Chapter 3

Conservation Physiology of Tigers in Zoos: Integrating Stress Physiology and Behaviour to Monitor Their Health and Welfare

Edward Narayan, Nagarajan Baskaran and
Janice Vaz

Additional information is available at the end of the chapter

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Abstract

Big cats in zoos can face challenges associated with captive environments such as inadequate biological adaptation, increased occurrence abnormal behaviour and health-related problems. Conservation physiology is an emerging theme and a dynamic field of research, which aims to reduce these challenges of big cats captive management programmes through new scientific research integrating physiology and behaviour. This field of research applies cutting-edge physiological tools (e.g. non-invasive reproductive and stress hormone monitoring) in combination with traditional methods of behaviour and veterinary health assessments to provide a holistic account of how big cats respond to the captive environment. This book chapter discusses the applications of conservation physiology tools in the captive management of tigers in zoos. Our goal is to bolster tiger captive management in zoos by studying their stress physiology. Overall, the application of conservation physiology tools into captive management programmes for tigers and other big cat species can provide valuable information for evaluating and managing stress, thus improving tiger welfare.

Keywords: conservation physiology, stress endocrinology, zoo biology, animal welfare, behaviour, human-animal interactions, breeding, management

1. Introduction

The world's largest feline species, the tiger, is on the brink of extinction. Tigers are globally listed as 'Endangered' on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [1]. Captive breeding programmes in zoos around the world

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have been established to create new home for tigers while also giving a unique opportunity for people and researchers to observe and study these majestic species from surrounds of large closed or open living enclosures. Tiger poaching and hunting has been happening from centuries long ago as ancestral humans became fascinated with their skin and body parts (e.g. tooth) as trophies and souvenirs. Humans are the single most serious threat for the survival of the species. Increasing human population around native tiger habitats (e.g. Southeast Asia) in addition to land clearing and the tiger territorial behaviour (the animal requires access to large habitats to source prey) have led to human-tiger conflicts [2]. In fact, tigers have lost over 90% of their native habitat within the last century, and the currently extant six tiger sub-species are all listed as endangered by the IUCN. Apart from the broader anthropogenic-induced factors that have caused the declines of wild tiger populations such as climate change, land clearing and poaching by humans, the proximate factors, such as diseases and health-related problems, should also be considered as major contributing factors that impact on the survival of tigers [3].

Captive breeding has been a key component of the Tiger Global Conservation Strategy (GCS), which was conceptualised during the international tiger workshop in 1992 at the Edinburgh Zoo, Scotland. The Tiger GCS is a plan for managing tigers internationally, linking captive breeding programmes (ex situ conservation) with in situ conservation [4]. The Tiger GCS supports the long-term management of captive as well as wild tiger populations. Captive populations of tigers should serve as genetic and demographic reservoirs, which will support the wild tiger recovery programmes. The Tiger GCS aims to identify research priorities and technologies that can be transferred between captive and wild conservation efforts. Captive breeding programme for tigers is one of the primary goals for the Tiger GCS, and long-term data on the health and welfare of tigers housed at individual zoos will be valuable resource for identifying the challenges and coming up with strategies to improve the success of tiger captive breeding.

Captive environment can provide safe havens for animals if appropriate environmental enrichment is available so that animals can adequately perform basic life history traits (e.g. foraging, territoriality, social behaviours, resting, mating and nursing young ones). In the absence of appropriate environmental enrichment (e.g. natural and complex enclosures), tigers tend to develop behavioural stereotypes, such as pacing, self-mutilation, aggressiveness, loss of appetite and increased reproductive failure [5]. There are evidences to show that animal-related factors such as sub-species, sex and age also can relate to differences in tiger behaviour and environmental need in captivity [6]. Therefore, the ultimate challenge with captive breeding programmes for large mammals like tigers is to manage the delicate balance between the species' psyche (psychological and physiological states), the phenotype (behaviour) and the environment (e.g. enclosure features, nutrition, human interactions). For example, scientific knowledge on the physiological reaction of the tigers to novel environments will be valuable for our better understanding of this majestic cat species' requirements for biological adaptation in captivity.

This chapter is based on the emerging and valuable science discipline of conservation physiology, which integrates the traditional fields of animal conservation ecology, behaviour, genetics, nutrition and species ethology to apply innovative physiological tools that can provide new knowledge on the species' biology [7]. The primary goal of conservation physiology is to support the on-ground conservation and management efforts through integration of physiological tools into target species conservation programmes. Conservation physiology research has been applied successfully to study the stress physiology and reproductive biology of numerous majestic and iconic animal species, and the technologies (e.g. non-invasive reproductive and stress hormone monitoring) have been immensely beneficial for wildlife captive breeding programmes and conservation translocations [7].

2. Stress physiology of tigers

Most wildlife animals like big cats perceive their environment or surroundings using a combination of behavioural and physical adaptations, such as excellent swimming capabilities, caring for young ones and skin coloration, which blends well into the grassland. Stress is an inbuilt component of the tiger's life history, and stress is not inherently bad because it prepares the animal for behavioural responses through energy mobilising processes, e.g. active hunting, mating, foraging and so forth. Glucocorticoids or stress hormones (e.g. corticosterone and cortisol in tigers) are released into systemic blood circulation during the initiation of the physiological stress response [8]. This occurs when a stimulus (e.g. ungulate prey source) is visualised by the tiger and the neuroendocrine signals in the brain are received by complex set of neurons in the hypothalamic brain region, the paraventricular nucleus (PVN). The PVN is responsible for the initiation of the physiological stress response through activation of the

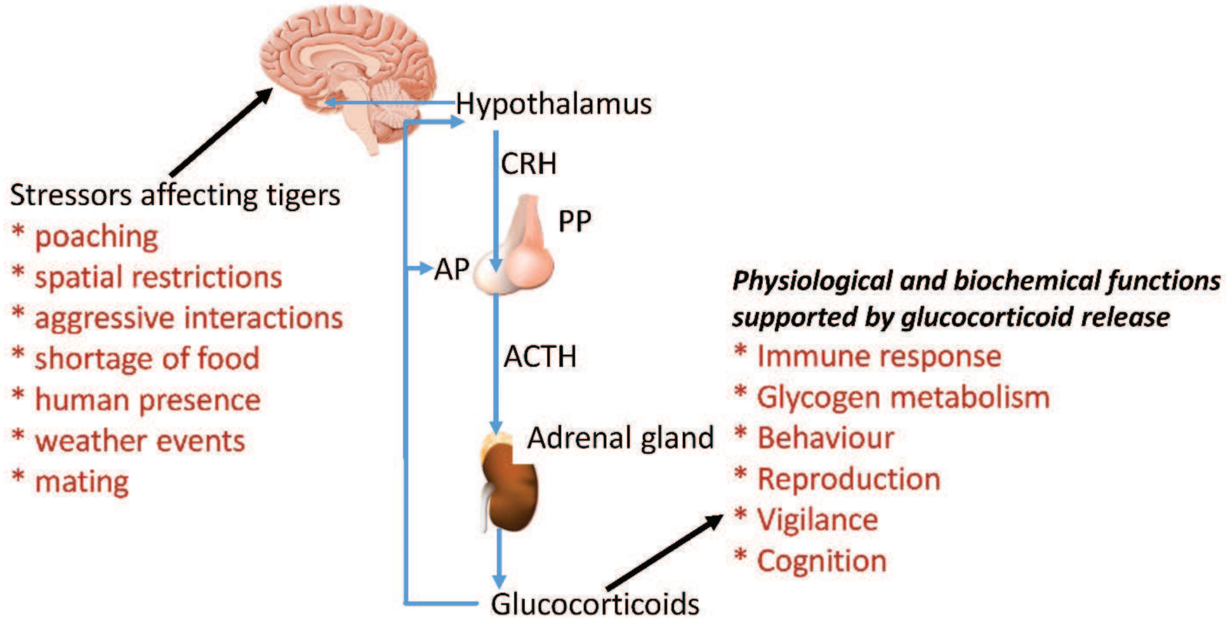

Figure 1. Pathway diagram showing the hypothalamus-pituitary-adrenal axis (HPA axis) and the feedback regulation of glucocorticoid hormone secretion by the HPA axis. CRH, corticotropin-releasing hormone; AP, anterior pituitary; PP, posterior pituitary; ACTH, adrenocorticotropic hormone.

hypothalamus-pituitary-adrenal (HPA) axis. Neural signals from the PVN lead to the secretion of corticotrophin-releasing hormone (CRH) from the hypothalamus and are released at the median eminence to the capillary beds of the hypothalamic-hypophyseal portal system. CRH stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, and ACTH ultimately stimulates the adrenal corticotrophes to secrete glucocorticoids (GCs). GCs are responsible for changing metabolic status of the animal (intermediary metabolism), as well as other key physiological processes, such as immune response, skeletal growth, cardiovascular function, reproduction and cognition. GCs perform physiological and biochemical actions in various cells in the body through the help of GC receptors. To maintain homeostasis, the GCs also regulate the higher brain centres, the hypothalamus and the anterior pituitary to stop CRH and ACTH release, thus enabling the levels of systemic GCs to return to basal levels once the stressor has been removed (e.g. few hours after the kill) (see **Figure 1**).

3. Tiger stress biology research in captivity

3.1. Conservation physiology approach: integrating stress biology, environmental factors and welfare

Capture and captivity of tigers can lead to physiological stress impacting on their wellbeing. Measurement of stress in tigers during human interventions such as capture is necessary to quantify potential impact of the external stimulus on the animal HPA axis. Stress could lead to negative consequences such as increased stereotypic behaviour, reproductive infertility and recurring health issues. Animals display a suit of behaviour in response to their environment to show emotional connectedness with their environment. These behaviours may either be signs of healthy animal or an indicator of poor welfare. Therefore, behaviour can be considered as the animal’s first line of defence in response to environmental change [9]. Stereotypic or repetitive behaviour in animals serves as indicators or stress, usually associated with elevated glucocorticoid levels [10]. Some of the stressors commonly faced in the captive environment include elements of the enclosure [11], certain aspects of animal care routines [8] and construction noise [12]. Assessment of the welfare of tigers in captivity will be more comprehensive through the integration of behavioural and physiological measures [13].

The captive environment has multiple extrinsic factors, such as duration and nature of light, sound, odours or temperatures, over which the tigers have little control [14]. Optimal psychological and physiological wellbeing of captive care will require the identification and provision of environmental conditions that can promote naturalistic behaviour and adaptation of tigers in captivity [15]. This is defined in animal husbandry principle as environmental enrichment. Therefore, by understanding the stress biology, performance (health and wellbeing) and environmental needs of tigers, zoo managers can identify why some individuals tend to perform poorly or show behavioural signs of stress more often than others. This integration of animal biology (e.g. stress physiology and reproductive behaviour) with environmental factors and monitoring of fitness directly highlights the concept of conservation physiology (see **Figure 2**).

Through the applications of conservation physiology tools, such as non-invasive reproductive and stress hormone monitoring, quantitative data related to tiger biology can be obtained and can be related to the environmental conditions available to them and their overall health and wellbeing. Zoos urgently need to apply these conservation physiology tools into animal welfare and health assessment programmes.

There has been a handful of research based on the stress biology of tigers in zoos, applying the conservation physiology framework (**Figure 2**).

Sajjad et al. [16] studied the effect of captivity on the blood plasma cortisol level and behavioural pattern of tigers managed in two local zoos (indoor versus outdoor enclosures) in Pakistan. The results showed no significant difference in plasma cortisol levels between the two enclosure types; however, behavioural analysis showed differences in pacing percentage between the wildlife park and the zoo (time spent in stereotyping was higher for tigers living in closed enclosures). Plasma cortisol levels were higher when blood collection was done using squeeze caging of the studied tigers.

In a recent research, Vaz et al. [17] applied the concept of conservation physiology to evaluate the stress biology and stereotypic behaviour of tigers (41 Royal Bengal tigers) across six zoos in India. We used non-invasive stress hormone monitoring using faecal corticosterone enzyme immunoassays to quantify the levels of stress in the studied tigers. Our research has found significant correlation between stereotypic behaviour and stress levels in healthy tigers. Furthermore, the stereotypic extent was reduced with environmental enrichment (e.g. increased enclosure size, presence of pools and stones, social interactions with conspecifics

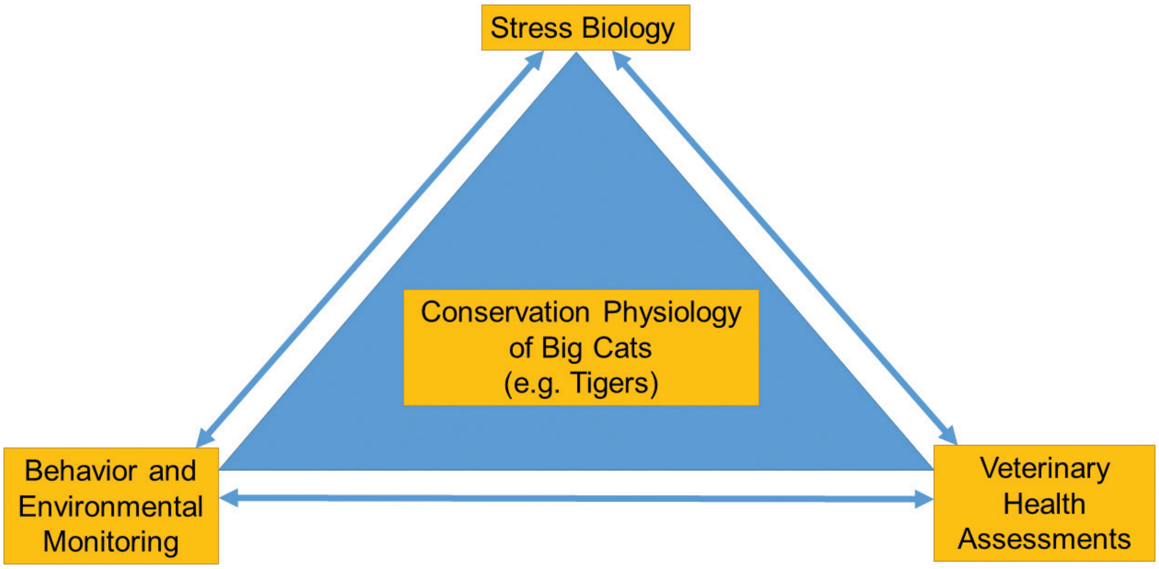

Figure 2. Conservation physiology conceptual framework that interconnects key components of research, including stress biology, behaviour and environmental monitoring and tiger veterinary health assessments.

and positive keeper interactions with the tigers). Stress levels indicated chronic stress in tigers with health problems.

Furthermore, Narayan et al. [8] used faecal cortisol monitoring to investigate the stress physiology of two tiger sub-species, the Bengal and Sumatran tigers, held in two Australian zoos. This study demonstrated that tiger stress levels were significantly different between sexes and zoos, matching with previous studies that also found higher average stress levels in females than males for other felids [18]. Narayan et al.'s [8] study also showed that individual tigers responded differently to the stressor type, intensity and duration. This highlights the phenomenon of unique personalities or coping abilities in animals [19]. Our 2013 study [8] showed that tigers participating in routine activities, such as visitor interactions, expressed higher levels of stress than those individuals that did not participate in these activities. However, tigers from another local Australian zoo showed lower levels of stress when they participated in display activities. These results highlighted that tigers have individual differences in their physiological stress responses, and it also shows that research should quantify stress biology as a quick way of determining the physiological health of the tigers. Variation in the physiological stress responses will indicate the physiological resilience or potential signs of sublethal stress in individual tigers. Non-invasive hormone monitoring provides an early window of opportunity for researchers to see into the stress biology of these majestic feline species.

Dembiec et al. [20] studied the effects of transportation on tigers using combination of stress biology measurements (faecal cortisol and respiration rate) and behaviour during and after transport (activity level, pacing behaviour, investigative behaviour and ear position). Both respiration rate and cortisol profiles showed significant changes after transport (respiration rate increased from 94.6 to 132.3 breaths/10 min after release into enclosures), while faecal cortisol levels remained elevated for up to 12 days in some tigers. Interestingly, the researchers also discovered that 'naïve tigers' showed higher incidence of pacing and higher faecal cortisol than conspecifics that were habituated to the procedure. The data highlighted requirement for appropriate enrichment training and controlled exposure to novel environments in captive tigers.

3.2. Key considerations for non-invasive endocrine monitoring in tigers

Traditional method of stress hormone evaluation in animals included blood collection; however, the process itself can be strenuous for the animal, and the pulsatile nature of systemic glucocorticoid concentrations makes interpretation very difficult. The dynamic field of conservation physiology research has vigorously tested and validated non-invasive methods of stress hormone quantification in wildlife, using excreta samples instead of blood. GCs are metabolised in the liver, and GC metabolites can be found in urine and/or faeces. Faecal-based GC metabolite testing has been used for felids, including tigers [20]. Faecal samples can be collected routinely as part of captive husbandry in zoos. Faecal glucocorticoid metabolites provide a pooled level of GCs that have already participated in the physiological stress response and ready for quantification through excreta analysis. Thus, faecal-based GC monitoring can be used to obtain longitudinal profiles of stress levels in captive animals [21]. One of the key considerations when non-invasive hormone monitoring is being attempted in a species (e.g. tigers) is to obtain data related to time lag of GCs between the activation of the physiological stress response and the collection of GC metabolites in excreta. Typically, hormonal challenge using ACTH is conducted with faecal collections for several days before and after the exogenous hormonal challenge. The peak response of FGMs is then used to identify the time lag, which is valuable information for designing the study. In the absence of ACTH challenge, other stressors such as pregnancy, veterinary anaesthesia can be used routinely to validate faecal-based hormone monitoring tools. Earlier, Narayan et al. [8] validated faecal-based cortisol monitoring in zoo tigers using veterinary check (blood collection) as a physical stressor. Narayan et al. [8] conducted biological validation by collecting faeces 5 days before and 5 days after blood was taken from four male and five female Bengal tigers from a local zoo in Australia. The results showed that the mean levels of faecal cortisol increased by 138 and 285% in the male and female tigers within 1 day after bloods were taken, returning to baseline in 5 days.

In earlier research, Powell et al. [12] validated faecal cortisol monitoring in the Siberian tiger (*Panthera tigris altaica*) using ACTH challenge, demonstrating 2 days time lag of faecal cortisol metabolites in tiger faecal extract after ACTH challenge. The other key considerations of designing comprehensive research in tiger conservation physiology are to consider the effect of natural environmental conditions on the decay of GC metabolites in faecal samples and also to consider the age of samples before analysis. Recently, research from my lab group has demonstrated that faecal cortisol metabolites are stable up to 2 days and homogenising fresh faecal pellets will help reduce the variation in faecal GC metabolite results when small sample sizes are used [22].

Furthermore, levels of GC metabolites in excreta can also be influenced by a suit of internal and external factors. It is well documented in wildlife studies that GCs are metabolic hormones, and the energy mobilisation hypothesis states that GC levels will be higher during more energy-demanding periods, such as breeding, foraging, migration and active search for food and substrate prior to hibernation [23]. Millspaugh and Washburn [24] reviewed the factors that can influence GC levels, such as season, food and habitat availability, reproductive variability, age and gender. Therefore, future research should combine intrinsic (animal related) and extrinsic (environment related) factors into stress hormone monitoring research to derive biologically meaningful interpretation and meaning of the hormonal data.

Furthermore, GC concentrations have been suggested to be significantly affected by reproductive status or cyclic phase of females in some species (e.g. carnivores [25]), which could be explained by increased metabolic demands associated with reproduction [26]. For example, Goymann et al. [25] found that GC levels in lactating female spotted hyenas (*Crocuta crocuta*) were higher than levels for non-lactating females in the wild. Natural variation in GCs tends to complicate the analysis and interpretation of large datasets; however, if researchers are able to obtain as much information on the biology and environmental factors surrounding their study subject, then appropriate statistical models can be applied to test the significance of GC data. Non-invasive reproductive and stress hormone monitoring has been successfully used to monitor tigers in managed habitats and zoos [27, 28].

4. Conclusions

Tiger conservation physiology is a useful new research theme that can significantly bolster captive breeding programmes for the species. Through the applications of non-invasive hormone monitoring tools into tiger health and welfare assessments, more detailed information can be obtained related to their physiological coping capacity towards the captive environment. Zoos can actively collaborate with university-based researchers and apply research students to develop new projects based on tiger conservation physiology. Conservation physiology research has plenty to offer to tiger biology and welfare research and now is the time to apply non-invasive physiological tools into zoo programmes, so that with access to new data on tiger conservation physiology, this majestic cat can be better managed and protected in zoos.

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Author details

Edward Narayan[1]*, Nagarajan Baskaran[2] and Janice Vaz[2]

*Address all correspondence to: e.narayan@westernsydney.edu.au

1 Western Sydney University, School of Science and Health, Locked Bag 1797,
Penrith, NSW, Australia

2 A.V.C. College (Autonomous), Mannampandal, India

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