Non-invasive hormone monitoring as a robust method for determining adrenocortical activity in injured, emaciated and oil-contaminated African penguins undergoing rehabilitation

Scheun, J.[1,2,3,*], Ludynia, K[4,5], Snyman A[4], Ganswindt, A. [2,3]

[1] Department of Life and Consumer Sciences, University of South Africa, South Africa

[2] National Zoological Garden, South African National Biodiversity Institute, South Africa.

[3] Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, South Africa

[4] Southern African Foundation for the Conservation of Coastal Birds (SANCCOB), Cape Town, South Africa

[5] Department of Biological Sciences, University of Cape Town, South Africa

*Corresponding author: Juan Scheun, Scheuj@unisa.ac.za

Permanent address: Department of Life and Consumer Sciences, University of South Africa, 28 Pioneer Ave, Florida Park, Roodepoort, South Africa

Highlights

• Rehabilitation stage affects the stress response in African penguins.

• Injury and emaciation affect the stress response in African penguins

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• High individual variation in the stress response to injury and emaciation.

Abstract

Anthropogenic activity is a major driver of seabird injury and mortality in the 21st century. Although most seabirds perish within the natural environment as a result of human activities, some are rescued and admitted to rehabilitation centres. Despite the considerable number of admissions, little is known regarding the physiological response seabirds have to specific admission reasons and the rehabilitation process. In this study, we aimed to determine the effect of injury, emaciation, oiling, individual removal from the natural environment and the rehabilitation process on the physiological stress response of the African penguin (*Spheniscus demersus*). Urofaecal samples were collected from African penguins throughout a three-stage rehabilitation process and quantified for glucocorticoid metabolites (ufGCM). The three stages included an initial ICU crate stage (Stage 1), an ICU pen stage (Stage 2) and a rehabilitation stage (Stage 3). Data were analysed using a generalised linear model in order to determine the effect of admission reason, age and rehabilitation stage (Stage 1, 2 and 3) on ufGCM levels. Although the model indicated that only Stage 1 was a significant driver of adrenocortical

activity in the study population, ufGCM levels of injured and emaciated animals within Stage 1 were considerably higher than those of birds with any other admission reason across all stages. This is the first study examining the causes and effect of rehabilitation on the physiological stress response in African penguins. Enhanced care and attention should be given to rescued individuals, especially during the first stage of rehabilitation, to reduce perception of additional stressors and thus increase the chance of full recovery.

Keywords: rehabilitation; seabirds; African penguin; glucocorticoid; injury; emaciation

1 Introduction

The dramatic increase in human populations, and the inevitable expansion into terrestrial and aquatic ecosystems, has resulted in the alteration of the natural environment and decline in wildlife populations due to anthropogenic activities (Bender et al., 2014; Daszak et al., 2000; Delach, 2006; Hannah, 2011; Köhler and Triebskorn, 2013). The effect of such activities is profound within the marine environment, where overexploitation of fish stocks, injury through direct human-animal interactions (e.g. longline fishing/tourism) and pollution (e.g. oil spills) have led to a rapid rise in mortality rates among marine birds (Brothers et al., 2010; Danckwerts et al., 2014; Dias et al., 2019; Munilla et al., 2011). Although most injured, oiled, and emaciated seabirds perish at sea, a small number do reach the coastline, where they are often transported to rehabilitation centres. Unfortunately, this does not guarantee survival; Montesdeoca et al. (2017) found that of the 1823 admissions of sea birds to a rehabilitation centre in Spain, 15% of the individuals needed to be euthanized, while 16% of animals died while in the rehabilitation programme (unassisted mortalities). Furthermore, high mortality rates and reproductive suppression are still observed in rehabilitated birds released from centres as a result of immune suppression and disease (Barham et al., 2007; Briggs et al., 1996; Fajardo et al., 2000; Newman et al., 2004; Parsons et al., 2018). In order to better understand the long-term physiological response marine birds may have to natural and anthropogenic stressors, allowing for improved animal care, rehabilitation, and release protocols, the implementation of physiological monitoring techniques would be vital (Kaleta and Kummerfeld, 2012; Mullineaux, 2014). In this regard, endocrine monitoring offers an ideal technique to assess general health and the physiological stress an individual is experiencing.

Both the sympathetic-nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis are of importance in restoring homeostasis within an individual when confronted with a stressor. The SNS releases catecholamines, which are important in the 'fight or flight' response of an individual (Dickens et al., 2010; Fair et al., 2014). The activation of the HPA axis leads to the elevated production and secretion of glucocorticoids (GCs) (Keay et al., 2006). This temporal elevation in GC concentration facilitates restoration of an organism's homeostasis through the adjustment in metabolism, enhanced cardiovascular activity, and altering behaviour (Busch and Hayward, 2009; Romero, 2002). Although elevated GC concentrations can be beneficial when acute in nature, a prolonged elevation can lead to a number of deleterious effects, including suppressed growth, reproductive and immune function, as well as a shortened life expectancy (Charmandari et al., 2005; Cohen et al., 2012; Möstl and Palme, 2002). As GCs are important mediators of the physiological stress response and can have a range of negative effects on an organism if chronically elevated, GC determination is often seen as an important marker of perceived stress (Palme, 2019). Despite blood being a robust matrix for determining real-time GC levels, the need for regular animal capture or restraint, along with an elevation in GC concentrations associated with such activities (Romero and Reed, 2005), renders this approach impractical for long-term studies on captive and free-ranging populations. Here, non-invasive hormone monitoring offers a suitable alternative. Circulating GCs within the bloodstream are processed by the liver and kidneys and excreted via the biliary and renal systems in metabolite form (Sheriff et al., 2011). As hormone metabolites are pooled within the gut prior to excretion, samples are less affected by episodic fluctuations or pulsatile secretions of hormones, thus providing a long-term (hours to days) metabolite pattern compared to the 'real-time' hormone pattern observed in blood samples (McEwen and Wingfield, 2003; Touma and Palme, 2005). Thus, GC metabolites in excreted urine and faeces can be quantified and used as a proxy of adrenocortical function. The non-invasive nature of the sampling technique reduces the need for human-animal interaction, and thus allows for long-term GC metabolite monitoring (Scheun et al., 2017; Wielebnowski and Watters, 2007).

Implementing non-invasive GC monitoring in injured, oiled, or starved animals, as an indicative measure for the severity and duration of the stress response, has only been conducted in a limited number of species. In terms of seabird oiling, no difference in GC concentrations were observed between oiled and non-oiled little penguins, *Eudyptula minor*, undergoing rehabilitation (Chilvers et al., 2016). In contrast, GC concentrations in domestic ducks (*Anas*

platyrhunchos) fed oil-contaminated food was lower than observed in control animals (Harvey et al., 1981), indicating a possible decrease in adrenal responsiveness (Rattner et al., 1984). In contrast, physical injury can lead to elevated GC production (Wolf et al., 2018) and a decrease in body condition (Ganswindt et al., 2010). Finally, starvation can have several effects on the HPA axis; in the Galapagos marine iguana, *Amblyrhynchus cristatus*, starvation resulted in a negative feedback loop being activated, decreasing the production and secretion of GCs (Romero, 2012). In contrast to this, rats starved for four days showed elevated GC levels (Makino et al., 2001). As the physiological response to injury, contamination and emaciation can be species-specific, researchers should aim to determine the nature of such response for each species. Further, it should be noted that, when all these potential stressors are present, a possible interaction between animal injury, emaciation and oiling can occur and drive adrenocortical activity in an organism. However, no study to date has attempted to determine if such an interaction occurs and, if so, what the effect on the HPA axis is.

Currently listed as endangered by the International Union for Conservation of Nature (Birdlife-International, 2018), the African penguin, *Spheniscus demersus*, is a seabird endemic to the coastline and islands of southern Africa (Shelton et al., 1984). Once abundant throughout its natural distribution range, the African penguin has declined from 147 000 to 13 000 breeding pairs between 1956 and 2019 (Department of Environment, Forestry and Fisheries, Unpublished data; Kemper et al., 2007; Koenig, 2007). A range of anthropogenic activities, including pollution (toxins/oil spills), habitat degradation, overfishing of preferred prey types, human-penguin interactions, disease and climate change, are all responsible for the ongoing decline in African penguin populations (Dias et al., 2019; Trathan et al., 2015). Many African penguins that survive the initial (e.g. injury) or long-term (e.g. contamination/reduced prey) contact with a stressor are collected and taken to a rescue centre within South Africa for care and rehabilitation (Parsons and Underhill, 2005; Wolfaardt et al., 2009; Yabsley et al., 2012). Several studies have implemented non-invasive urofaecal glucocorticoid metabolite (ufGCM) monitoring in the species, specifically to determine the effect of a visitor/tourism environment on adrenocortical activity (Ozella et al., 2015; Ozella et al., 2017; Scheun et al., 2020b). However, no study to date has attempted to monitor the causes and effect of rehabilitation on adrenocortical activity in African penguins.

Thus, in the current study we aimed to monitor ufGCM concentrations, as a marker of physiological stress, in injured, emaciated (starvation/illness) and oiled African penguins through the stages of rehabilitation at a rescue centre in South Africa.

2 Methods and materials

2.1 Study Site and animals

The Southern African Foundation for the Conservation of Coastal Birds (SANCCOB; -33.833629, 18.491212) is an internationally recognised centre that focuses on the rehabilitation of African penguins and other seabird species. Individuals admitted to SANCCOB present with a range of injuries/statuses (termed here "**admission reason")**, including (i) **injury** to the chest, flipper, eye, leg or foot, (ii) covered in petrochemical products ("**oiled**"), or (iii) weak and lethargic due to starvation or dehydration ("**emaciated**"). Furthermore, SANCCOB staff members frequently conduct environmental "**sweeps"**, removing any penguins deemed to be in a hazardous area due to natural or anthropogenic factors.

Upon arrival at SANCCOB, all individuals are assessed by trained staff members and a veterinarian in order to determine admission reason severity, as well as the most appropriate rehabilitation protocol to be implemented. All animals are initially placed into Stage 1 (S1) for monitoring, before moving on to Stage 2 (S2) and then 3 (S3) (Tab. 1; supplementary figures 1-3); individual recovery rate will determine the duration spent within each rehabilitation stage. Individuals admitted to SANCCOB are marked with coloured bands to ensure individual identification. Individual age (**adult/juvenile**) was determined by trained SANCCOB staff assessing body weight, feather plumage and physical development. As African penguins are sexually monomorphic, individual sex could not be determined during the rehabilitation process.

Table 1. Description of the three rehabilitation stages present at SANCCOB.

Stage	Description
Stage 1 S1	Admitted individuals require intensive care offered by a registered veterinarian as a result of admission reason severity. This may include prolonged human-animal interaction, including feeding, surgeries and medical care. Individuals are housed in separate crates throughout this period.
Stage 2 S2	Animals found to have recovered sufficiently from S1 are placed into S2. Individuals are housed in small groups throughout this period. Although human-animal interactions may be less than S1, animal handling and monitoring, as well as surgeries and medical care are still required.
Stage 3 S3	Individuals are deemed to have recovered from the injury/status observed upon admission and moved from S2 to S3. Individuals within S3 are regularly handled to assess pre-release health; this includes general health checks and bleedings, as well as feeding events. Limited medical care is required. Animals are group-housed and prepared for group-release into a suitable habitat.

2.2 Urofaecal collection and storage

In birds, where urine and faeces are excreted in unison, and the complete separation of matrices is not possible, the entire sample can be collected and analysed (Scheun et al., 2020b; Vidal et al., 2019). Urofaecal sampling was conducted by SANCCOB staff members from 12 March – 6 October 2018. Upon witnessing an excretion event, the staff member would enter the enclosure to collect the urofaecal sample, making note of animal ID, as well as the date and time of collection. Samples were collected using a clean syringe or tongue depressor and placed into a 5 ml microcentrifuge tube. All samples were frozen at– 4 ℃ immediately following collection in order to minimise any degradation. Reoccurring sampling from specific individuals throughout the three stages was attempted; however, group housing, individual injury/status severity and rehabilitation length made long-term, repeated sampling impractical. As such, samples were grouped into 'admission reason' (**"injury", "oiled", "emaciated", "sweep"**) for each of the three rehabilitation stages for statistical analysis. A total of 128 urofaecal samples (S1: 38, S2: 46, S3: 43) were collected across all three stages for injured (S1: 17, S2: 24, S3: 18), emaciated (S1: 16, S2: 17, S3: 16), oiled (S1: 3, S2: 3, S3: 3) and sweep (S1: 2, S2: 2, S3: 5) animals. The study was performed with the approval of the NZG ethics committee (P18/47) and SANCCOB's Research and Ethic Committee (REC2017/06). Sampling at SANCCOB was conducted under the DEA permit RES2018/33.

2.3 Urofaecal sample extraction and enzyme immunoassay

All urofaecal samples were shipped on dry ice to the National Zoological Garden, South African National Biodiversity Institute, South Africa, for processing and extraction. Samples were lyophilised, pulverised and sieved through thin mesh to remove any undigested materials (Fieß et al., 1999). Subsequently, 0.050 – 0.055 g of urofaecal powder was extracted with 1.5 ml 80% ethanol following Scheun et al. (2020b). After vortexing for 15 min, the suspension was centrifuged at 1,600 g for 10 min; and the resulting supernatant transferred into a microcentrifuge tube and stored at – 20 ℃ until analysis.

Urofaecal extracts were analysed for ufGCM concentrations using a tetrahydrocorticosterone enzyme immunoassay (EIA) previously validated for the African penguin (Anfossi et al., 2014; Ozella et al., 2015). Details about the assay, including antibody cross-reactivities, are given by Quillfeldt and Möstl (2003). Serial dilutions of extracted African penguin urofaecal samples gave displacement curves that were parallel to the respective standard curves (relative variation in the slope of respective trendlines < 5%). Assay

sensitivity was 9.0 ng/g dry weight (DW). Intra-assay coefficients of variance (CV), determined by repeated measurements of high- and low-value quality controls, were 6.33 % and 6.64 %, respectively. Equally determined inter-assay CVs, utilizing high and low quality controls, were 11.68 % and 13.40 %, respectively. EIA analysis was performed at the Endocrine Research Laboratory, University of Pretoria, South Africa.

2.4 Data analysis

All data were analysed using R (R Core Team, 2019). Data exploration was conducted following the protocols described by Zuur et al. (2010). Initial descriptive and graphical data analyses were performed. The assumption of normality was checked by examining probability plots and conducting a Shapiro-Wilk test ($W = 0.55$, $p < 0.001$); subsequently, data were $\log^{10}$ transformed to conform to data normality. A generalised linear model (GLM) was conducted to determine the effect of **admission reason, age,** and **rehabilitation stage** on ufGCM concentrations in African penguins undergoing rehabilitation. However, due to the low sample numbers across all three stages, sweep and oiled ufGCM concentrations were not included in the GLM analysis, but included in the graphical analysis. Full factorial and additives models were conducted; however, none of the mentioned models had significant interaction terms and were thus removed from the analysis. As only rehabilitation stage showed significance, the marginality rule was applied (Crawley, 2014); here, a one-way analysis of variance (ANOVA) was conducted including ufGCM concentration found in S1, S2 and S3. Following this, a *post hoc* Tukey HSD test was conducted.

3 Results

Only rehabilitation stage and not admission reason or age significantly affected ufGCM levels in individual African penguins at SANCCOB ($n=109$, $F = 5.24$, $p < 0.001$, $R^2 = 0.14$, Tab. 2). The one-way ANOVA test conducted in line with the marginality rule supported the GLM results, indicating rehabilitation stage as a significant driver of ufGCM pattern in the study population ($F_{(2,106)} = 21.45$, $p < 0.001$). The subsequent *post hoc* Tukey HSD test showed that ufGCM levels observed in S1 (3.31 ± 3.36 µg/g SD DW) were significantly higher than for individuals in S2 (1.10 ± 1.13 µg/g SD DW, $p < 0.001$) and S3 (0.88 ± 0.31 µg/g SD DW, $p < 0.001$, Fig. 1).

Table 2. The estimates, standard errors, t values, significance and 95 % confidence intervals of all predictor variables in the generalised linear model for $\log^{10}$ urofaecal glucocorticoid metabolite concentrations in African penguins undergoing rehabilitation.

	Estimate	**Std Error**	**t value**	**Pr(>|z|)**	**Confidence intervals**	
	ß	*SE*			*2.5 %*	*97.5 %*
Intercept	3.321	0.634	5.242	**<0.01**	2.065	4.577
Stage 2	-2.600	0.458	-5.680	**< 0.001**	-3.508	-1.692
Stage 3	-2.780	0.475	-5.854	**< 0.001**	-3.722	-1.838
Reason – Injury	-0.094	0.543	-0.173	0.863	-1.170	0. 982
Age – Juvenile	0.617	0.544	1.135	0.259	-0.461	1.696

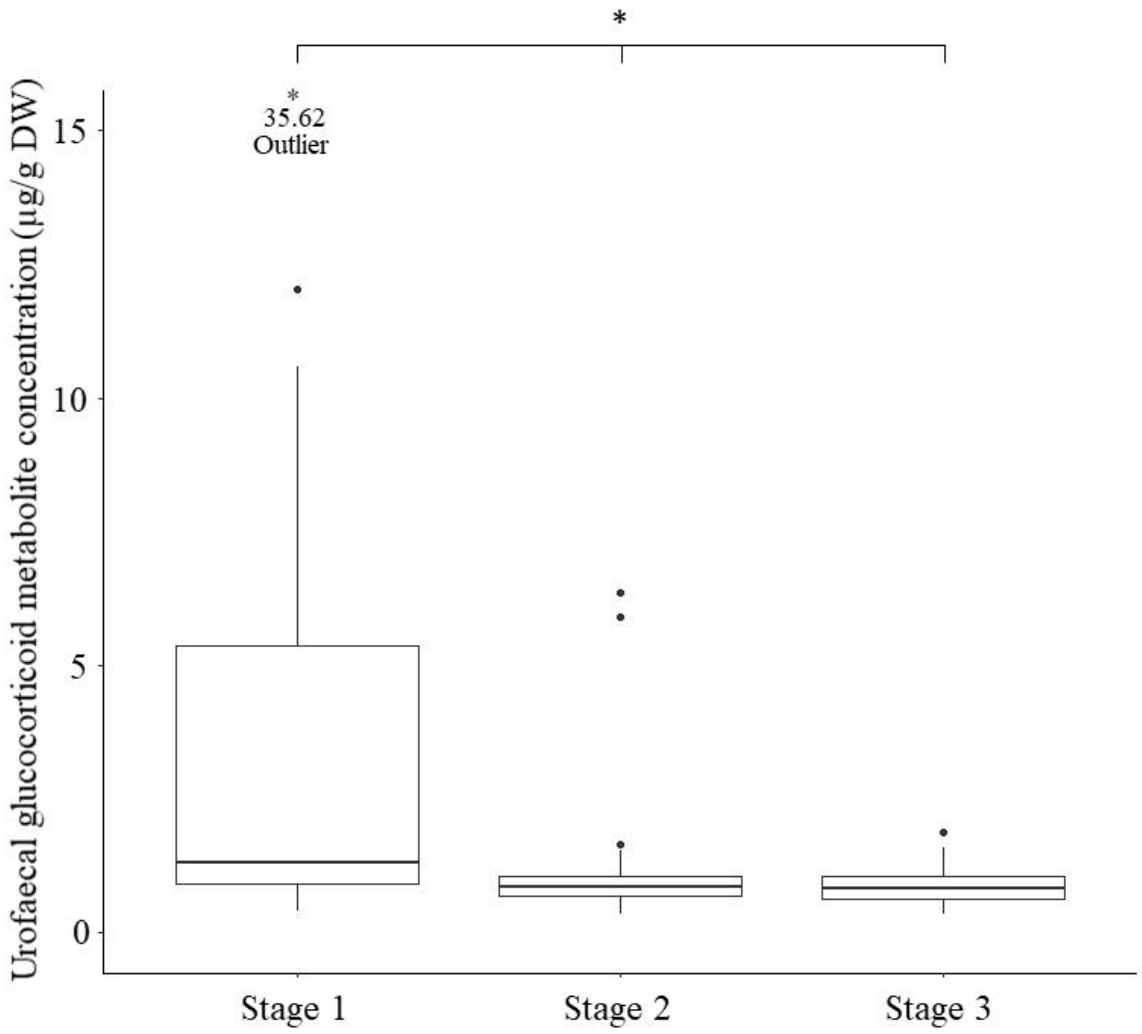

Figure 1. Boxplot (median, 25 % percentile, 75 % percentile) of ufGCM concentrations (µg/g dry weight) across the three rehabilitation stages. Significance is indicated by *.

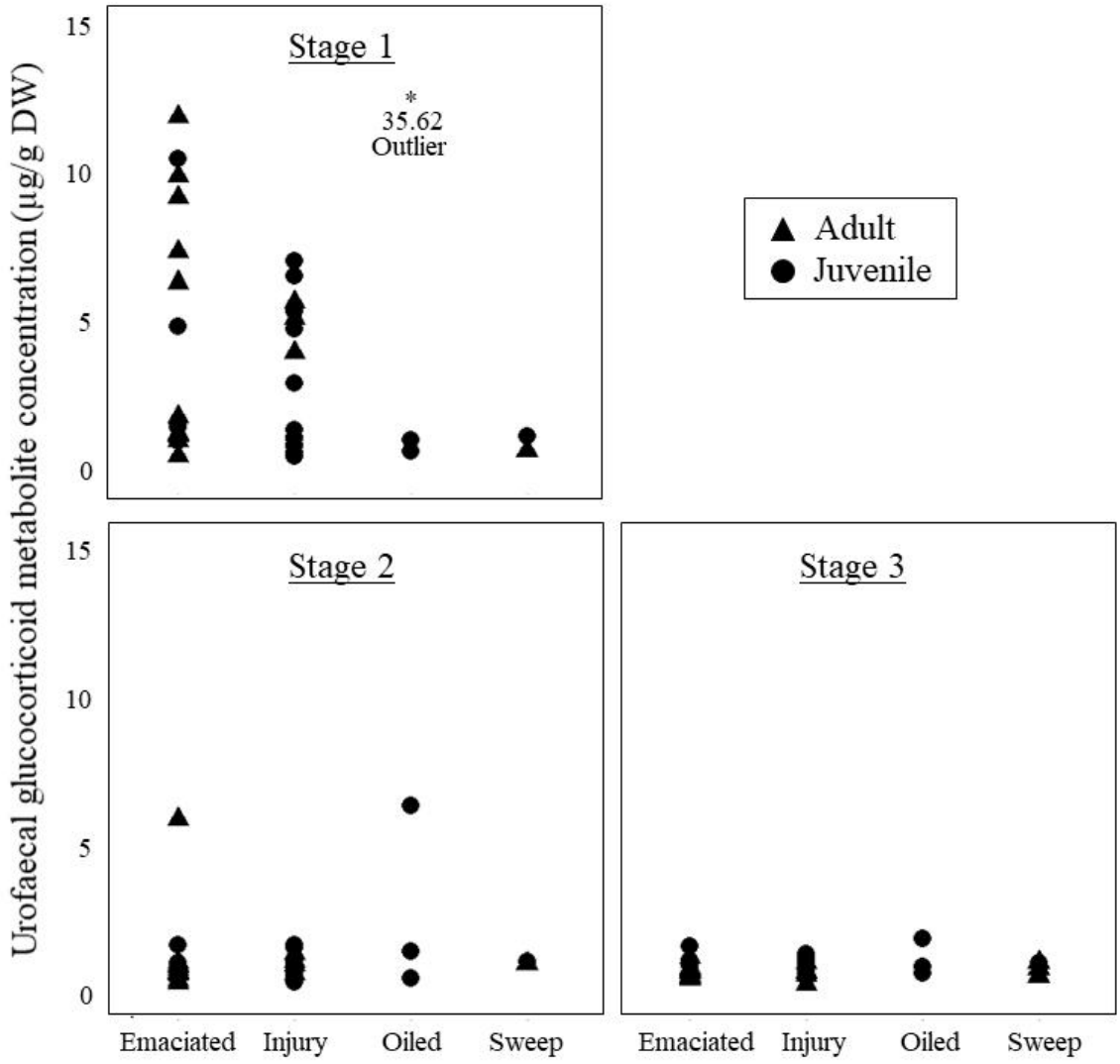

Figure 2. Dotplot figures indicating ufGCM levels (µg/g dry weight) of each admission reason throughout Stage 1, Stage 2 and Stage 3 of the rehabilitation process. Adult and juvenile animals are indicated by ● and▲ respectively.

Data exploration showed comparable ufGCM levels for adult and juvenile individuals within each stage for all admission reasons (Fig. 2). Despite the lack of significance for admission reasons, average ufGCM levels in S1 for emaciated individuals (4.42 ± 4.18 µg/g DW SD) were considerably higher than respective hormone metabolite levels revealed for emaciated animals in S2 (1.11 ± 1.27 µg/g DW SD) and S3 (0.82 ± 0.28 µg/g DW SD). Similarly, injured penguins within S1 (2.84 ± 2.45 µg/g DW SD) had considerably higher ufGCM levels than their injured counterparts in S2 (0.89 ± 0.31 µg/g SD DW) and S3 (0.90 ± 0.29 µg/g SD DW, Fig. 2). The ufGCM levels of oiled (0.76 ± 0.26 µg/g SD DW; outlier: 35.62 µg/g SD DW) and sweeped (0.83 ± 0.34 µg/g SD DW) birds in S1 were considerably lower

than their injured and emaciated counterparts (Fig. 2). In contrast to this, ufGCM levels of oiled (S2: 2.77 ± 3.13 µg/g SD DW; S3: 1.17 ± 0.62 µg/g SD DW) and sweeped (S2: 1.06 ± 0.04 µg/g SD DW ; S3: 0.83 ± 0.23 µg/g SD DW; Fig. 2) birds were comparable to ufGCM levels from injured and emaciated animals in S2 and S3.

4 Discussion

This is the first study to implement non-invasive hormone monitoring in order to determine the adrenocortical activity in African penguins undergoing rehabilitation due to injury, emaciation, oiling or being removed from their habitat due to environmental threats.

In contrast to the notion that the monitored admission reasons should lead to significantly elevated GC levels in an individual, the results of this study showed that injury, emaciation, oiled and removal from a chosen habitat were not statistically significant drivers of adrenocortical activity in African penguins undergoing rehabilitation at SANCCOB. The absence of significance to all admission reasons may be explained by three factors within the current study.

Although it is assumed that wildlife species will respond to certain stressors in a more or less similar fashion (Schoenle et al., 2018), differences in the physiological stress response commonly exist between species as well as between individuals (Bonnot et al., 2018; Cockrem, 2007; Koolhaas et al., 2010). This may explain the large variation in ufGCM levels seen between and within different admission reasons. For example, petrochemical contamination of an individual can have a suppressive or enhanced effect on the activation of the HPA axis and the resulting production of GCs (Chilvers et al., 2016; Rattner et al., 1984). Thus, such individual and species-specific variation should be taken into consideration when monitoring the physiological response to the admission reasons included in this study.

Further, admission reason severity at the time of admission may be an important factor responsible for driving fGCM patterns in the current study. Prolonged secretion of elevated GC levels can result in the activation of a GC negative feedback loop responsible for reduced reactivity and sensitivity of the HPA axis (Herman et al., 2012); this mechanism is driven by the downregulation and desensitisation of GC, mineralocorticoid and corticotropin-releasing hormone (CRH) receptor regulation (Aguilera et al., 2004; Gjerstad et al., 2018; Ladd et al., 2004). A reduction in GC production due to the activation of such a mechanism, along with veterinary and general care of animals, may result in the decreased fGCM levels observed. Furthermore, the expression of GC-mediated traits between and within a species rely on several

physiological components (immune function and cells, nutrient availability) and the variation in physiological states (injury, starvation, dehydration, pregnancy) present (Schoenle et al., 2018); in this regard, little is known of the physiological state and components within newly admitted animals.

Finally, although both injury and emaciation were found to be statistically non-significant, likely due to the high SD values detected, both factors are biologically meaningful in driving fGCM levels within S1. The information on the effect of severe injury on wildlife physiology is currently limited, largely due to the inability to encounter and monitor such individuals within the wild. This is especially true for injured and emaciated seabirds. Research on the African elephant, *Loxodonta africana*, showed that injury severity and length are important factors driving adrenocortical activity (Ganswindt et al., 2010). Similarly, Wolf et al. (2018) found that injury severity was an important driver of GC levels; here, only deep tissue injuries resulted in elevated GC levels in free-ranging giraffe, *Giraffa camelopardalis*. These findings support the elevated ufGCM levels observed in injured African penguins within S1 of the current study. Furthermore, research by Crossey et al. (2020) on African wild dogs, *Lycaon pictus*, showed that injury did not activate the physiological stress response in all individuals; this highlights the variance in injury-related fGCM alteration and may explain the high SD values of injured African penguins in S1. As theorised by Sapolsky (2002), the pain stimuli inherent to severe and prolonged injury may be an important factor driving adrenocortical activity in wildlife species. However, as defining pain and wound severity for an individual is subjective, additional monitoring techniques should be employed to determine injury severity and the effect thereof on penguin physiology.

Furthermore, the inability to meet energy demands is a constant threat to wildlife populations; to survive during periods of reduced nutrient intake, individuals often lower their metabolic rate until sufficient nutrient sources can be located (Schwartz and Seeley, 1997). Metabolic optimisation during nutrient-poor periods is regulated by the HPA axis (Dallman et al., 1993; Kadmiel and Cidlowski, 2013). However, during such periods, elevated GC production is not driven by CRH, but rather several neuropeptides. In the absence of elevated CRH levels the negative feedback mechanism responsible for regulating GC levels within an organism remains inactive, resulting in chronically elevated GC levels until the nutrient imbalance is corrected (Makino et al., 2001; Schwartz and Seeley, 1997). This elevation in GC concentration interact with insulin to metabolise alternative energy sources (e.g. fat) (Schwartz

and Seeley, 1997); although adaptive in the short term, prolonged GC elevation, and the metabolism of stored energy reserve, will result in a low body index and ultimately death.

In this study, rehabilitation stage, specifically S1, was the only significant driver of ufGCM levels in African penguins. Close proximity to anthropogenic activities, or direct human-animal interaction (**handling/captivity**), has been shown to act as a psychological stressor, resulting in the activation of the physiological stress response (Cañadas Santiago et al., 2020; Ciuti et al., 2012; Merrill et al., 2012). In this regard, activities such as colour banding of naïve birds could be perceived as a stressor, potentially resulting in an increase in glucocorticoid output in birds at SANCCOB, though additional research would be required to confirm this.

Furthermore, stage-related **housing conditions** might play an important role in altering glucocorticoid output in the study animals. As African penguins are highly social birds (Wilson et al., 1986), the separation and individual housing of animals in Stage 1 may act as an additional stimulus for the HPA axis. Such an elevation in GC production has been shown to occur in other social wildlife species following social separation and single housing (Hennessy, 1997; Scheun et al., 2020a; Serres et al., 2020). Required handling for Stage 1 animals, e.g. due to more frequent tubing, can be higher compared to their Stage 2 and 3 counterparts, potentially leading to the significantly higher ufGCM levels observed. Finally, free-ranging African penguins usually spent a lot of time walking/swimming/foraging on a daily basis (Figel, 2020).Thus, the limited mobility of Stage 1 animals due to the crate housing may be perceived as an additional stressor, as animals within Stage 2 and 3 are free to walk in larger pens and spend prolonged periods free-swimming. Elevated GC concentrations in response to smaller enclosure size and increased human proximity has been observed in several wildlife species, including the Persian onager (*Equus hemionus onager*) (Vick et al., 2012), Pere David's deer stags (*Elaphurus davidianus*) (Li et al., 2007) and the pygmy rabbits (*Brachylagus idahoensis*) (Scarlata et al., 2013). However, in order to pinpoint the role housing conditions play for the physiological stress response of penguins atSANCCOB , additional research would be required.

Furthermore, individual state at admission, both physical and physiological, may result in an enhanced or suppressed physiological reaction to initial human-animal interaction and captivity (Schoenle et al., 2018). For example, injured and emaciated individuals may have an enhanced physiological response, whether it be due to the severity of human-animal interaction

required or the confinement thereafter, during S1. Furthermore, a decrease in ufGCM levels during S2 and S3 can likely be attributed to individual recovery from the original injury/ailment or habituation to the captive environment and human presence. With regard to the latter, it should be noted that a decrease in ufGCM levels during S2 and S3 might be an indication of neuroendocrine factors acclimating to the captive environment; despite this, downstream physiological measures, such as immune response, might not be as adaptable as neuroendocrine endpoints (DuRant et al., 2020). Finally, it is unknown whether a possible interaction in admission reason is present, altering the possible adrenocortical response in respective individuals. With so many factors still unknown, it is imperative that additional monitoring techniques be implemented during each stage to ensure individual recovery and health prior to release.

This study has added valuable information on the effect of rehabilitation, injury and emaciations on adrenocortical function in the African penguin. Injured and emaciated individuals show elevated ufGCM levels, especially during S1. Every effort should be made to enhance the care of these animals and ensure little to no downstream effects, especially following the release of individuals. Although this study has added invaluable information on the physiological response African penguins have to a range of admission reasons and the rehabilitation process, several questions remain unanswered and open to future research. Firstly, addition monitoring techniques (e.g. behavioural/physical) should be implemented during each stage to ensure optimum animal care. Next, individual sex should be included in studies such as this, as the physiological response to a stressor, and the subsequent hormone metabolism, can differ between sexes (Goymann, 2012). Finally, although logistically difficult, long term monitoring of ufGCM levels in specific individuals, throughout the three stage rehabilitation process, should be attempted to confirm the possible physiological response to the monitored admission reasons in the species. Despite the importance of this study, caution is required when assessing these results due to the low number of samples collected throughout this study, especially for oiled and sweep animals. We hope that future research on this topic will be able to increase the samples analysed and support the findings of this study.

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Declarations of interest

None

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