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Effect of weather conditions and presence of visitors on adrenocortical activity in captive African penguins (*Spheniscus demersus*)

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ABSTRACT

A number of potential stressors are present in captive environments and it is critically important to identify them in order to improve health and welfare in *ex situ* animal populations. In this study, we investigated the adrenocortical activity of a colony of African penguins hosted in an immersive zoo in Italy, with respect to the presence of visitors and local microclimatic conditions, using the non-invasive method of assessing faecal glucocorticoid metabolites (FGMs). The penguins' exhibit is a large naturalistic outdoor enclosure, which closely reproduces the natural habitat of this species. Data collection took place from the beginning of June to the end of August 2014, during the period of maximum flow of visitors. We carried out 12 sampling periods, each involving 2 consecutive days; during the first day we counted the visitors and we registered the meteorological data, and on the second day, we collected the faecal samples, which amounted to a total of 285 faecal samples. Our results showed that the number of visitors did not influence the adrenocortical activity of the African penguins. Conversely, the local microclimatic conditions did influence the physiological stress on these birds. We found that an increase of the daily mean temperature induced a significant increase in FGM concentrations, although humidity and wind speed had a moderating effect on temperature and reduced the heat-induced stress. Moreover, we calculated two climatic indices, commonly used to assess the thermal discomfort in animals, namely the THI (Temperature-Humidity Index) and WCI (Wind Chill Index), and we detected a positive relationship between their values and the FGM levels, demonstrating that these indices could be useful indicators of weather discomfort in African penguins. Our study shows that the simulating naturalistic conditions could have significant benefits for zoo animals, such as reducing the negative effect of visitors. Nevertheless, it should be taken into account where the zoological facility is located and if the local microclimatic conditions are compatible with the hosted species, to ensure that they do not differ greatly from their natural habitat.

1. Introduction

A wide range of environmental challenges threatens the welfare of animals housed in artificial habitats. For zoo animals, the number of potential environmental stressors can be numerous, and the effects are often species-specific (Scarlata et al., 2013). Improving health and welfare of *ex situ* populations is one of the main goals of zoological facilities worldwide (Melfi, 2009; Howell-Stephens et al., 2012). Therefore, identifying these conditions, which are associated with high levels of stress in captive animals, is critically important to achieve these aims. There are a number of potential stressors in captive environments, such as inadequate housing conditions and inappropriate social interactions, which could restrict the natural behaviour of zoo animals (Scarlata et al., 2013; Khonmee et al., 2014). In addition, captive animals are exposed to a continuous human presence. The presence of people at zoo exhibits could be perceived by animals as a negative influence, a form of enrichment, or simply a changing variable with no evident effect (Hosey, 2000; Davey, 2007; Hosey, 2008). Whether the zoo visitors have a stressful effect on the animals seems to depend on many factors, such as the temperament of the species or individuals (Fernandez et al., 2009), the animals' body size (Davey, 2007), and the enclosure design (Davey, 2007; Hosey, 2000). Particularly, it is known that the zoo exhibit design influences the well-being of captive animals (Morgan and Tromborg, 2007; Young et al., 2013). Due to the logistics of space and enclosure size, the variety of environmental conditions available for zoo animals is

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normally reduced compared to the wild (Young et al., 2013). Moreover, animals in zoos are often subjected to microclimatic conditions that can differ greatly from their natural habitat, and to which their species is not usually adapted (Morgan and Tromborg, 2007). While the effect of visitors on the welfare of captive animals has been widely studied for more than four decades (Hosey, 2000; Davey, 2007; Hosey, 2013), few studies have investigated the microclimatic conditions that result from outdoor zoo enclosures (Young et al., 2013) and their effect on captive species.

Assessing hormone levels in animals is a widely accepted method to determine the sources of stress, which could influence the welfare of captive animals (Wielebnowski, 2003; Howell-Stephens et al., 2012). The physiological stress response in vertebrates results in an increased secretion of glucocorticoid hormones (GCs) subsequent to the activation of the hypothalamic–pituitary–adrenal (HPA) axis (Möstl and Palme, 2002; Touma and Palme, 2005; Sheriff et al., 2011). The main GCs produced by the adrenal gland are cortisol and corticosterone, with the latter being predominant in birds (Möstl et al., 2005; Palme et al., 2005; Cockrem, 2007). If exposure to a stressor persists, chronic GC secretion can lead to deleterious consequences on animal behaviour, individual fitness and reproductive success, and can induce immunosuppression (Munck et al., 1984; Liptrap, 1993; Munck and Naráy-Fejes-Tóth, 1994). One way to evaluate physiological responses to environmental stressors is by analysing the excretion of GC metabolites in faeces (Schwarzenberger, 2007; Scarlata et al., 2013). This non-invasive method permits the monitoring of the animal's endocrine status without the necessity to capture or handle the animals, which may compromise the accurate assessment of stress (Millspaugh and Washburn, 2004; Goymann, 2012). In addition, faecal glucocorticoid metabolites (FGMs) represent an average, pooled value of excreted steroids, and are therefore less affected by diurnal fluctuations and pulsatile hormone secretion (Palme, 2005). Monitoring adrenal activity through non-invasive faecal hormone sampling is rapidly gaining popularity as a tool to assess zoo animal welfare (Clark et al., 2012) in order to develop the best management practices, also with respect to endangered species.

The African Penguin (*Spheniscus demersus*) is an endemic seabird of the coastal region of South Africa and Namibia, and is the only member of the Order *Sphenisciformes* which breeds in Africa (Shelton et al., 1984). African penguins are found from central Namibia (Hollam's Bird Island) to South Africa's Eastern Cape province (Bird Island) (Crawford et al., 2011). African penguins at this latitude have developed adaptations for cold marine environments, due to the presence of cold up-welling currents, while the air temperatures, modified by cool sea breezes, are generally moderate (Frost et al., 1976). The current conservation status of this species is "Endangered", and is included in the Red List of Threatened Species of the IUCN (International Union for Conservation of Nature). The population in the wild of this species has dramatically decreased in recent years to less than 75,000–80,000 mature individuals (BirdLife International, 2013). This decline is mainly due to loss of habitat, reduction of fish stocks, environmental pollution (including oil spills), and egg collection (Barham et al., 2006; Crawford et al., 2011). African penguins are exhibited in zoos and aquaria all over the world, and as they face an elevated risk of extinction, *ex situ* conservation programs are becoming increasingly crucial.

Here, we investigated the physiological stress related to environmental conditions of a colony of African penguins hosted in an immersive zoo (Zoom Torino, Cumiana, Italy), using the non-invasive method of assessing faecal glucocorticoid metabolites (FGMs). Previous studies have demonstrated that this method is useful for detecting and highlighting potentially stressful situations, which may influence the welfare of penguins, and it allows the detection of individual differences of FGM concentrations (Anfossi et al., 2014; Ozella et al., 2015). At the time of study, there were 54 penguins (26 females and 28 males) in the colony. We carried out 12 sampling days of faecal collections; during each sampling day the collection of samples took place from 9:00 AM to 8:00 PM. The faeces collected during the different sampling days did not belong to the same penguins, and we considered the FGM average that related to the penguin colony as a whole. Moreover, there was variability concerning the number of samples, collected on each sampling day, between males and females, penguins' age, and time of day (morning, afternoon and evening). During the 12 days before the faecal collections, we registered the number of visitors and the microclimatic conditions. The data collection took place during summer 2014 (from the beginning of June until the end of August), when the weather conditions in Cumiana are usually warm and muggy. This period included the maximum flow of visitors for the zoological facility, mainly due to the presence of a swimming pool for the use of visitors, adjacent to the penguins' pond.

The main objectives of this study were: (1) to evaluate any differences in FGM concentrations between penguins of different gender and ages, and between time of day and sampling days; (2) to assess if the presence of visitors and the microclimatic conditions could influence the adrenocortical activity in these birds; (3) to evaluate if two climatic indices, namely the THI (Temperature-Humidity Index) and WCI (Wind Chill Index), are related to FGM levels.

2. Materials and methods

2.1. Study site and animals

The study was conducted at an immersive zoo called Zoom Torino (44°56′N, 7°25′E) located in Cumiana (Italy), and accredited by the EAZA (European Association of Zoos and Aquaria). The biopark covers an area of about 16 ha with naturalistic enclosures that closely reproduce the natural habitat of the different species. The exhibits provide an opportunity for visitors to view the various animal species through the use of open-faced exhibits and glass panels, without evident barriers or cages. All the exhibits have a large outdoor area, with flooring and plant elements designed to simulate natural habitat conditions, and an indoor area for the night shelter.

The penguins are housed in an exhibit, which reproduces the habitat of "Boulder Beach", a natural nesting site in South Africa, near Cape Town (Fig. 1). The exhibit covers an area of 1500 m^2, including a pond of 120 m^2 (water depth maximum 3 m; temperature constantly maintained at 15 °C). The exhibit substrate is made from sand and pebbles; trees and bushes are present to serve as hiding places or cover for penguins, and artificial nests are present in sufficient numbers to accommodate each pair. An indoor area is used only as a quarantine zone, and these animals are therefore exposed both day and night to the weather. Visitors can view the penguins from three points of the exhibit: a wooden footbridge that crosses the enclosure, where they are separated from the animals by a low wooden fence; from two glass panels, which allow the underwater viewing of the animals; and from two other glass panels, which separate the penguins' pond from the swimming pool for visitors, which is adjacent to the penguin exhibit. This point also allows the underwater viewing of the animals.

At the time of the study there were 54 African penguins (26 females and 28 males) in the colony, all of which were born and reared in captivity; 28 penguins were transferred from another European zoo in 2009, while 26 penguins were born at Zoom Torino. Penguins of age 3–12 months were classified as "juveniles";

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