ANIMAL SCIENCE

Blood biochemistry and antioxidant status altered by anthropogenic impact in Adélie penguins (*Pygoscelis adeliae*)

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Abstract: Human activities are increased in Antarctica during decades, primarily due to the logistic and tourism operations, which consequent negative impact on penguin populations, altering their physiological responses. Therefore, we aimed to assess the blood biochemistry and oxidant/antioxidant balance of Adélie penguins (*Pygoscelis adeliae*) inhabiting two selected colonies: Potter Peninsula, considered as a low impacted colony, and Esperanza/Hope Bay as a high impacted colony. The levels of calcium, uric acid, and fructosamine, showed significant high values (p<0.05) for the Potter Peninsula´s penguins. Besides, this population showed high levels of plasma protein oxidation and erythrocyte lipid peroxidation (p<0.005) while the Esperanza/Hope Bay population presented high levels of erythrocyte protein oxidation and plasma lipid peroxidation (p<0.005). The oxidative damage values were similar in the Potter Peninsula population and slightly lower in the Esperanza/Hope Bay penguins if the results were compared to previous reports. The enzymes superoxide dismutase and glutathione peroxidase had significantly (p<0.005) high activity in the Esperanza/Hope Bay population, which also showed high reduced glutathione levels. The glutathione S-transferase activity was significantly high (p<0.005) in the Potter Peninsula population. The obtained results might take into account for making decisions about management and protection plans for the different penguin nesting areas in Antarctica.

Key words: Adélie penguins, Antarctica, Anthropogenic impact, Blood biochemistry, Oxidative stress biomarkers.

INTRODUCTION

Humans depend on marine ecosystems, but due to their improper use, they finally have been directly or indirectly altered. Halpern et al. (2008) analyzed the human impact in the 20 marine ecosystems and reported that no area had not been impacted. They also found that the least impacted areas were found at the poles, approximately 3.7% of the oceans, where seasonal or permanent ice limits human access. Marine biotas worldwide are being globalized and degraded by biological invasions, climate change, pollution, and a host of other anthropogenic perturbations (Aronson et al.

2011). Particularly, Antarctic ecosystems can no longer be regarded as pristine. Although it is an isolated continent, human activity is increasing (Aronson et al. 2011, Znój et al. 2017, Golubev 2021). The potential effects of this activity on wildlife populations have thus become an important issue in Antarctica because the activities of both, humans and wildlife, tend to converge on a small fraction of the coastal landscape that is free of ice (Patterson et al. 2003).

In addition to the above, Antarctic environments now confront the double threat, in some cases synergistic, of direct human impact coupled with climate change in the

region. Antarctica is one of the region's most seriously affected by climate change, it is projected to warm by approximately 4 C by the end of the century (Clarke et al. 2007, Hughes et al. 2021). Over the last 50 years, the western Antarctic Peninsula has experienced a rapid increase in temperature (especially in winter), accompanied by a marked glacial retreat, and an evident decrease in sea ice cover, considering its duration and extent (Turner et al. 2009, Ducklow et al. 2013, Convey & Peck 2019, Hughes et al. 2021).

Seabirds are essential components of aquatic ecosystems and their status as environmental health sentinels were well established. They were used to assess the effect of pollution, the size of fish populations, and the effects of fisheries management practices, as well as making visible changes in aquatic ecosystem productivity or climate change. Consequently, monitoring bird populations or their reproductive success may show any disturbance in marine ecosystems (Mallory et al. 2010 and references therein).

It was reported that penguin health status or its disorders may reflect local and/or regional oceanic conditions (Dee Boersma 2008, Barbosa 2011, Trathan et al. 2015). This ability was related to their high specialization in swimming and diving. Moreover, they were highly restricted in their foraging habitat, particularly during the breeding season (Trathan et al. 2015). Consequently, through their metabolic state, penguins reflect changes in ocean productivity, and human-induced changes in the environment, including pollution and climate variation, (Dee Boersma 2008). As was commented above, the human activity developed in Antarctica were increased primarily due to logistic and tourism operations (Patterson et al. 2003, Lynch et al. 2019). There was evidence that both activities negatively influence the penguin populations in Antarctica (Patterson et al. 2003, Bricher et al. 2008, Ropert-Coudert et al. 2019).

The use of physiological markers as indicators of the health of animal populations, including those related to the oxidative status, was shown by Beaulieu et al. 2013. Natural factors such as physical activity, potential pathogens, and changes in air temperature could generate alterations in the oxidant/antioxidant balance, generating oxidative stress which, in turn, could have consequences on longevity, reproduction, and immune responses (Costantini 2008, 2010, Ibañez et al. 2021). Besides, there is a long list of factors generated by the anthropogenic impact, which can directly or indirectly affect the oxidative/antioxidant status of the exposed animals. These include human presence *per se*, tourism, light and noise pollution, fumes, pesticides, metals, radiation, air warming, drought, variations in food availability, pathogens, and emerging diseases (Beaulieu & Costantini 2014, Ropert-Coudert et al. 2019).

Variations in the oxidative/antioxidant balance will result in oxidative stress (OS). Increases in the level of OS can ultimately result in reversible or irreversible damage to essential molecules such as lipids, proteins, and DNA, leading to cell death (Halliwell & Gutteridge 2015). If the damage is low, many cell types tend to adapt to the stress and proliferate by regulation of their defence and/or repair systems. If the damage is moderate, cell cycle arrest or senescence may occur, with the cell surviving but unable to divide. In the case of severe oxidative damage, cells can lead to death by apoptosis, necrosis, or mechanisms with characteristics of both (Halliwell 2006). Consequently, the organisms' response may be adaptive and maintain or restore the body's homeostasis, or be inadequate with detrimental effects on the health and biological fitness of the individual such as decreased fertility and

decreased likelihood of survival (Costantini 2008, Beaulieu & Schaefer 2013).

Approximately 4 km far away from Carlini Station, and within the Antarctic Specially Protected Area 132, there is an important colony of Adélie penguins, with a population of approximately 3000 pairs (Antarctic Treaty 2013). Because of its character as a protected area, there are no tourist arrivals or other logistical activities, then the penguin colonies located there are considered not impacted by human activity.

At Esperanza/Hope Bay, the penguin colonies' situation is completely different because the nesting area is close to where the normal activity of the Esperanza Station takes place. Near the Station, there is part of approximately 104,139 breeding pairs of Adélie, one of the largest colonies of this species (Santos et al. 2018). Moreover, it should be noted that Esperanza/Hope Bay is not a protected area so tourism is allowed.

During the penguin sampling season, according to the International Association of Antarctica Tour Operators (IAATO) report, some 3,477 people visited the area, of which 792 visited Esperanza Station and the penguins' colony area (IAATO 2013). Also, that season, historical trash and materials of various kinds were scattered in the vicinity of the Station, generated in the years before Argentina's international commitments under the Antarctic Treaty System, particularly the Madrid Protocol. In general, among the historical trash, there were diesel drums with a high degree of deterioration. All these particularities mean that this penguin colony was considered to suffer a chronic anthropogenic impact.

Knowing that: a) there is a progressive increase of anthropogenic activity in Antarctica, and b) penguins are considered good sentinels of the marine ecosystem, the present study aimed to assess the biochemistry and blood oxidant/antioxidant balance of Adélie penguins inhabiting selected colonies with different human impact. This method of assessment facilitated a knowledge of the physiology of the penguins at the time of sampling, thus allowing us to know and compare their health over the years. Based on the data obtained from such monitoring, decisions about management and protection plans for the species in the different nesting areas would be possible.

Taking into account that the blood biochemistry and oxidant/antioxidant status vary in response to the anthropogenic impact in different penguin populations, we predict that the penguin rookeries at Hope Bay should have augmented oxidative damage, with an increment of the antioxidant defenses, and their biochemical values outside of those expected.

MATERIALS AND METHODS

Study area and sample obtaining

The study was conducted in two penguin rookeries. One is located at Potter Peninsula (62°14'S 58°40'W, Antarctic Specially Protected Area (ASPA) N° 132, 25 de Mayo Island, South Shetland Islands) near the Carlini Station and the other at Esperanza/Hope Bay near the Esperanza Station (63°23'S 56°59'W, Esperanza Peninsula, Antarctic Peninsula) (Fig. 1). Breeding penguins were sampled during the austral summer of 2012-2013. All samples were taken on the same day, for each location. At that time, intensive care of chicks was taking place. A total of 30 penguins: 15 from Potter Peninsula and 15 from Esperanza/Hope Bay were sampled not considering the sex.

Approximately 3 mL of blood was drawn from the metatarsal vein of the birds' feet using a syringe with a 23-gauge sterile needle. Heparin was added to the syringes to avoid coagulation
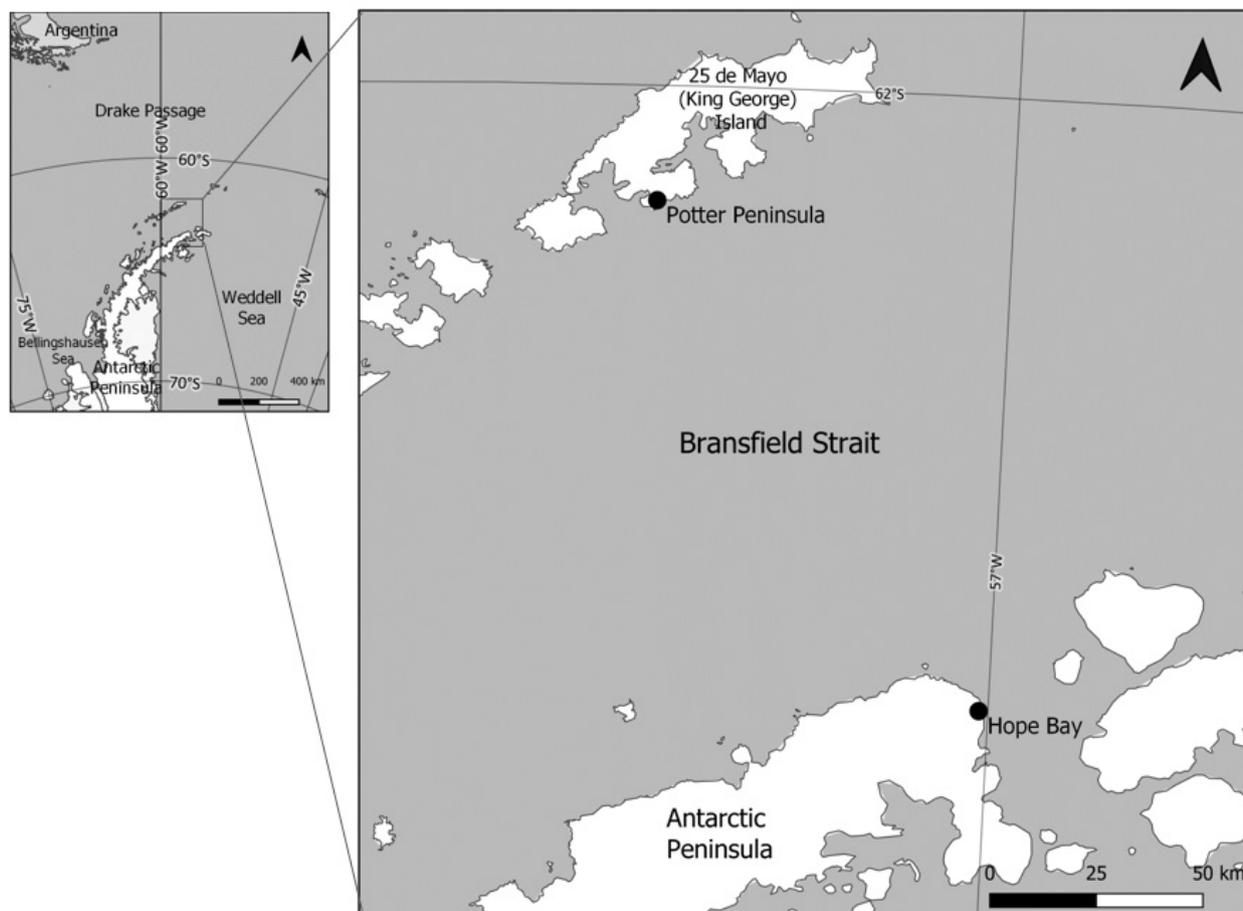
Figure 1. Location of the study sites at Potter Peninsula (62°14'S 58°40'W, ASPA 132, 25 de Mayo Island, South Shetland Islands) and at Esperanza/Hope Bay (63°23'S 56°59'W, Esperanza Peninsula, Antarctic Peninsula). Image made with Quantarctica 3 (Quantum GIS [Since 2.0 just QGIS] + Antarctica = "Quantarctica") (Norwegian Polar Institute, 2018).

and favoring the plasma - erythrocyte separation and it was not used for serum separation. Samples were centrifuged at 1500 g for 10 minutes to separate erythrocytes from plasma or serum. Plasma and serum samples were stored in liquid N_2 until their analysis. The erythrocytes were washed twice with cold saline solution, centrifuged to remove the leucocytes layer, and then hemolysis was carried out through the freeze/thaw procedure. After that, samples were centrifuged at 14,000 x g for 10 min and the supernatants were stored in liquid N_2 until they were processed in the laboratory.

The specimens´ body mass was measured as an index of the body condition. Besides, the haematocrit was also measured at the time of sampling by the Microhematocrit method. Briefly, blood samples were taken using a heparinized capillary tube. Then, they were centrifuged and haematocrits were calculated by measuring with a digital caliper the ratio of the column of packed erythrocytes to the total length of the sample in the capillary tube (Campbell & Ellis 2007).

Compliance with ethical standards

The authors declare to have no conflicts of interest. All applicable international, national, and institutional guidelines for sampling, care, and experimental use of animals for the study were followed as established by the Article III, Annex II of the Madrid Protocol, Law 24.216

(Taking, Harmful Intrusion and Introduction of Species) within the framework of the projects evaluated by the Environment Office of the IAA and Dirección Nacional del Antártico (DNA).

Determination of blood oxidant/antioxidant markers

The analyzed parameters were all measured using spectrophotometric techniques. All the reagents and solvents used in the processing of samples and measurement techniques were of analytical grade. The enzyme activities of superoxide dismutase (SOD), catalase (CAT), glutathione s-transferase (GST), and glutathione peroxidase (GPx), as well as the levels of reduced glutathione (GSH), lipid oxidation (LPOe), and protein oxidation (POe), were measured in the erythrocyte fraction. Besides, in the plasma fraction, the levels of lipid oxidation (LPOp) and protein oxidation (POp), and total proteins (TPp) were measured.

Before the SOD activity measurement, a chloroform: ethanol (3:5) extraction from the erythrocyte fraction was done followed by centrifugation at 5000 g (for 15 min) to get rid of hemoglobin (Hb$^+$) interference. Then, the aqueous supernatant was saved for determining the SOD activity by the Misra & Fridovich (1972) protocol. One SOD unit was the amount of enzyme necessary to inhibit 50% of the rate of autocatalytic adrenochrome formation, measured at 480 nm (e_{480} = 2.96 M^{-1}cm^{-1}).

The CAT activity was measured sensing the decrease in hydrogen peroxide concentration (H$_2$O$_2$ 10 mM solution) at 240 nm (e_{240} = 40 M^{-1}cm^{-1}) (Aebi 1984). For that, the erythrocyte samples were diluted (1:500 v/v). One CAT unit was the amount of enzyme necessary to degrade 1 µmole of H$_2$O$_2$.

The GST enzyme activity was determined according to Habig et al. (1974) protocol using 1-chloride-2.4-dinitrobenzene (CDNB) 100 mM as

substrate and reduced glutathione (GSH) 100 mM. One GST unit represents the amount of enzyme required to conjugate 1 µmole of 1-chloro-2.4-dinitrobenzene to GSH in the erythrocyte's fraction, at 340 nm (e_{340} = 9.6 M^{-1}cm^{-1}).

Both SOD, CAT, and GST units were expressed as enzyme units per mg protein per minute of assay reaction (U enzyme/mg protein. min).

The enzyme GPx catalyzes the H$_2$O$_2$ reduction reaction and other peroxides by oxidation of GSH. Physiologically it acts coupled to the enzyme glutathione reductase (GR) which in turn catalyses the reduction of oxidized glutathione (GSSG) using NADPH as a cofactor. The consumption of NADPH vs time was recorded spectrophotometrically at 340 nm (e_{340} = 6.22 mM^{-1}cm^{-1}) according to the method of St Clair & Chow (1996). The activity of GPx is expressed as µmol NADPH mg/ protein. min.

For the GSH determination, the sample was treated with 400 µl of trichloroacetic acid (TCA) 12%. The mixture was centrifuged, the supernatant was separated and 5.5-di-thio-bis-2-nitrobenzene (DTNB) was added to react with GSH to form a yellow thiolate anion (TNB) (ε_{412}=13.6 mM^{-1}cm^{-1}) according to Moron et al. (1979). The results were expressed as µmoles GSH/mL hemolysate.

The LPO levels were quantified using a colorimetric technique where thiobarbituric acid (TBA) binds to form a pink Schiff's base (e_{535} = 153 M^{-1} cm^{-1}) and is stable for a few hours (Buege & Aust 1978). The results are expressed as mmol TBARS /mL erythrocyte´s haemolysate (LPOe) or plasma (LPOp).

The PO levels were assessed according to Ansaldo et al. (2007). The assay is based on the detection of the formation of protein hydrazones as a result of the reaction of 2,4-dinitrophenyl hydrazine (DNPH) with the carbonyls of oxidized proteins. The carbonyl content is calculated from the measurement of the protein hydrazones

(ε_{375} = 22000 M^{-1} cm^{-1}). The results are expressed in nmoles Carbonyls/mg erythrocyte proteins (POe) or mg plasma proteins (POp).

The TP concentration was measured by the Lowry et al. (1951) protocol, using bovine serum albumin as standard. The results are expressed as mg protein/ mL plasma (TPp).

Determination of plasma biochemistry

Biochemical parameters were measured in plasma using spectrophotometric techniques. Those were the concentration of glucose (Glu), calcium (Ca), inorganic phosphorus (Pi), triglycerides (Tri), fructosamine (Fru), and uric acid (UA). For this purpose, the Wiener Lab standard haematology kits were used (reference number: 1400106, 1152002, 1382321, 1780111, 1400050, and 1840101). And Fe-s levels were measured in serum (kits Wiener Lab, ref.: 1492001).

The data of air temperature was obtained from data provided by the Servicio Meteorológico Nacional of the Argentinean Air Force at Carlini and Esperanza Stations.

Statistical analysis

The InfoStat software version 2018 (Di Rienzo et al. 2018) was used for all the statistical analyses employed. The t-test for independent samples was used to analyze the results of Adélie penguin blood measurements. Results were expressed as means ± standard error and the significance level was set at α = 0.05.

RESULTS

The average body mass and the haematocrit values were similar for the two colonies. Body mass: Potter Peninsula: 4.23 ± 0.17 kg and Esperanza/Hope Bay: 4.35 ± 0.16 kg (mean ± standard error). The haematocrit values fluctuated between 57% (minimum value) and 69% (maximum value) at Potter Peninsula while, in Esperanza/Hope Bay, the minimum value was 51% and the maximum 70%.

Blood biochemistry values varied in the studied penguin populations (Table I). Ca, UA, and Fru levels showed significant differences (p < 0.05) between the Esperanza/Hope Bay and Potter Peninsula colonies, with the highest values recorded in the latter. The Glu, Pi, and Fe-s levels were similar and did not differ between colonies (Table I).

Adélie penguins showed variations in the oxidant/antioxidant balance according to the considered parameter and the studied colony. The Potter Peninsula population showed higher levels of POp and LPOe, while higher levels of POe and LPOp were recorded in the Esperanza/Hope Bay population (p < 0.005) (Fig. 2). Considering the enzymes, both SOD and GPx had significantly higher activity (p < 0.05) in penguins from the Esperanza/Hope Bay population, which also had higher GSH levels. On the other hand, penguins from the Potter Peninsula population had higher GST activity (Fig. 3).

Table I. Adélie blood biochemistry values from adults nesting at Potter Peninsula and Esperanza/Hope Bay.

	POTTER PENINSULA			**ESPERANZA/ HOPE BAY**		
	n	**Mean**	**S.E.**	**n**	**Mean**	**S.E.**
Glu	15	241.3[a]	12.3	15	223.44[a]	8.13
UA	15	13.25[a]	0.39	15	5.8[b]	0.41
Ca	15	14.55[a]	0.53	15	5.46[b]	0.33
Pi	15	3.72[a]	0.32	15	4.19[a]	0.55
Fru	15	1.23[a]	0.03	15	0.36[b]	0.01
Fe-s	10	597.36[a]	95.74	8	593.55[a]	94.62
TPp	15	47.02[a]	2.65	15	54.5[a]	2.87

Concentration of Glu - Glucose (mg/dL), UA - Uric acid (mg/dL), Ca - Calcium (mg/dL), Pi - Inorganic phosphorus (mg/dL), Fe-s - Iron (ug/dL), Fru - Fructosamine (mmol/L) and TPp - Total plasma protein (mg/mL). Different letters indicate significant differences (p-value < 0.05).
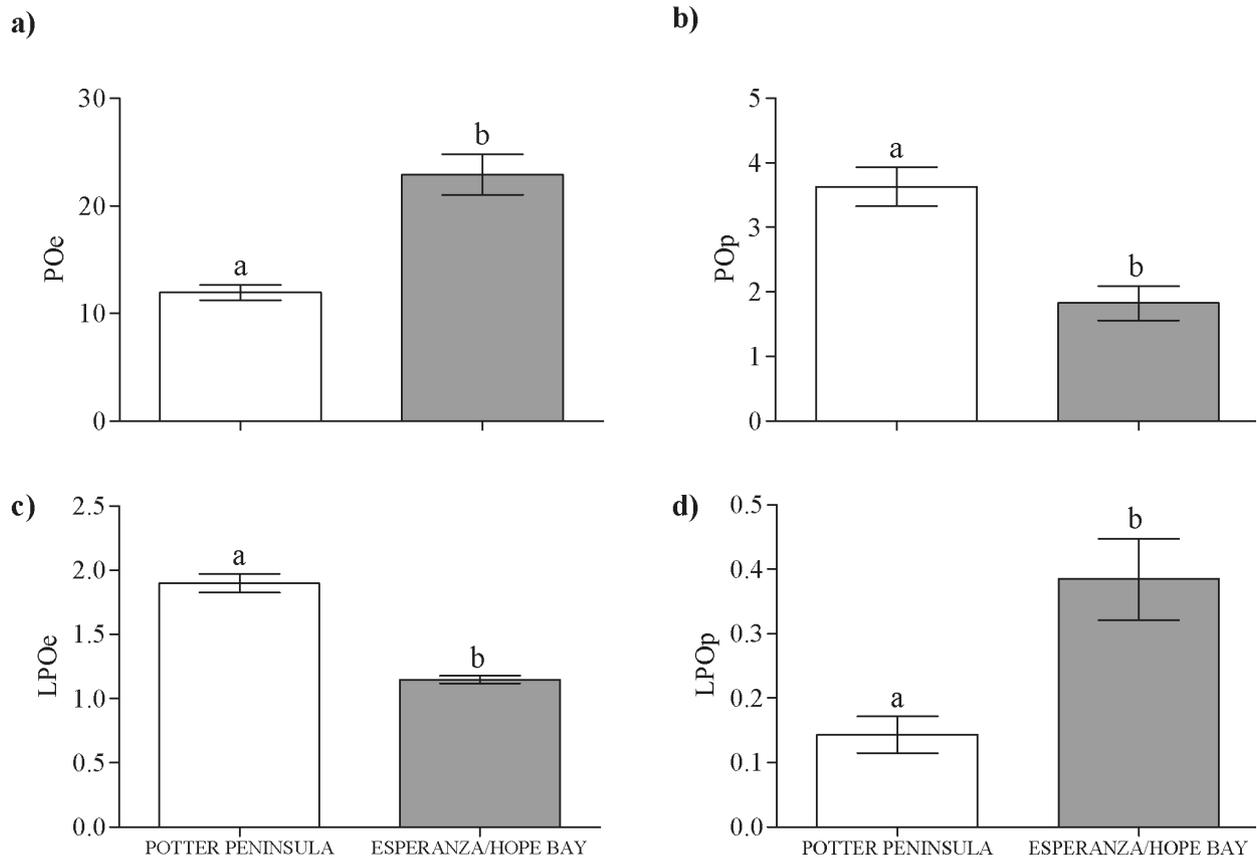
Figure 2. Adélie blood oxidative damage from Potter Peninsula (n = 15) and Esperanza/Hope Bay (n = 15) populations. Levels of a) POe - Erythrocyte protein oxidation (nmoles carbonyl/mg erythrocyte protein), b) POp - Plasma protein oxidation (nmoles carbonyl/mg plasma protein), c) LPOe - Erythrocyte lipid oxidation (mmol TBARS /mL haemolysate) and d) LPOp - Plasma lipid oxidation (mmol TBARS /mL plasma). Values are expressed as mean ± standard error. Different letters indicate significant differences (p-value < 0.005).
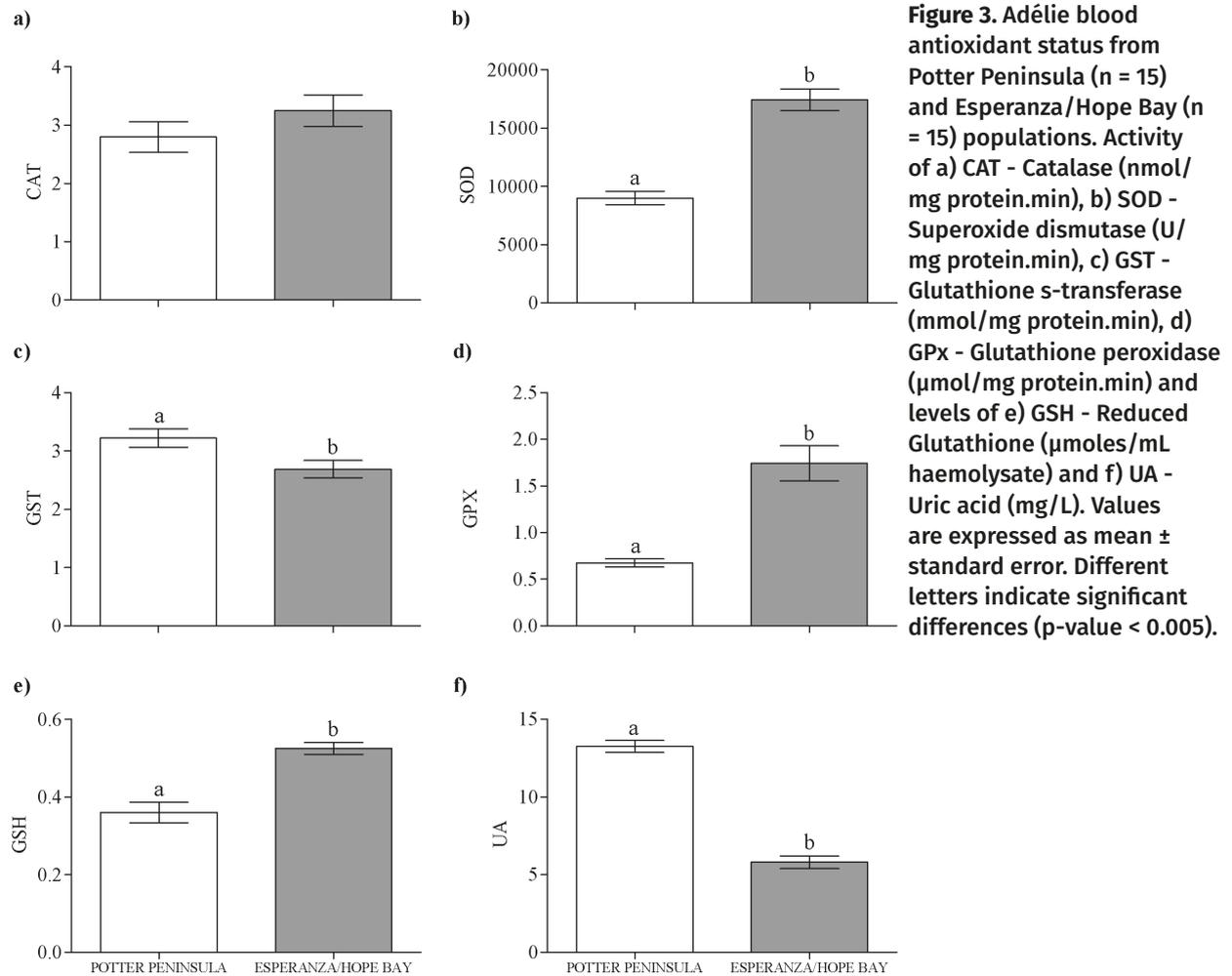
DISCUSSION

Analyzing the present results, we observed that the blood biochemistry parameters recorded in the studied populations of Adélie penguins were within the range of values reported for birds (Aguilera et al. 1993, Sakas 2002) and, in particular, for these species (Ibañez et al. 2015). Significant differences were only observed in Ca, Fru, and UA levels, with higher values in the Potter Peninsula population (Table I).

The Adélie penguin has a prolonged fasting stage at the beginning of the breeding season (Ainley 2002). Then, and during the incubation period, both males and females take turns to forage and hydrate themselves. Besides, there

is a marked difference between the analyzed colonies in the length of time they leave the nest for foraging trips during the period of intensive chick care. They leave the nest for an average of 36 hours at Esperanza/Hope Bay, while, they leave the nest every 24 hours at Potter Peninsula (Trivelpiece et al. 1987, Ainley 2002). In short fasting periods, as in the present case of the study, plasma Glu levels did not change and UA levels remained low (Table I) (Vleck & Vleck 2002).

As mentioned above, natural and anthropogenic factors could modify the physiology of penguins. In the case of natural factors like air temperature and precipitation

Figure 3. Adélie blood antioxidant status from Potter Peninsula (n = 15) and Esperanza/Hope Bay (n = 15) populations. Activity of a) CAT - Catalase (nmol/mg protein.min), b) SOD - Superoxide dismutase (U/mg protein.min), c) GST - Glutathione s-transferase (mmol/mg protein.min), d) GPx - Glutathione peroxidase (μmol/mg protein.min) and levels of e) GSH - Reduced Glutathione (μmoles/mL haemolysate) and f) UA - Uric acid (mg/L). Values are expressed as mean ± standard error. Different letters indicate significant differences (p-value < 0.005).

increasing may affect them as was reviewed by Ropert-Coudert et al. (2019). The Adélie penguins from Esperanza Peninsula were exposed to more extreme environmental conditions than penguins from Potter Peninsula. During summer 2013, when Adélie blood samples were taken, average air temperatures (Esperanza/Hope Bay: 0.7 ºC and Potter Peninsula: 1.5 ºC) were also similar to the 10-year (2001-2010 data) weather climatological norm recorded by the Servicio Meteorológico Nacional of Argentina (SMN) in those areas. However, precipitation, both in the form of rain and accumulated snow in January 2013 (63.3 mm), was much higher than the climatology (2001-2010, SMN) in the Esperanza Station area. Because of this, the Adélie penguins and their chicks had to cope with extreme environmental conditions that imposed greater stress on them in terms of temperature maintenance and feeding. Patterson et al. (2003), Bricher et al. (2008), and Juáres et al. (2015) all provided evidence that wind exposure and snow accumulation predict Adélie penguin population trends.

For those causes, foraging trips turn more difficult to do and this was reflected in lower UA levels (Fig. 3f) probably due to a longer and more costly fasting period. In comparison, the Potter Peninsula population had not the same difficulties because the registered amount of accumulated snow was lower (16.3 mm, SMN). In captive conditions, Cape penguins (*Spheniscus*

demersus) were deprived of food for 11 hours, then fed and 2 hours later a blood sample was taken. In this experiment, Cray et al. (2010) recorded a more than threefold increase in UA levels. They also observed a 30% decrease in UA after a daytime fast of just over 5 hours. These reports are consistent with what we recorded for Adélie, indicating that blood biochemistry reflected differences in access to food between the Potter Peninsula and Esperanza/Hope Bay colonies.

Besides, in the Potter Peninsula population, the increased Ca levels (Table I) may be related to some level of dehydration. As mentioned above, Adélie penguins leave the nest about 36 hours during incubation and usually do not have access to freshwater unless they use the snow surrounding the nest (Vleck & Vleck 2002). In the case of the Potter Peninsula population, there was no snow around the nests, with a consequent elevation of blood Ca levels, indicating dehydration. However, this was not severe dehydration, because other parameters such as haematocrit or TPp did not show significant differences between colonies. In addition, body mass, an indicator of dehydration and fasting, showed no differences between the colonies studied and the values were similar to those reported previously (Vleck & Vleck 2002).

Several factors generated by the anthropogenic impact may be acting at the same time on the metabolism of penguins in the colonies studied, so it is difficult to determine which of them is the most important factor to which the alterations found in the blood oxidant/antioxidant balance should be associated.

As just was mentioned, air temperatures during December, January, and February 2013 were similar to the climatological norm for the areas studied (SMN). However, snow accumulation at Esperanza Station was higher

and implied greater foraging effort for the penguins. The latter is associated with increased metabolism with higher ROS production and, consequently, increased antioxidant defenses. Both SOD and GPx activity (Fig. 3b and d respectively), two of the enzymes of the first line of antioxidant defense (Ighodaro & Akinloye 2018), showed increased activity in blood samples from Esperanza/Hope Bay colonies, as well as higher GSH levels (Fig. 3e). Also, the enzyme CAT showed similar patterns between colonies, but no significant differences (Fig. 3a). Beaulieu et al. (2011) reported that Adélie penguins subjected to restrictions during chick-rearing prioritized physical self-maintenance with increased antioxidant capacity, as we observed in the present work.

On the other hand, both colonies differed in oxidative damage values. Potter Peninsula population had high OPp and LPOe levels (Fig. 2b and c respectively), while OPe was lower and OPp was higher. At this place, the registered Adélie LPO levels were similar to those observed by Di Fonzo (2019) when studying the same penguin species for 6 consecutive years. On the other hand, oxidative damage in Adélie penguins from Esperanza/Hope Bay was slightly lower than previously shown in other works (Cebuhar et al. 2017).

At the same time, the Potter Peninsula population had high GST activity (Fig. 3c). In contrast, the Adélies from Esperanza/Hope Bay showed high OPe levels (Fig. 2a), high LPOp levels (Fig. 2e), and low GST activity (Fig. 3c). In a study carried out on Gentoo penguins (*Pygoscelis papua*), the blood oxidative damage was compared in three colonies with different exposure to anthropogenic impact, one in the vicinity of the Carlini Station (considered to have the very low impact), and the others in the Esperanza Station (medium impact) and the Almirante Brown Station (high impact) (Di

Fonzo 2019). As in the present work, oxidative damage and GST activity levels were found to be high in Potter Peninsula penguins and similar to those of the colony with the highest disturbance. It would therefore appear that the Potter Peninsula penguin colony was more disturbed than expected and mainly due to exposure to xenobiotics, as evidenced by increased GST activity. This analysis could be alerting us to a high anthropogenic impact on the penguins' foraging area or to which their prey was exposed. Therefore, it is considered very helpful to evaluate different contaminants present in the penguins' possible prey in the area, to make a better assessment of the state of the ecosystem in which they live, and to be able to make decisions regarding the conservation and protective plans.

Another point to be taken into account is that the presence of pathogens. These can also reduce an individual's ability to respond to other stressors like pollutants or extreme environmental changes (Ropert-Coudert et al. 2019). Ibañez et al. (2021) noticed that immune responses were elevated in Adélie penguins from the disturbed area in Esperanza Bay. In agreement with the data presented here in, they also observed that individuals breeding under anthropogenic pressure overexpressed proteins with immune, antioxidant, and metabolic functions. Although the immune response has not been studied in the present work, human activity could be influencing the spread of new pathogens on high-impact penguin colonies with the consequent increased oxidative stress observed. Moreover, there is great controversy about the disturbance caused by the human activities in the colonies, as some studies indicated that they did not seem to have a detectable impact at the population level, while others expressed that they directly affect the reproductive success (eggs hatching and chicks survival) (Giese 1996, Patterson et al. 2003).

Based on our results and as was mentioned by Beaulieu et al. (2013), it is clear that, at the physiological level, penguins are undergoing alterations that could lead to long-term problems such as accelerated cellular aging, reduced fertility, and reduced probability of survival. Finally, it is important to understand the physiological capacities of organisms at environmental stressors to predict their response to changing environments.

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