suggesting a similar health status and immune profile for the Adélie penguin during the 2017–18 summer season. Concerning ENAs, only NWC was significantly higher in penguins from EdPo (Table 1).

The most recurrent ENAs were TN (average % values >21.18 ± 9.00) followed by LN (>17.58 ± 8.49), NWC (>14.76 ± 5.69), BN (>11.21 ± 5.77), UMN (>10.18 ± 6.90), KSN (>6.17 ± 4.96), while MN and TLN were the lowest with average values between 2.7 ± 2.8 % in individuals from EdPo and 5.2 ± 4.9 % in those from Inls (Table 1). Mean total ENAs values were similar in AdCo and EdPo and lower in Inls, probably due to the high variability observed among individuals belonging to the same colony (Table 1).

The results of MNs for the EdPo individuals were in line with those previously found in the same colony (Olmastroni et al., 2019), where similar percentages of MNs were observed. However, the number of ENAs found at EdPo by Olmastroni et al. (2019) was lower than that found in the present work. This difference may be explained by the higher number of erythrocytes counted at EdPo in the present study compared with that counted previously (see Table 1 and Olmastroni

Table 3
Comparison of physiological parameters (mean ± SD; *mean ± SE) measured in adult Adélie penguins at different locations of the Antarctic Peninsula (De Mas et al., 2015; D'Amico et al., 2014, 2016a) and in the Ross Sea (Olmastroni et al., 2019 and the present study). n: number of samples; nd: not determined.

Location	ENAs	MNs	H:L ratio	WBCs	n	Reference
King George I.	72.0 ± 35.3	0	nd	nd	10	De Mas et al. (2015)
Torgersen I.	46.9 ± 43.5	1.3 ± 1.5	nd	nd	10	De Mas et al. (2015)
Yalour I.	109.9 ± 80.0	5.2 ± 4.1	0.4 ± 0.04*b	183.4 ± 12.9*b	10; 25b	De Mas et al., 2015; b) D'Amico et al. (2016a)
Avian I.	41.2 ± 40.1	3.25 ± 3.7	0.5 ± 0.05* b	267 ± 23.2 *b	10; 23b	De Mas et al. (2015); b) D'Amico et al. (2016a)
Stranger Point	26.2 ± 3.2	nd	1.10 ± 0.20* a	49.2 ± 3.7* a	20	De Mas et al., 2015; a) D'Amico et al. (2014)
			10.7 ± 0.11 *b	197.5 ± 16.1* b	25	b) D'Amico et al. (2016a)
Edmonson Point	43.11 ± 6.33	4.53 ± 0.52	3.08 ± 0.87	34.63 ± 3.20	19	Olmastroni et al. (2019)
Adélie Cove	109.12 ± 57.40	4.21 ± 7.44	1.99 ± 0.92	82.00 ± 33.16	26	This study
Edmonson Point	110.62 ± 70.48	4.88 ± 5.43	1.73 ± 0.89	82.45 ± 34.24	42	This study
Inexpressible Island	77.05 ± 46.93	5.15 ± 4.94	1.97 ± 1.43	67.05 ± 32.63	19	This study

Table 2
Immunological parameters as total and detailed WBCs, and H:L ratio from Adélie penguins from the three colonies (n = sample size). Reported values are mean ± standard deviation and [range]. H: test statistic *$p < 0.05$.

	Adélie Cove (AdCo) (n = 26[a])	Edmonson Point (EdPo) (n = 42)	Inexpressible Island (Inls) (n = 19)	H (p-value)
Total WBCs/10,000 RBCs	82.00 ± 33.16 [37.00–148.00]	82.45 ± 34.24 [25.00–206.00]	67.05 ± 32.63 [24.00–173.00]	H = 5.01 (p = 0.082)
% Heterophils (HE)	45.25 ± 8.48 [31.25–63.16]	40.92 ± 9.94 [19.30–60.00]	41.21 ± 11.73 [25.00–67.12]	H = 3.34 (p = 0.19)
% Eosinophils (EO)	19.57 ± 8.90 [6.12–35.94]	20.94 ± 8.77 [3.51–39.00]	19.08 ± 9.79 [5.17–41.86]	H = 1.16 (p = 0.56)
% Lymphocytes (LY)	25.27 ± 6.30 [10.88–35.14]	27.17 ± 9.48 [10.99–64.91]	26.72 ± 9.54 [6.98–43.10]	H = 0.196 (p = 0.91)
% Basophils (BA)	5.26 ± 4.24 [0.00–17.24]	5.47 ± 3.69 [0.00–14.46]	8.01 ± 3.85 [2.04–18.18]	H = 6.95 (p = 0.031) *
% Monocytes (MO)	4.64 ± 3.31 [0.00–12.35]	5.50 ± 3.03 [0.00–16.28]	4.98 ± 4.63 [0.00–17.07]	H = 2.55 (p = 0.28)
H:L ratio	1.99 ± 0.92 [1.09–4.38]	1.73 ± 0.89 [0.30–5.00]	1.97 ± 1.43 [0.60–6.00]	H = 2.02 (p = 0.36)

[a] n = 25 for WBCs categories and H:L ratio, because WBCs could not be classified for one sample.

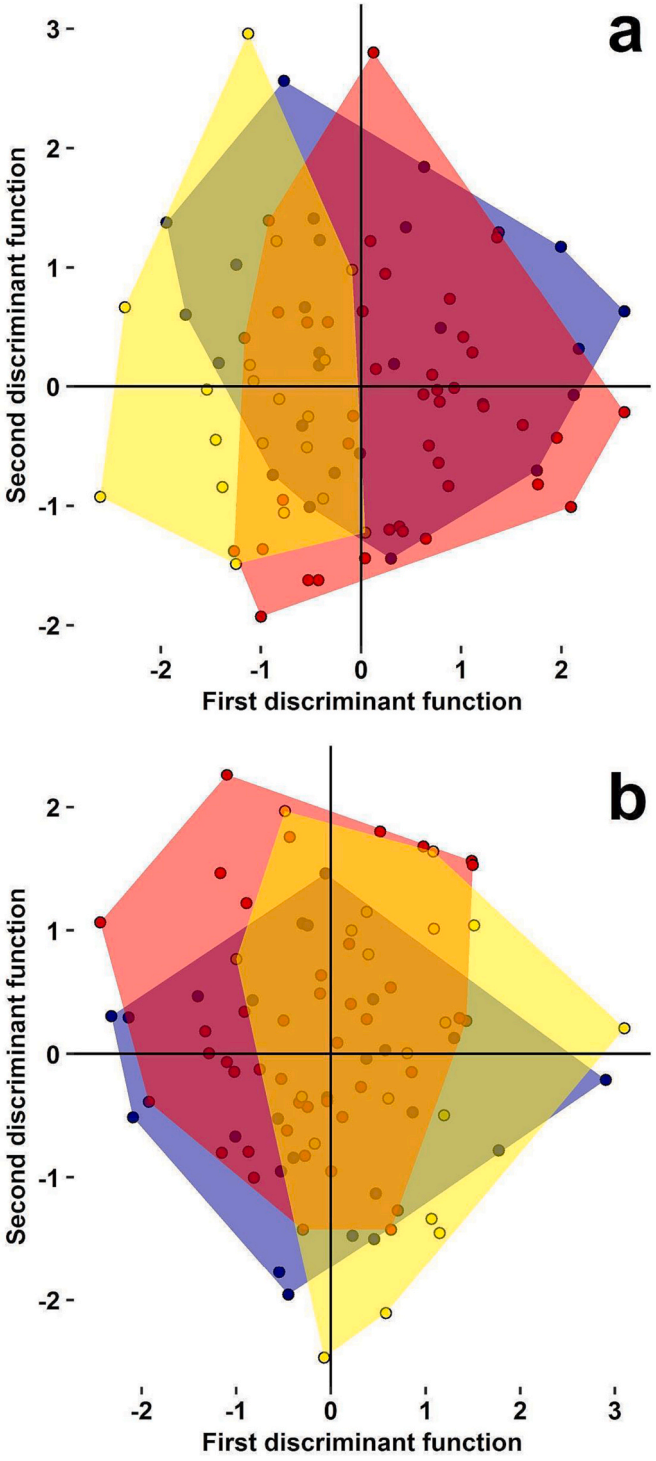

Fig. 2. Overlap between study colonies (blue: AdCo, red: EdPo, yellow: InIs) in discriminant scores explained by the linear combination of (a) haematological and (b) immunological responses measured in Adélie penguins.

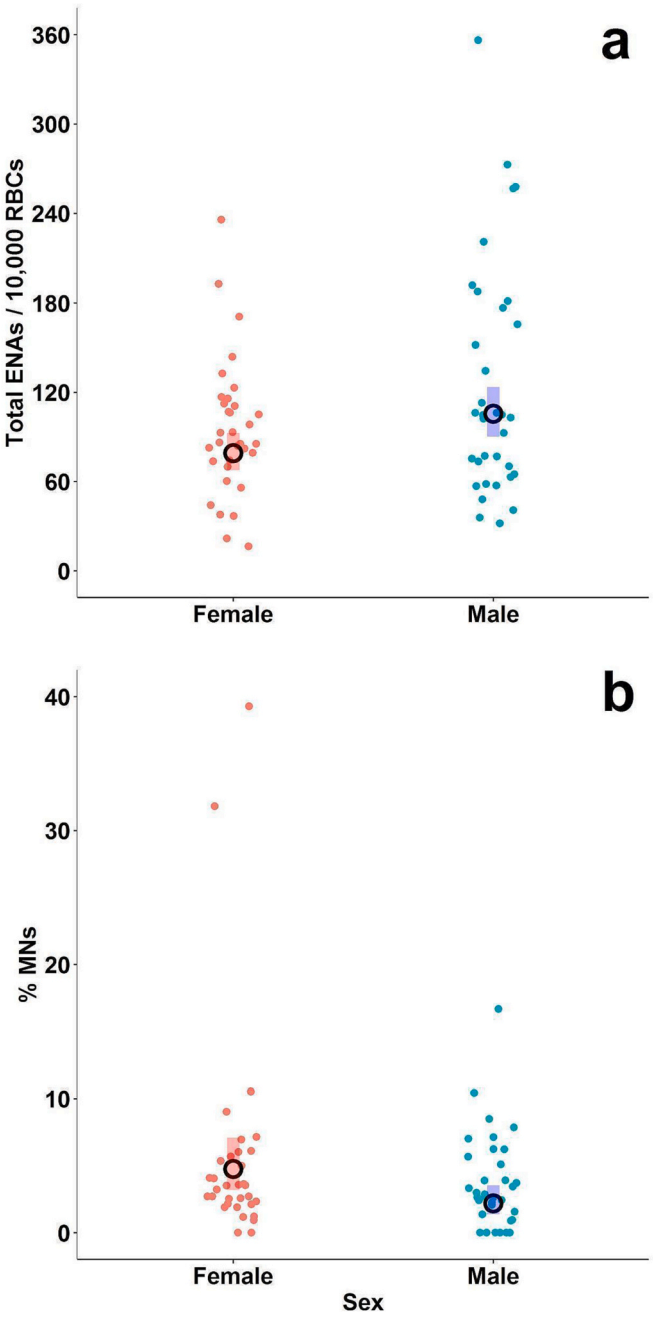

Fig. 3. Predicted (a) number of ENAs and (b) % MNs in relation to sex. Circle and bar depict the predicted mean and 95 % confidence interval. Dots show observed values.

et al., 2019, see also Menéndez-Blázquez et al., 2021). Our results show that the ranges of MN values for Adélie penguins are comparable to those from other Antarctic areas more affected by anthropogenic impacts, such as the Antarctic Peninsula (see Table 3). The high similarity of MN values to those reported for samples collected at Edmonson Point in 2014–15 (Table 3) supports the hypothesis of low genome instability in adult penguins breeding in Mid-Victoria Land (Olmastroni et al., 2019).

Total WBCs and leukocyte types did not show significant variation among colonies except for basophils (B) which were significantly higher in specimens from InIs compared to EdPo and AdCo (Table 2). Among WBCs, HE was the most abundant, followed by LY and EO, while BA and MO were the lowest (Table 2). Antarctic penguins generally have very low numbers of basophils (D'Amico et al., 2016a). The number of basophils in the smears varies greatly between species (Maxwell and Robertson, 1995). Adélie penguins had the highest average counts compared to Chinstrap (*Pygoscelis antarcticus*) and Gentoo (*Pygoscelis papua*) penguins along the Antarctic Peninsula (D'Amico et al., 2016a). In northern Patagonia, Magellanic penguins (*Spheniscus magellanicus*) also had very low numbers of basophils (range: 0–1) (D'Amico and Bertellotti, 2019). Variations in basophil numbers have been reported to be due to unspecified factors such as age, sex, environment, hormones, and diet. However, an earlier study has shown that the number of

basophils is relatively high just after hatching in chicks of *Gallus gallus domesticus* (Burton and Harrison, 1969). Together with the high number of heterophils observed at hatching, it has been suggested that this cellular response is part of a defence mechanism that supports the chick until its immune system is sufficiently active to produce lymphocytes at about 3 weeks of age (Burton and Harrison, 1969). In addition, Maxwell et al. (1990) found more basophils in younger birds than in older birds. Similar results were found by D'Amico et al. (2016b) for adult Adélie and Gentoo penguins compared to their chicks in Antarctica, with higher levels in both adults and chicks of Adélie than Gentoo penguins. Finally, basophils appear to be involved in the initial phase of the acute inflammatory response in birds, but this does not always show up as basophilia in the leukogram (Campbell, 1995).

The discriminant functions reflecting immuno-haematological profiles showed a low discriminatory power (ENAs, cross-validated discriminant rate: 37.9 %; WBCs, cross-validated discriminant rate: 40.7 %), and their scores overlapped substantially among colonies (Fig. 4). This implies that both haematological and immunological responses failed to explain the separation among colonies. This was further confirmed by PERMANOVA, which did not detect significant differences between colonies in both multivariate datasets of immuno-haematological parameters (ENAs: $F = 2.073$, $p = 0.118$; WBCs: $F = 1.386$, $p = 0.236$; see Table S2, for post-hoc comparisons) (Fig. 2). Our multivariate analysis thus confirms the lack of differences in the immune-haematological profiles of penguins from different colonies.

3.2. Individual life-history traits affected immuno-haematological parameters

Although it is relatively easy to sample and measure these parameters (Owen, 2011), their interpretation is more difficult, as individual characteristics (*e.g.*, health, age, sex, reproductive stage) can also influence them (Vleck et al., 2000; Barbosa et al., 2013; De Mas et al., 2015b). Hence, possible intraspecific differences were analysed by using samples from the two colonies (AdCo and EdPo), where penguins were monitored at their nest and simultaneous data on their breeding biology and individual life-history traits (*i.e.*, sex, nest position, body condition and chick-rearing period) could be collected and accounted for.

Among all predictor variables investigated, models highlighted sex as the most frequent predictor influencing immuno-haematological parameters. In particular, the total number of ENAs and the total number of WBCs were respectively ~30 % and ~20 % higher in male than in female penguins (Table S3; Fig. 3a) and males showed a ~50 % lower proportion of MNs (Table S3; Fig. 3b). Males' higher energetic efforts than females at some stage of the breeding cycle (*e.g.*, territorial fighting for nest defence, and longer fast at the beginning of the breeding season) may explain differences between sexes.

Our best model did not support the study colony as an influential predictor of immuno-haematological parameters, therefore the lack of differences between the two colonies (EdPo and AdCo) confirms our result found when comparing all three colonies and indicates that the "colony" *per se* does not have a significant impact on the determination of immuno-haematological parameters/profiles of Adélie penguins sampled in 2017–18. This suggests similar environmental conditions in this area, whatever the colony.

Body condition influenced the total number of WBCs, with penguins in the best conditions having a ~22 % higher level of WBCs than those in the worst conditions within the sample (Table S3; Fig. 5). This result supports previous findings by Tella et al. (2001), who showed a positive relationship between immune response and body condition in Magellanic penguins *Spheniscus magellanicus*, suggesting that immunocompetence, influenced by colony size, density-dependent factors and food limitation, is an important component of individual fitness.

Nest position only affected the proportion of MNs, with inner nesting penguins showing more than a threefold increase in the proportion of MNs compared to penguins nesting in peripheral positions (Table S3;

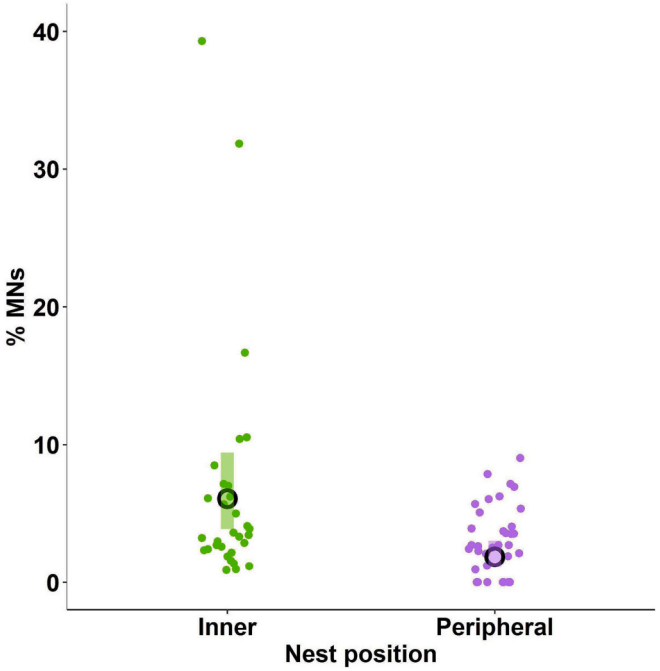

Fig. 4. Predicted % MNs in relation to nest position. Circle and bar depict the predicted mean and 95 % confidence interval. Dots show observed values.

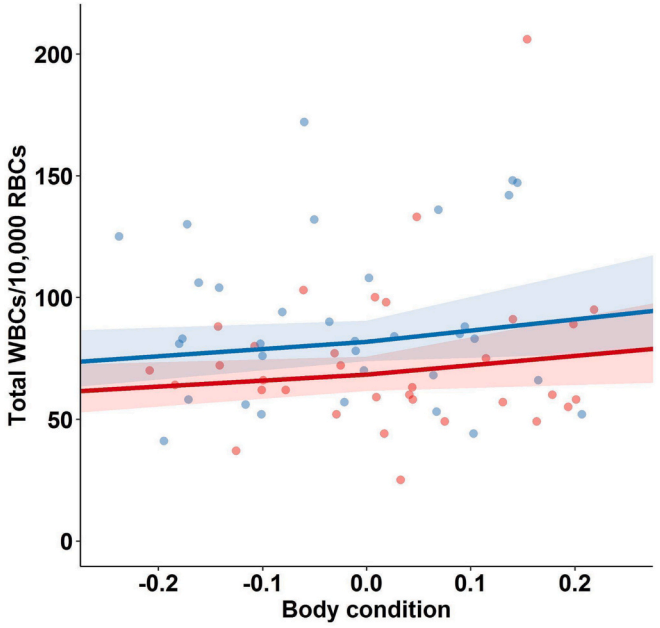

Fig. 5. Predicted number of WBCs in relation to body condition and sex (red: female; blue: male). Line and band depict predicted values and 95 % confidence interval. Dots show observed values.

Fig. 4), suggesting that central individuals were more stressed. For colonial seabirds, nest position may play an important role in nesting success. In Adélie penguins, central nests (high-quality nests) usually tend to be associated with individuals in better conditions and/or more experienced birds (Tenaza, 1971; Morandini et al., 2021), including individuals better able to withstand nest defence through territorial aggression (Spurr, 1974). Peripheral nests have been described as higher effort/cost maintenance, since more individuals attempt to steal stones, thus increasing intraspecific competition (Tenaza, 1971; Morandini et al., 2021). Also, peripheral nests are more susceptible to skua's

Table 4
Feather Hg concentrations, and carbon- ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values measured in female and male Adélie penguins from three colonies in Terra Nova Bay (Ross Sea, Antarctica). Values of $\delta^{13}C$ and $\delta^{15}N$ are proxies of the feeding habitat and trophic position for the pre-moulting period, respectively. Values are mean ± SD [range]; *n* indicates sample sizes.

Colony	*n*	Feather Hg (μg g^{-1})	Feather $\delta^{13}C$ (‰)	Feather $\delta^{15}N$ (‰)
AdCo				
All individuals	15	0.92 ± 0.25 [0.49–1.35]	−24.4 ± 0.8 [−25.6, −22.5]	11.0 ± 1.0 [9.9–12.2]
Females	8	0.91 ± 0.27 [0.50–1.35]	−24.2 ± 0.9 [−25.3, −22.5]	11.0 ± 1.2 [9.9–12.8]
Males	7	0.94 ± 0.26 [0.49–1.27]	−24.7 ± 0.6 [−25.6, −24.0]	10.9 ± 0.8 [10.1–12.1]
EdPo				
All individuals	15	0.83 ± 0.18 [0.50–1.18]	−24.5 ± 0.7 [−25.8, −23.6]	10.6 ± 0.6 [9.8–11.6]
Males	8	0.75 ± 0.12 [0.50–0.93]	−24.8 ± 0.5 [−25.5, −24.1]	10.3 ± 0.5 [9.8–11.0]
Females	7	0.92 ± 0.19 [0.63–1.18]	−24.2 ± 0.8 [−25.8, −23.6]	11.0 ± 0.4 [10.4–11.6]
InIs				
All individuals	15	0.83 ± 0.26 [0.50–1.33]	−24.5 ± 0.9 [−25.6, −22.8]	10.9 ± 1.0 [9.0–12.8]
Females	7	0.70 ± 0.24 [0.50–1.22]	−24.6 ± 1.0 [−25.6, −22.8]	10.7 ± 1.3 [9.0–12.8]
Males	8	0.95 ± 0.23 [0.66–1.33]	−24.3 ± 0.8 [−25.3, −22.9]	11.2 ± 0.8 [10.1–12.2]

attacks and predations (see Olmastroni et al., 2022, for our study colonies), and their occupants are both subject to stress due to interspecific and intraspecific competition (Giese, 1996; but see also Spurr, 1974).

Surprisingly, the H:L ratio was not explained by any predictors, suggesting that other, unaccounted factors may be involved in shaping this parameter (*e.g.*, fast duration or age, see Vleck et al., 2000). Eventually, investigating immuno-haematological parameters throughout the breeding season did not evidence a linear trend from incubation to crèche. Thus, when other individual characteristics are accounted for, variations in reproductive biology may not have such an important effect in determining these parameters, differently from what is described for other seabird species (Colominas-Ciuró et al., 2022).

3.3. Hg contamination and trophic ecology

Results from the two-way ANOVA revealed that (1) feather Hg concentrations did not differ between colonies ($F_{2,39} = 0.78$, $p = 0.47$), but did between sexes ($F_{1,39} = 4.97$, $p = 0.03$), with females showing on average lower Hg concentrations than males (Table 4), especially in EdPo and InIs (Table 4). No effect was detected for the sex:colony interaction ($F_{2,39} = 0.92$, $p = 0.41$). In general, seabirds, including penguins, considerably reduce their Hg body burden thanks to the moult, with up to 90 % of the Hg load being excreted in the new plumage (Honda et al., 1986; Albert et al., 2019). Female penguins possess an additional excretion route of Hg through egg laying (Braune and Gaskin, 1987; Bond and Diamond, 2009). This could thus explain the lower Hg concentrations observed in female Adélie penguins in Terra Nova Bay. Overall, Hg concentrations measured here in penguin feathers (Table 4) were similar to those reported >25 years ago at Terra Nova Bay (0.83 ± 0.13 μg·g^{-1}; Bargagli et al., 1998), suggesting that Hg contamination remained fairly stable over time in this Antarctic region (but see Seco et al., 2020a, 2020b who showed a decreasing trend over the last decade). Results also showed that (2) there was no difference in feather $\delta^{13}C$ values between colonies ($F_{2,39} = 0.06$, $p = 0.94$) nor between sexes ($F_{1,39} = 0.38$, $p = 0.54$), and no effect of their interaction ($F_{2,39} = 1.83$, $p = 0.17$). (3) Similarly, there was no difference in $\delta^{15}N$ values between colonies ($F_{2,39} = 0.64$, $p = 0.54$) nor between sexes ($F_{1,39} = 1.62$, $p = 0.21$), and no effect of their interaction ($F_{2,39} = 0.78$, $p = 0.47$). These results suggest that Adélie penguins from Terra Nova Bay share similar feeding habitat and trophic position (as indicated by $\delta^{13}C$ and $\delta^{15}N$ values, respectively) whatever the colony or sex during the pre-moulting period of building-up energy reserves at sea. However, mean $\delta^{15}N$ values were lower in females than males at all colonies, matching the lower Hg concentrations in females. This slight, even though not statistically significant difference in trophic position, could also account for the sex differences in Hg discussed above as Hg biomagnifies in food webs (Bargagli, 2008).

We did not find any linear or non-linear correlations between immuno-haematological parameters and Hg concentrations (ENAs: $r = 0.14$ and $p = 0.386$, $r_s = 0.10$ and $p = 0.511$; WBCs: $r = 0.20$ and $p = 0.184$, $r_s = 0.21$ and $p = 0.189$; % MNs: $r = -0.10$ and $p = 0.558$, $r_s = 0.01$ and $p = 0.943$; H:L ratio: $r = -0.11$ and $p = 0.482$, $r_s = 0.05$ and $p = 0.758$). The lack of relationship between blood stress biomarkers and feather Hg concentrations seems consistent, since they provide different information on both the temporal and spatial scales (Albert et al., 2019). Feathers indicate Hg accumulation in the whole body since the previous moulting episode (*i.e.*, one year ago in adult penguins), a temporal period that includes different stages of their life cycle (Furness et al., 1986). In contrast, blood informs about the recent Hg exposure (*i.e.*, a few weeks/months during the breeding season). Unfortunately, blood samples were limited here, preventing Hg measurements in this tissue. Future Hg analyses on blood samples would enable further investigation of the association between stress biomarkers and Hg contamination in Adélie penguins at a shorter period scale.

4. Conclusions

Multiple factors acting as stressors are expected to increase more prominently in Antarctica and its wildlife in the near future. At present, it is timely and important to detect significant alterations or changes at an early stage in Antarctic seabirds. Immuno-haematological parameters investigated in females and males of Adélie penguins during chick-rearing showed no substantial differences among individuals from the three colonies, suggesting a similar immune and stress profile during the 2017–18 summer season. In addition, the non-destructive method used here provides a useful tool for broader monitoring both in spatial and temporal terms capable of identifying stress events useful for conservation purposes, with particular regards to polar areas. However, our findings suggest that stress indicators are dependent on individual characteristics, therefore balancing the collection of non-destructive samples spatially across the colony, as well as between sexes, could help us to design more robust monitoring programs that would account for such drivers. Our rarefaction analysis also shows that variability in the population-level estimates of such stress indicators increases when the number of individual samples collected is reduced (Fig. S1, Supplementary Material), meaning that, when sample size is small, general differences among colonies may not be detected even when they occur in reality. Year-round Hg exposure was also similar among colonies as indicated by feather Hg concentrations. Trophic ecology during the previous pre-moulting period (post-breeding) was also similar among colonies, as indicated by feather $\delta^{13}C$ and $\delta^{15}N$ values. Although immuno-haematological parameters during the chick-rearing period

were not influenced by year-round Hg exposure, they are confirmed to be useful proxies for characterising the health status of Adélie penguin populations.

To secure Antarctic ecosystem resilience and manage the impact of the human footprint, extended protection of Antarctic biota from the risks of climate change, pollution and other anthropogenic disturbances should be claimed. Monitoring programs coupled with targeted research on seabirds' health are mandatory in the upcoming scenario because "effective surveillance begins in the field" (Wells et al., 2023). By identifying key drivers of immuno-haematological parameters, our results provide a valuable baseline to improve wildlife risk assessment in future health and stress ecology studies.

CRediT authorship contribution statement

Silvia Olmastroni: Writing – original draft preparation, review & editing, Funding acquisition, Conceptualization, Investigation, Supervision, Data curation. **Silvia Simonetti:** Writing-review & editing, Methodology, Formal analysis. **Niccolò Fattorini:** Writing-review & editing, Investigation, Methodology, Formal analysis, Data curation. **Verónica D'Amico:** Writing review & editing. **Fanny Cusset:** Writing-review & editing, Investigation, Formal analysis. **Paco Bustamante:** Writing review & editing. **Yves Cherel:** Writing review & editing. **Ilaria Corsi:** Writing – original draft preparation, review & editing, Conceptualization, Investigation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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