Body temperature and thermoregulatory behaviour in the Endangered African Penguin *Spheniscus demersus*

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Summary

Heat stress from high temperatures has been recognised as a threat to several bird species and one that is likely to increase in severity and frequency as a result of global environmental change. Many seabirds are particularly vulnerable as their adaptations to forage in cold water can make it hard for them to resist heat gain while on land. The African Penguin *Spheniscus demersus* forages in cool water (10–20°C) but breeds and moults on land where temperatures can exceed 30°C. Little is known about its thermal physiology and when heat stress will occur. Here, we provide the first account of changes in body temperature and behaviour in African Penguins in response to an experimental increase in ambient temperature from 20°C to 35°C. Surprisingly, the highest body temperature observed (39.2°C) was recorded at the start of the study. Presumably, this response in body temperature was due to the stress of transport and handling. Penguins returned to normal body temperature (37.3°C) after 3.5 hours and maintained normothermia for roughly an hour. We then observed the onset of heat storage at an ambient temperature of 29°C, whereafter body temperature increased steadily at a rate of *ca*.0.2°C per 1°C increase in ambient temperature. Panting commenced at an ambient temperature of 31.4°C, when body temperature was 37.8°C. In addition to panting, penguins changed their posture to facilitate heat dissipation by standing, raising their heads, and extending their flippers. Our results corroborate field observations and support the argument that African Penguins are highly vulnerable to heat stress in the near future as extreme heat events become more severe and frequent due to climate change. Our results also confirm that penguins are sensitive to handling, which elicits a hyperthermic response. Given the general sensitivity of penguins to disturbance, from a conservation perspective, we advise that cautionary measures be implemented at colonies during critical life-history stages.

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Introduction

The impact of high ambient temperatures (T_a) on biodiversity and ecosystems is currently under scrutiny, as researchers seek to understand the consequences of global environmental change (Conradie et al. 2019; Urban et al. 2016). For endothermic animals such as birds, studies typically seek to understand thermal tolerance in terms of their physiology (e.g. McKechnie, Gerson et al. 2021; Noakes et al. 2016), behavioural responses (e.g. Conradie et al. 2019; du Plessis et al. 2012), and implications for fitness (e.g. Carroll et al. 2018; Conradie et al. 2019), all of which have been used to establish when the animals are subjected to heat stress from high T_a. Based on Schmidt-Nielsen (1997)'s simple heat balance equation, we define heat stress as the physiological strain associated with exposure to temperatures sufficiently high to increase the total heat load experienced by an animal to the point where passive heat dissipation alone is insufficient to avoid heat storage. Our definition accommodates a gradient from low to severe heat stress without prescribing specific temperatures at which this is expected because perceived heat stress, thermal tolerance, and survival at high T_a vary greatly between avian species (reviewed by McKechnie and Wolf 2019).

The onset of heat stress is expected to occur at relatively moderate temperatures in cold-adapted avian species (Murrish 1982; O'Connor et al. 2021) compared with arid-adapted (McKechnie et al. 2016; Smith et al. 2015), tropical (Ellis et al. 1995), and temperate zone species (Nzama et al. 2010; Zheng et al. 2008). For example, studies examining the physiological response of seabirds to variable T_a have shown that the onset of heat stress-related panting begins at T_a = 22°C in an arctic diving seabird, the Thick-billed Murre *Uria lomvia*, normal T_b = 38.6°C, mass =

1 kg (Choy et al. 2021) compared with $T_a = 36°C$ in the tropical Brown Noddy *Anous stolidus*, normal $T_b = 40.3°C$, mass = 142 g (Ellis et al. 1995). This is because cold-adapted avian species have evolved efficient heat-retention mechanisms to minimise heat lost in frigid environments (Dawson and Whittow 2000; Luna-Jorquera 1996; Williams 1995) and have very small thermal windows (McCafferty et al. 2013). One such adaptation is the heat-exchanger mechanism – the Rete Tibiotarsale – in their legs that allows them to retain 25–66% of their metabolic heat production while their feet are in contact with frigid surfaces (Kazas et al. 2017). This and other types of arteriovenous heat-exchange mechanisms, in combination with a relatively thick layer of fat, are essential for diving seabirds. The reason being that the thermal conductivity of water is *ca.* 24 times greater than air meaning that diving seabirds would experience much higher rates of heat loss while diving compared with their non-diving counterparts (Schmidt-Nielsen 1997). While such a high level of heat retention is advantageous in the cold, it does pose a severe impediment to off-loading excess body heat at warm temperatures (Gaston et al. 2002; Holt and Boersma 2022).

O'Connor et al. (2021) showed that the Snow Bunting *Plectrophenax nivalis*, an arctic passerine, has a low capacity for evaporative heat loss, with 40 of 42 birds unable to dissipate their own metabolic heat (i.e. evaporative heat loss capacity EHL/MHP <1) at T_as of approximately 25–42°C. An even lower evaporative heat loss capacity was observed in the previously mentioned Thick-billed Murre, which was unable to dissipate more than 34% of its own metabolic heat at T_as of approximately16–38°C (Choy et al. 2021). These two examples show that cold-adapted avian species are inefficient at off-loading excess body heat, and an argument can be made that diving seabirds are particularly vulnerable to heat stress as they may have evolved more efficient heat-retention adaptations to deal with cold subsurface water while foraging (Thomas et al. 2011). An exemption to this could be the small-sized (150 g) Dovekie *Alle alle* (see Beaman et al. 2024), but the general inability to off-load excess body heat makes it difficult for cold-adapted diving seabirds that forage in cold waters to resist heat gain during warm weather while breeding or moulting on land (Ganendran et al. 2016; Oswald and Arnold 2012). This notion is corroborated by the heat-related mass mortality events in Thick-billed Murre (Gaston et al. 2002) and Magellanic Penguin *Spheniscus magellanicus* (Holt and Boersma 2022), making it clear that they are extremely vulnerable to the direct effect of heatwaves due to global climate change.

Although most penguin species reside at or near Antarctica, *Spheniscus* penguins comprising the Magellanic Penguin, African Penguin *S. demersus*, Galapagos Penguin *S. mendiculus*, and Humboldt penguin *S. humboldti*, breed in hot climates while on land but feed in relatively cool waters (10–20°C). Thus, they experience selective pressure to maintain adequate insulation to avoid hypothermia while foraging, placing them at high risk of heat stress while ashore (Holt and Boersma 2022; Simeone et al. 2004; Welman and Pichegru 2023). As an adaptation, these penguins breed in burrows dug in guano, which have buffered microclimates protecting them from high T_a, shading them from direct sunlight, and providing protection from predation and storms (Frere et al. 1992; Frost, Siegfried and Burger 1976). Unfortunately for African Penguins past human exploitation has depleted this resource (Frost, Siegfried and Cooper 1976), which contributed to the 90% decline in population size in the twentieth century (Crawford et al. 1995). Habitat loss and heat stress have been implicated in recent further declines (Ludynia et al. 2014) and large incubation failure in hot conditions has been recorded (Traisnel and Pichegru 2018). For example, daily temperatures during the austral summer and early breeding season at the second largest breeding colony of African Penguin globally at Bird Island, Algoa Bay, South Africa routinely approaches or exceeds 30°C, and many breeding sites do not offer much natural shade (Lei et al. 2014; Welman and Pichegru 2023). Consequently, the species was listed as "Endangered" in 2010 (BirdLife International 2020), and the on-going population decline suggests that the African Penguin should now be considered as "Critically Endangered" (Sherley et al. 2024).

The listing of the African Penguin as Endangered led to a number of management actions (DFFE 2013), including the provision of artificial burrows of different designs. Those burrows which effectively buffer extreme T_a can improve reproductive success (Pichegru 2013; Sherley et al. 2012; Welman and Pichegru 2023), whereas low breeding success in other designs of artificial burrows has been attributed to the very high temperatures observed within them, often even greater than exposed nests (Lei et al. 2014). A key limitation is that assessments of the exposure of African Penguins to heat stress in their burrows and elsewhere on shore has been made without specific information on their thermal biology. Improving the understanding of the species' thermal tolerance is urgently needed to inform conservation efforts such as prioritising where to place artificial nests, knowing when to intervene in cases of nest abandonment to reduce breeding failure, and potentially using remotely operated unmanned aerial vehicles (UAVs) with thermal imaging capabilities with artificial intelligence to monitor individuals (*sensu* Santangeli et al. 2020). Remote monitoring and deploying people only when necessary will benefit penguins as they are sensitive to human-related disturbance (Lewden et al. 2024; Scheun et al. 2021). Thus, our study aimed to estimate the T_a at which African Penguins begin to experience heat stress in the absence of direct solar radiation as this would offer insight into when they may become heat stressed in shaded artificial nests. The objectives were to record (1) the change in body temperature of penguins exposed to incremental increases in ambient temperature and (2) to describe the associated thermoregulatory behaviour.

Methods

Animal collection and transportation

All experimental procedures were approved by the University of Cape Town's Animal Ethics Committee (2013/V4/LP) and the study was undertaken with the permission of the Department of Environmental Affairs. Five healthy African Penguins (two males and three females) that were resident at the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) facility in Rietvlei, Cape Town were used in this experiment. These birds were all in good condition at the time of the experiment but deemed not suitable for release into the wild due to past injuries to their limbs (flippers or legs). As resident individuals at the SANCCOB facility, these birds were used to being hand-fed and interacting with SANCCOB staff and were expected to be relatively acquiescent to the conditions associated with transport and experimentation. Penguins were transported in individual ventilated cardboard penguin carriers by air-conditioned car to the laboratory at the University of Cape Town on the morning of the study (11 March 2013). Travel time between the SANCCOB facility and the laboratory was approximately 30 minutes.

Experimental protocol

Upon arrival at the laboratory, a 2-mm diameter sterilised and lubricated K-type thermocouple wire was inserted approximately 6 cm into the penguins' cloaca. The wire was held in place with waterproof adhesive tape (Tesa 4651) wrapped around the long tail feathers. Further tape attached the wire to feathers on the back, up to the shoulders of the bird, which ultimately fed up to the ceiling of the climate-controlled room where the experiment was conducted. Handling time to attach the thermocouples was approximately 5 minutes per penguin. All five penguins were held together in the climate-controlled room for the duration of the experiment in individual enclosures made from PVC tubing and translucent mesh. Penguins had room to turn around, lie down, and move in their enclosure and could see and interact with the other penguins. The five thermocouple wires led to a central hook in the ceiling and then to a portable data logger (Omega, OM-DAQPRO-5300). The positioning of the wire allowed penguins to move freely within their enclosures. Three other thermocouples were placed around the climate-controlled room to monitor and record T_a. In addition to the climate-controlled room's recirculating temperature and humidity control mechanism, the room was equipped with a fan to aid air circulation and a lamp to provide dim lighting to allow observation. Penguins were left undisturbed in the climate-controlled room throughout the experiment and were observed through a small window in the door of the room.

The penguins were exposed to a temperature regime consistent with similar studies (Choy et al. 2021), designed to mimic temperature change in an African Penguin nest on a typical hot day (Lei et al. 2014). After an initial acclimation period of 30 minutes at 20°C, the temperature of the climate-controlled room was increased by 1°C every 20 minutes to a maximum temperature of 35°C, which marked the end of the experiment. We added 20 minutes of cooling time, resulting in penguins spending a total of six hours in the climate-controlled room, which was the maximum period allowable under our permit. Previous work by Lei et al. (2014) and Welman and Pichegru (2023) showed that relative humidity typically remained above 70% in natural burrows, with penguins generally experiencing mean hourly wet-bulb temperatures of 18.4–23.2°C. The desired humidity in the climate-controlled room was set at 80% during the acclimation period, corresponding to a wet-bulb of 17.5°C. However, subsequent validation of the climatic conditions experienced by penguins showed that the climate-controlled room was only capable of generating a relatively humidity of 50% at T_a = 20°C (wet-bulb temperature = 13.7°C), which decreased to a relative humidity of 20% at T_a = 35°C (wet-bulb temperature = 19.3°C). Subsequent validation was done by repeating the experimental protocol once per day over a three-day period and using eight Hygrochron temperature and humidity sensitive data loggers (DS1923-F5, Maxim Integrated, Dublin, Ireland) placed in pairs against all sidewalls of the climate-controlled room at a height of ≤1 m from the floor to better estimate the condition penguins would have experienced. Given the air flow created by the fan and room recirculation, it was assumed that any evaporative water loss contribution from the penguins would have been adequately mixed with the rest of the air within the room and detected by the room's sensors used to regulate humidity within the room. Thus, the penguins would not have had any significant impact on the level of humidity within the room. At the termination of the experiment, all penguins were removed from the climate-controlled room, their thermocouples removed, and they were given an oral electrolyte hydration solution as a precautionary measure. They were returned to SANCCOB, released to their enclosure, and offered food. On-going monitoring of the birds revealed no apparent immediate or longer-lasting effects of the experiment.

Behavioural observations

During the experiment, penguins were continuously observed to monitor behaviour and detect signs of distress or discomfort. At 10-minute intervals we noted each penguin's posture, feet, flipper, and head position to assess their thermoregulatory behaviour (*sensu* Bartholomew and Dawson 1979). This approach was based on previous observations on the congeneric Magellanic Penguin (Simeone et al. 2004). Following Simeone et al. (2004), we calculated a Thermoregulatory Index (TRI) for the group of birds as follows:

$$TRI = \sum TRBS_i n_i / N$$

where $TRBS_i$ is the score assigned to a specific thermoregulatory behaviour (TRB) (Table 1), n_i is the number of individuals exhibiting the TRB, and N is the number of penguins under observation (five at all times in this case). TRI values were assigned a score from -1 to +3, where negative scores indicate heat-conservation behaviour, a zero score indicates neutral behaviour, and positive values indicate heat-dissipation behaviour.

Statistical analyses

We performed a piecewise regression analysis in R version 4.2.2 (R Core Team 2022) using the R software package "segmented" (Muggeo 2008) to identify objectively any inflection points in the

Table 1. Scoring system for thermoregulatory behaviour (TRB) in African Penguins *Spheniscus demersus* used to determine the Thermoregulatory Index (TRI) while exposed to increasing ambient temperatures in a climate-controlled room

Posture	Feet	Flippers	Beak position	Panting	TRB score
Lying	Covered	Folded	Down	No	−1
Lying	Covered	Folded	Upward	No	0
Lying	Covered	Extended	Down	No	0
Lying	Exposed	Folded	Down	No	0
Standing	Exposed	Folded	Down	No	0
Lying	Exposed	Extended	Down	No	1
Lying	Exposed	Folded	Upward	No	1
Lying	Covered	Extended	Upward	No	1
Standing	Exposed	Folded	Upward	No	1
Standing	Exposed	Extended	Down	No	1
Lying	Exposed	Extended	Upward	No	2
Lying	Covered	Extended	Upward	Yes	2
Standing	Exposed	Extended	Upward	No	2
Lying	Exposed	Extended	Upward	Yes	3
Standing	Exposed	Extended	Upward	Yes	3

thermoregulatory profiles of T_a vs T_b and TRI. Identified inflection points mark a change in the slope of the relationship between T_a and the variable of interest and each segment of data identified by the analysis was treated as independent. We described the overall relationship between T_b and T_a in penguins for each segment with a mixed-effect model using the R package "nlme" (Pinheiro et al. 2019), including individual ID as a random factor to account for repeated measurements. We used the R package "MuMIn" (Bartoń 2023) to calculate the *marginal* R^2, which represents the variance explained by the fixed effect. We then determined the repeatability via the *R*-statistic, using the R package "rptR" (Stoffel et al. 2017), which in our case provided an estimate for the contribution of individual differences to the overall variation in body temperature. As observations for TRI were scored as a group, for each segment, we described the relationship using simple linear regression analysis. Individual penguin body temperatures are provided in Supplementary Table S1. Given the significant contribution of individual differences in both segments (see Results), we further explored the data describing the relationship between T_b and T_a for individual penguins for each segment (see Supplementary results). To do so, we used a linear model and included individual ID as an interaction term in the model instead of a random effect.

All eight thermocouples were calibrated against each other in a well-mixed bucket of cooling water. This helped to account for potential inconsistencies between the individual probes, which differed from the mean by, on average, 0.22°C at $T_a = 20$°C and 0.14°C at $T_a = 35$°C. Adjusted values were used in subsequent analyses. The data logger recorded the temperature from each thermocouple at 1 Hz. A mean temperature was calculated every five minutes for each penguin thermocouple and to define T_a a grand mean temperature was taken for every five minutes from the three probes that were in air during the experiment. Hygrochon data loggers were factory calibrated against a National Institute of Standards and Technology (NIST)-traceable chamber. However, each logger was tested before installation, using a water bath and a precision mercury-in-glass thermometer (resolution = 0.5°C) at temperatures from 4°C to 40°C.

Results

During the experiment, the penguins remained calm and moved very little, with no visible signs of distress or discomfort. No birds attempted to leave their enclosure or remove their thermocouples at any time during the experiment and thus all birds remained undisturbed throughout.

Body temperature showed a point of inflection at $T_a = 29.0 \pm 0.4$°C ($\pm 95\%$ CI). The time elapsed since the start of the experiment until the inflection was approximately 215 minutes and the mean ($\pm$ SEM) T_b at the inflection was 37.1 ± 0.1°C (T_b range: 36.7–37.2°C). Body temperature had a negative relationship with T_a below the inflection ($F_{1,219} = 689.58$, $P < 0.001$, *marginal* $R^2 = 0.65$) and a positive relationship with T_a above the inflection ($F_{1,104} = 176.97$, $P < 0.001$, *marginal* $R^2 = 0.56$) (Figure 1). The repeatability test was significant in both segments of the T_b profile, showing individual variability. The *R*-statistic was 0.40 ($P < 0.001$) for the segment below the inflection and 0.21 ($P < 0.001$) for the segment above the inflection.

The mean ($\pm$ SEM) highest $T_b = 39.2 \pm 0.2$°C was observed at the start of the experiment immediately after the penguins were introduced into the climate-controlled room. This coincided with a neutral TRI score (0). TRI showed a point of inflection at $T_a = 23.4 \pm 0.8$°C ($\pm 95\%$ CI) and had a negative relationship with T_a below the inflection ($F_{1,10} = 21.55$, $P = 0.001$, $r^2 = 0.70$) and a positive relationship with T_a above the inflection ($F_{1,22} = 257.38$, $P < 0.001$, $r^2 = 0.92$) (Figure 2). The time elapsed since the start of the experiment until the inflection was approximately 100 minutes. Panting was first observed at a T_a of 31.4°C, which was approximately 260 minutes since the start of the experiment. The mean ($\pm$ SEM) T_b coinciding with the onset of panting was 37.8 ± 0.1°C (T_b range: 37.6–37.9°C).

Discussion

The results show two distinct periods of hyperthermia in the African Penguins. Despite any visible signs of distress, we attribute the initial period of hyperthermia to handling stress, whereas the
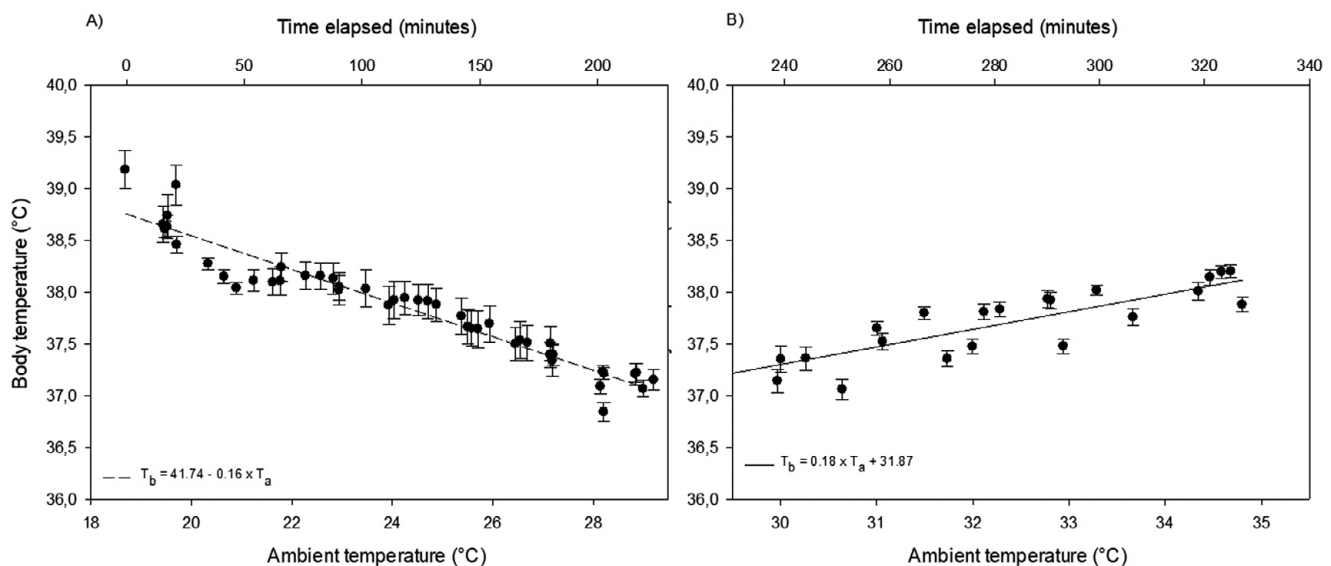

Figure 1. Body temperature in African Penguins *Spheniscus demersus* exposed to increasing ambient temperatures in a climate-controlled room. (A) The negative relationship between body temperature and ambient temperature below the inflection point. (B) The positive relationship between body temperature and ambient temperature above the inflection point. Plotted regression lines indicate the best fit for the data based on a piecewise regression analysis, as described by the equations provided in the figure.

Figure 2. Thermoregulatory Index (TRI) in African Penguins *Spheniscus demersus* exposed to increasing ambient temperatures in a climate-controlled room. The scoring system of individual behaviour used to determine the TRI is provided in Table 1. Plotted regression lines indicate the best fit for the data based on a piecewise regression analysi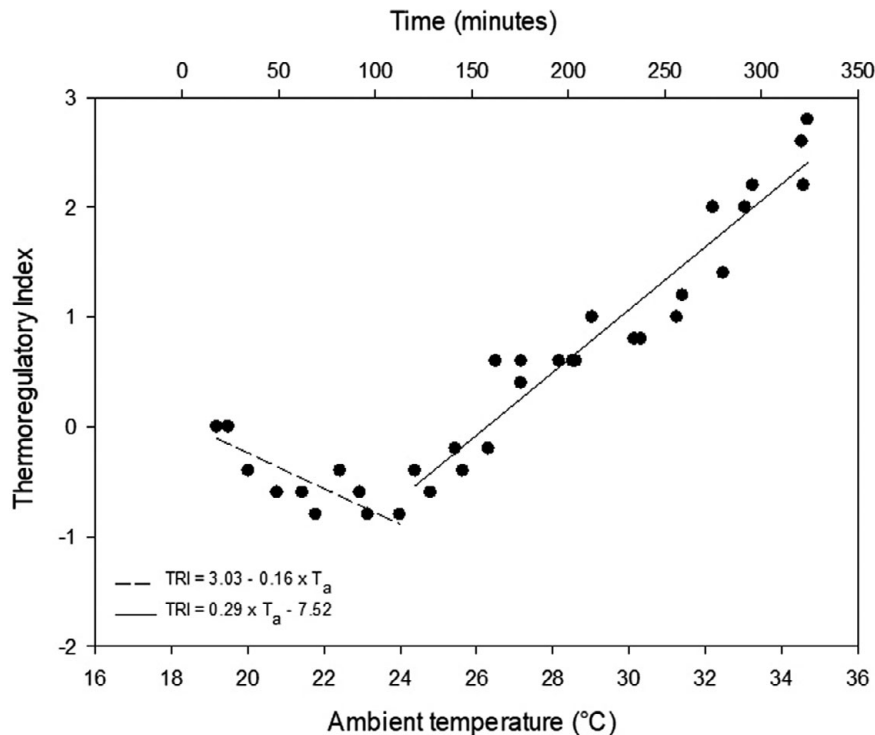s, as described by the equations provided in the figure. As TRI is based on arbitrarily assigned values the equations are purely illustrative.

latter period of hyperthermia was in response to increasing ambient temperatures. Both of these observations have implications for conservation in this endangered species. The elevated T_bs observed during the first half of the experiment were unexpected given that these animals routinely interacted with staff at their captive facility and the five individuals used were specifically chosen as they seemed to be the most comfortable around humans. Yet, the response observed corroborates previous studies showing that penguins are sensitive to human-related disturbances (Lewden et al. 2024), suggesting that wild African Penguins would show a greater or more sustained increase in T_b due to human-related disturbance. Elevated T_bs due to human-related disturbance have also been reported in Yellow-eyed Penguins *Megadyptes antipodes* (Farner 1958), Humboldt Penguins (Drent and Stonehouse 1971), and Emperor Penguins *Aptenodytes forsteri* (Regel and Pütz 1997). The recovery time needed by penguins for their T_b to return to normothermic level could span several hours, but this may also depend on the duration of the disturbance (Regel and Pütz 1997) and the individual, as suggested by the observed intraspecific variability. Thus, despite a relatively short period of disturbance (approximately one hour from capture at SANCCOB to the beginning of the experiment), the observed 3.5 hours recovery time in African Penguins aligns with the general observations of 3–4 hours recovery time reported by Drent and Stonehouse (1971) and Regel and Pütz (1997). Collectively these findings show that penguins are highly sensitive to disturbances and suggests that these well-insulated species may struggle to offload any heat generated due to stress or movement associated with disturbance or handling long after the event.

In contrast to the first segment of the penguins' T_b thermal profile, the latter segment (i.e. after the point of inflection) showed

that penguins began to store heat at $T_a \approx 29°C$. This is supported by the fact that penguins had already returned to their normothermic T_b (37.3°C; Wilson and Grémillet 1996) by this time and were able to defend normothermia for approximately one hour. Yet, based on their behaviour, penguins seemingly only showed signs of heat stress at $T_a \approx 31°C$ with the onset of panting at this temperature and birds also adopting postures, such as extended flippers, which is favourable for heat dissipation. At first glance, African Penguins seem to be more tolerant of heat compared with Chinstrap Penguins *Pygoscelis antarcticus* and Adélie Penguins *P. adeliae* where panting starts at $T_a \approx 26°C$, and they have a similar tolerance as Gentoo Penguins *P. papua* where panting began at $T_a \approx 30°C$ (Murrish 1982). However, the risk of heat stress is comparatively much higher for African Penguins which breed in a warmer climate compared with *Pygoscelis* penguins, which breed on the Antarctic Peninsula where temperatures are typically subzero and the highest recorded temperature anomaly to date is 13.7°C recorded in the Austral summer of 2022 (Gorodetskaya et al. 2023). This is not to say that *Pygoscelis* penguins are not negatively affected by warming temperatures (see for example Cimino et al. 2016; Krüger 2023; Peña et al. 2014) or that they may not experience heat stress due to heat loading from direct solar radiation (*sensu* O'Connor et al. 2022), simply that they have a lower chance of hyperthermia from exposure to heat in their natural environment compared with African Penguins.

Panting behaviour in nesting wild African Penguins was observed at much lower T_a compared with the current study. Welman and Pichegru (2023) observed penguins panting at T_a as low as 22°C when nesting fully exposed to sunlight, i.e. subjected to direct solar radiation, at 25°C when nesting in double-layered ceramic artificial nests, and at 29°C when nesting in open-ended

tunnel-shaped artificial nests. For penguins in artificial nests, the authors argued that convective cooling likely contributed to the delayed onset in panting in the tunnel-shaped nests as they allowed better through-flow of air compared with the ceramic nests which have only a single entrance and small ventilation holes at the back. Perhaps the air movement within the climate-controlled room also facilitated convective cooling in penguins in the current study, leading to panting at a relatively high T_a compared with nesting birds in the aforementioned study. Even so, as with the cold-climate residing *Pygoscelis* penguins (Murrish 1982), the results show a clear trend of increasing T_b after panting commenced albeit that one individual (penguin #3) appeared to heat more slowly (Supplementary Figure S2). Whereas facultative hyperthermia seems to be a viable strategy for some birds to save on body water during acute heat exposure (Freeman et al. 2022; Gerson et al. 2019), given that their high level of insulation would make it challenging to later off-load excess heat, it is unlikely that penguins adopt this approach. Thus, we argue that the increase in T_b observed at temperatures above 31°C is a true response to heat stress. As mentioned previously, in the wild, heat loading is exacerbated by direct solar radiation. The results therefore support the argument that while ashore, African Penguins are extremely vulnerable to heat stress, which is particularly concerning in the context of future climate change scenarios, as extreme heat events become more severe and frequent (McKechnie, Rushworth et al. 2021).

Despite the small sample size our observations provide valuable insights into the thermal tolerance at high temperatures of African Penguins, and a human-related stress response, both of which are important for conservation. Increased T_as are associated with reduced inter-annual survival in Little Penguins *Eudyptula minor*, which also nest in burrows, particularly during extended periods ashore (Ganendran et al. 2016). For African Penguins, such effects may be hard to avoid, but direct and indirect impacts of heat stress during nesting can be mitigated to some degree by using artificial or modified burrows, rehabilitation of nesting habitat with natural vegetation, and early warning indicators based on an improved understanding of thermoregulation to inform intervention actions. Furthermore, Dunn et al. (2019) found a correlation between increases in tourist numbers at colonies and a decline in breeding success in Gentoo Penguins. Thus, based on our observation of stress-related hyperthermia, together with the body of evidence showing that penguins are sensitive to human disturbance (Bender et al. 2016; Dunn et al. 2019; Ellenberg et al. 2006, 2007; Lewden et al. 2024; Scheun et al. 2021), we recommend that management authorities increase precautionary measures at African Penguin colonies to reduce stress at critical stages of their life-history. For example, although penguins and their chicks show some level of habituation, it has been suggested that incubation and moulting should be considered as the more vulnerable stages (Villanueva et al. 2014; Walker et al. 2005).

With hindsight, an optimised experimental design would allow the penguins a longer period of time to acclimatise in the climate-controlled room before initially increasing T_a (e.g. Choy et al. 2021). However ethical limitations prevented either a longer duration or a repeat of the experiment. While it would have been ideal to record concurrent measures of metabolic rate and evaporative water loss alongside our recordings of T_b to gain a more holistic understanding of the risk of heat stress in the African Penguin (McKechnie, Gerson et al. 2021; Mitchell et al. 2018; Wolf et al. 2017), our observations of thermoregulatory behaviour are nevertheless valuable to conservation efforts. African Penguin numbers continue to

dwindle (Sherley et al. 2020) and the species faces extinction within the next 20 years (Sherley et al. 2024). Among other drivers, heat stress while on land is a contributory factor in this decline (DFFE 2013; Ludynia et al. 2014), and the understanding of thermoregulatory behaviour in this species will improve interpretations of observations that conservation practitioners and researchers make of populations of African Penguins at their colonies and assess temporal or spatial changes in behaviour and the likelihood of heat stress (Cook et al. 2020; Simeone et al. 2004).

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