WELFARE STATUS ASSESSMENT OF CAPTIVE ASIATIC LIONS IN GUJARAT: AN INTEGRATED APPROACH

A THESIS

Submitted by

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for the award of the Degree of

DOCTOR OF PHILOSOPHY

IN

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Under the guidance of

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DECLARATION

I hereby declare that the work conducted under the thesis titled **"Welfare status assessment of captive Asiatic Lions in Gujarat: An integrated approach"**, is a record of original and independent research work done by me and submitted for the award of the degree of **Doctor of Philosophy in Wildlife Science** to the **Saurashtra University, Rajkot, Gujarat**. This research work has been carried out under the guidance and supervision of Dr. Samrat Mondol, Scientist-E, Wildlife Institute of India, Dehradun. The work has not formed the basis for the award of any other degree, diploma or any other qualification. I also declare that the thesis embodies my work, analysis, observation, understanding and the particulars given in it are true to the best of my knowledge.

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CERTIFICATE

This is to certify that the thesis by **Mr. Sitendu Goswami** titled **"Welfare status assessment of captive Asiatic Lions in Gujarat: An integrated approach"** is an original and independent research work submitted to the **Saurashtra University, Rajkot, Gujarat**, for the award of the degree of Doctor of Philosophy in Wildlife Science.

Mr. Sitendu Goswami has put in more than six terms of research work embedded in this thesis under my guidance and supervision. The work presented in this thesis has not been submitted to any other University or Institute for the award of any degree, diploma or distinction.

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I certify that the research work was appreciated by all who were present, and the comments made by the faculty and researchers have been appropriately included in the thesis.

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1.	Animal welfare and the Asiatic lion conservation breeding programme	4%
2.	Role of enclosure complexity and visitor presence on Asiatic lion welfare	4%
3.	Role of personality and rearing-history on Asiatic lion welfare	8%
4.	Effects of enrichment interventions on Asiatic lion welfare	0%
5.	Synthesis and recommendations	1%
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1

Animal welfare and the Asiatic lion conservation breeding programme

1.1 Introduction

Our present knowledge of the natural world is a thread in the tapestry of human-animal interactions accumulated over millions of years (Veissier et al., 2008). Since the middle of the twentieth century, animal welfare has become a dominant theme in our relationship with captive animals. Hence, it is important to document our present understanding of animal welfare in the context of our past mistakes as a lesson for future generations (Rothfels, 2002). Broom (1986) defined animal welfare as the state of the animal as it tries to cope with its internal and external environment. This thesis aims to a. address the welfare state of Asiatic lions (*Panthera leo persica*) based on environmental factors and individual-level differences, and b. propose a methodological framework for evaluation and improvement welfare in captive environments. In this chapter, I will set up the context for this study with a brief chronology of our understanding of the origin of animal welfare and the science of zoo biology and their relevance to the Asiatic lion conservation breeding programme (CBP).

The Asiatic lion is an interesting species that has witnessed several upheavals throughout its history, with the most recent ones being largely anthropogenic (Jhala et al., 2019). Once at the brink of extinction, this species has benefitted from extensive conservation and management efforts both *in-situ* and *ex-situ*. The *ex-situ* management of the species has primarily focused on managing its genetic diversity, with very little being said on the welfare status of the captive population and the management of its behaviour diversity and fitness (Kaumanns et al., 2015). Since the future of this species will depend on successful repatriation to lost ranges (Jhala et al., 2019). There is an urgent need to evaluate and improve incumbent management practices for the captive population to improve their present welfare state and increase post-release fitness (Rabin, 2003; Reading et al., 2013).

1.2 Animal welfare, and sentience

Although humans have hunted, domesticated, captured, and exploited animals for several millennia (Vigne, 2011), our understanding of their psychology remains limited. Anthropocentrism and utilitarianism are the two most prominent themes that define our modern outlook towards the natural world. Animal welfare and animal ethics share a common history of public concern for the well-being of animals under human care (Fraser, 1999). The early discourse on animal welfare was primarily concerned with whether animals were sentient beings, worthy enough to bear the gift of emotions, pain and suffering, hitherto, a holy birthright of humans. History is rife with examples of how humans of the past and present ignored (or continue to ignore) the humanity of their conspecies, based on distinctions of race, sex, class, religion, caste and geography. Hence it is only logical that we would pause for confirmation before attributing such treasured qualities to other members of the animal kingdom.

Descartes (1596-1650), described animals as non-sentient beings that cannot reason or feel pain (Proctor et al., 2013). In contrast, Voltaire (1694-1778), Hume (1711-1776) and Rousseau (1712-1778) noted the pain and suffering of animals around them (Singer, 1979). In 1635, Ireland enacted the first set of laws prohibiting cruelty towards farm animals (Lucht, 2012). The quest for animal sentience as the basis for their right to humane treatment continued until, the eighteenth-century philosopher Bentham (1789), posed a different question. He asked if animals needed to have sentience or reasoning to have the capability to feel emotions or suffer from pain? The British Parliament passed the Richard Martin's Act (1822), which deemed it illegal for any person to wantonly and cruelly beat a horse, mare, and other livestock. This legislation also laid the foundation of the Royal Society for Prevention of Cruelty to Animals (Favre et al., 1993). British politicians James Burgh and Richard Martin were some of the earliest proponents for the prevention of animal cruelty (Englefield et al., 2019; Francione, 2010; Jamieson, 1990; Sweeney, 2017). However, animal welfare legislation in the nineteenth century was primarily directed towards the prevention of cruelty towards domesticated animals.

Some argue that the earliest enactments of animal welfare legislation concerning farm animals may have been driven by the concern for the quality of the produce.

The period between the latter part of the nineteenth century, to the years leading up to the first world war, was illuminating for the animal welfare revolution. Charles Darwin's book on the origin of species positioned humans as a part of the natural world (Darwin's, 1859). Darwin's book on "The expression of the emotions in man and animals" (Darwin, 1872) advanced the cause for animal welfare by showcasing that animals were capable of feeling complex emotions that percolated to their body language and facial expressions. The theory of evolution had a profound impact on the field of psychology. With the "continuity of species" established, psychologists reasoned that akin to biological traits one could compare behavioural traits across species to understand their origins. Watson argued that psychology should be defined as the science of behaviour rather than the science of mind, thereby sowing the seeds of behaviourism (Morris et al., 1987). Behaviourism in principle divorces the internal workings of the animals and treats it as a machine, a mediator between the behaviour and the stimulus (Skinner, 1963). However, the pragmatic nature of behaviourism when combined with ethology can be greatly beneficial towards understanding animal welfare (Duncan, 2006a).

The first world war (1914-1918) led to an epidemic of post-traumatic stress disorder among humans and animals leading to advancements in the field of psychology and animal behaviour (Crocq et al., 2000). These learnings were also later applied to understanding the stress psychology of animals in the livestock industry (Flandreau et al., 2017). The field of comparative psychology was born in the 1950s which led to a surge in animal welfare research (Hinde, 1966; Hodos et al., 1969). During this time, Hediger conceptualized the field of zoo biology and established it as a science to understand and meet the biological requirement of wild animals in captivity (Hediger, 1964).

1.2.1 Emergence of proliferation of Animal welfare science

By the middle of the twentieth century, animals were considered sentient beings capable of feeling pain and suffering, and thus there was a need to measure their subjective well-being in captivity (Broom, 2011).

Although welfare was a prevalent concept in the twentieth century, it was primarily used in the context of prevention of cruelty to captive animals especially livestock. It was during the middle of the twentieth century that the idea of animal welfare was concretized as something more than prevention of cruelty and providing for the physical and psychological wants of captive animals. During the 1960s, the animal rights movement probably reached its peak that was spearheaded by animal rights pioneers like "Animal Machines" by Harrison (1964) was pivotal in garnering public attention towards the welfare status of farm animals. Harrison's book pointed to the common occurrence of stereotypy, like feather pecking in poultry and excessive licking of calves as a significant symptom of welfare deficits in the livestock industry. Animal rights activism during this time was based on the twin principle of moral improvement and welfare, which posited that our mistreatment of farm animals was an insult to our humanity. Animal rights activism was crucial to the development of the legal framework that led to the improvement of farm animal welfare (Rollin, 1990). In response, the UK government formed the Brambell commission that included prominent ethologists, Thorpe and Brambell as prominent committee members (Brambell, 1965).

In 1965, the Brambell commission report on farm animal welfare was published in the UK and, this was one of those watershed moments for animal welfare science (Brambell, 1965). This report not only defined animal welfare but also framed the basis for welfare evaluations.

> "welfare evaluations must include scientific evidence available concerning the feelings of the animals that can be derived from their structure and functions and also from their behaviour."

According to Gonyou (1994), this was that moment when applied ethology and animal welfare science were interlinked together.

This report also envisaged that captive animals should have the "five freedoms":

1. Freedom from hunger and thirst (nutrition).
2. Freedom from discomfort (environment).
3. Freedom from pain, injury and disease (health).
4. Freedom to express normal behaviours (behaviour).
5. Freedom from fear and distress (mental state).

The "five-freedoms" concept provides a reasonable template to provide for the requirement of captive animals and it continued to be the norm until the 1990s. This was a watershed moment in the history of animal welfare science and created modern-day legal precedence to ensure that the welfare of animals was considered in the commercial livestock industry.

However, there are a few disadvantages to the five-freedoms model, which as pointed out by Mellor (Mellor et al., 1994; Mellor, 2016). first the use of the word "freedoms" led to animal rights activists claiming that all captive animals should be free from hunger and thirst, which is biologically unachievable and paradoxical. Second, the five-freedoms model sought to reduce the number of negative experiences and few accommodations were made for the inclusion of positive affective states. In 1994, the five domains model was proposed that has been used widely for animal welfare evaluations (Mellor et al., 1994). The five domains proposed in this model include

1. Nutrition
2. Physical environment
3. Health
4. Behavioural interaction
5. Mental state

The five domains model has since gone through periodic changes to incorporate the newest findings in animal welfare science (Mellor, 2016, 2017; Mellor et al., 2015; Mellor et al., 2020). Our understanding of animal welfare has since broadened to include viz.,

1. Opportunity express specie-typical behaviour patterns (Miller et al., 2020a)

2. Opportunity to have meaningful animal-keeper interactions (Mellor et al., 2020)

3. Opportunity to choose (Dawkins, 2021) - Are the animals happy?

The inclusion of positive markers of welfare is noteworthy and indicative of the present research focus in this field.

1.2.2 Modern interpretation of animal welfare

Animal welfare has several definitions which include the following types

1. Lexical: The Brambell Commission report defined welfare as a "wide term that embraces both the physical and mental well-being of the animal."

2. Explanatory: "The welfare of an individual is its state as regards its attempts to cope with its environment" (Broom, 1986). Coping is then used to describe the efforts made by the animal to adapt to the environment.

3. Operational: Third, operational definitions mention the parameters by which animal welfare shall be assessed.

Proctor et al. (2013) discovered in a comprehensive assessment of animal sentience studies that the majority of material relating to animal welfare and sentience focused largely on negative outcomes. Animal welfare in the modern era should aim not just to reduce the allostatic load on captive animals, but also try to provide enough possibilities for positive affective states (Miller et al., 2020a; Whitham et al., 2013). Broom (2011) on the other hand have equated animal welfare with adaptive responses that animals use to cope with the stressors of captivity. According to Broom, health and animal welfare cannot be dealt with separately, because affective states or emotions have a physiological basis and hence cannot be divorced from overall health and coping mechanisms. Extensive studies have been undertaken in recent years to better understand animal cognition, which should not be mistaken with sentience.

While sentience refers to an animal's ability to experience emotions like pleasure and pain (Broom, 2007), cognition refers to the process through which animals receive, process, and retain information (Dawkins, 2017). In broad terms, while animal cognition deals with the objective confines of behaviourism, animal sentience research can address other subjective ideas like affective states (Mellor, 2019). Rather than using a teleological approach to behaviourism (Rachlin, 1999), we need a more holistic view of animal psychology that takes into account both the internal and external environment that drives behaviour patterns.

A biopsychosocial approach, as advocated by Engel (Engel, 1980), is best summed up by the tenets of theoretical behaviourism, which accounts for the animal's internal condition while watching and analysing behaviour, which may be thought of as the context around the occurrence. Lately, the conversation about animal sentience has been replaced with "affective states" and "positive emotions" to define the concepts of subjective well-being (Crump et al., 2018; Mellor, 2019). Dawkins (Dawkins, 2017) opined that rather than stress on debating the presence of animal sentience or consciousness, we should only look at two factors, viz., is the animal healthy and does it have what it wants. Dawkins acknowledged the relevance of subjective experiences and emotional states in animal welfare studies by inserting the second sentence. Dawkins' definition of animal welfare also underlines the need for understanding the entire gamut of animal emotions. Fortunately, the modern narrative on animal welfare unanimously agrees on animal sentience and is committed to the mitigation of negative emotions in captivity (Boissy et al., 2007; Crump et al., 2018; Dawkins, 2021; Fraser, 2009; Harding et al., 2004). Animal welfare literature is filled with several methods to measure welfare. According to Fraser (1995), animal welfare as a concept is an admixture of several concepts that come together to serve a common purpose. Since welfare is not a singular concept, it requires a multifarious approach for measurement (Mason et al., 2007). Past research on animal welfare has focused primarily on the mitigation of pain, suffering and negative emotions (Ahloy-Dallaire et al., 2018; Moe et al., 2006). However, most experts accept that welfare is a spectrum that encompasses both the mitigation of suffering as well as the opportunity to experience positive affective states (Hill et al., 2009; Mason et al., 2007). That is probably why recent animal welfare literature focuses on finding a mix of welfare indices that can summarise the entire gamut of the biopsychosocial state of the animal. Some of the most commonly useed indicators of welfare are behavioural in nature, which may include,activity budgets (Rose et al., 2013), play behaviour (Ahloy-Dallaire et al., 2018; Van der Harst et al., 2007), reward related behaviours (Van der Harst et al., 2007), cognitive tests (Clegg, 2018), behaviour diversity (MacIntosh et al., 2011), enclosure usage or enclosure space utilization patterns (Bashaw et al., 2007; Lukas et al., 2003; Mallapur, Waran, et al., 2005; Ross et al., 2009). Physiological measures often employ endocrinological tools (Mitra et al., 2009; Tilbrook et al., 2018; Welberg et al., 2006), heart rates (Visser et al., 2002), body condition scores (Valle et al., 2017), eye blink rates nand ear movements (Marcet-Rius et al., 2019; Merkies et al., 2019) among others. In some cases, subjective evaluation of welfare by keepers is a reliable measure of the welfare of captive animals (Canino et al., 2010; Patel et al., 2019). The literature suggests using multiple types of positive and negative indicators of welfare for any modern animal-welfare study design (Miller et al., 2020a).

1.3 Zoos and conservation breeding programmes

1.3.1 Origin of zoos

Zoos are human constructs, originally designed to portray our control over nature and other members of the animal kingdom. The origin of zoos and their form in Europe in the middle of the nineteenth century is indeed controversial (Hanson, 1996; Kistler et al., 2010). In "A history of zoological gardens in the west" Baratay et al. (2004) wrote that the origin of the modern zoo can be traced back to the rise in European colonialism, which served as propaganda about far away colonies and their inhabitants (human and non-human) to the home crowd. Several researchers have termed the collaboration between early zoos and European colonists as a form of "epistemological genocide" that was perpetrated against the subaltern and the biodiversity of the colonies (Braverman, 2014; Rashkow, 2015). This exemplifies one of the primary ethical concerns regarding the continuance of zoos in the modern era. In the past few decades, zoos have undergone a makeover and attempted to obscure their past by the addition of *ex-situ* conservation and conservation education as primary goals in their manifestos. Elizabeth Hanson (1996) in her thesis " Nature Civilized: A Cultural History of American Zoos, 1870-1940" argues that the goals of a modern zoo viz., recreation, education and advancement of science and conservation are conflicted.

Modern zoos are often criticized for perpetuating a culture of exotic collection planning that puts the visitor's needs above that of animal welfare. As Hutchins points out, responsible collection planning in zoos is of critical importance, yet often conveniently overlooked (Hutchins et al., 1995).

Zoo biology as a subject joined the public discourse by the middle of the twentieth century through the work of Heini Hediger (1908-1992). Heini Hediger considered the father of modern "Zoo-biology" worked extensively to understand the impact of captivity on the inner world of animals (Hediger, 1968). Hediger emphasized strong interlinkages between ethology, zoology and psychology as immutable components of zoo biology (1968). While animal sentience was a debatable topic till the late nineteenth century (Duncan, 2006a; Preece, 2006), we have made significant progress to address concerns about animal psychology through the collective efforts of the twentieth-century ethologists and behaviourists, Watson (1913), Tinbergen and Lorenz (1963).

Despite being written several decades apart, the writings of Rambrahma Sanyal (1892) Hediger (1955) and other experts (Broom, 2014; Broom et al., 2008; Fraser et al., 1997; McFarland et al., 2009) agree that zoo biology should be multi-discilplinary science. Recent studies on animal welfare seem to take a multifarious approach to measuring welfare using, endocrinology (Mitra et al., 2009) and animal psychology

(Watanabe, 2007). Hediger preferred animal psychology or the subjective evaluation welfare as compared to causal analysis of behaviour through mechanistic ethology (Chrulew, 2018). The contemporary definition of welfare includes pathophysiology as well as affective states experienced by an animal or its condition while coping with its surroundings (Fraser, 2009; Mellor, 2019; Wolfensohn et al., 2018). Thanks to advancements in neuroscience and endocrinology, we can now ascertain the state of welfare of animals based on several markers (Marinath et al., 2019; Narayan et al., 2017; Vaz et al., 2017). Holistic welfare evaluations are the necessary first step to welfare improvement, which should be the ultimate goal of any *ex-situ* conservation breeding centre for endangered animals. Although animal welfare as a science has made significant progresses, thanks to its linkages with the economics of the livestock industry. The uptake of pro-welfare practices has been slow at many *ex-situ* institutions where the benefits are less tangible. This thesis hopes to tip the balance in the favour of animal-centric welfare evaluations at zoos and conservation breeding programs with a focus on individual needs.

1.3.2 Conservation breeding programmes

The human urge to maintain traditions across social, cultural, political, and ecological settings for future generations has frequently been linked to our proclivity for biodiversity conservation (Breithoff et al., 2020). Nature conservation as a method of maintaining traditions, religious practises, and people's identities have impacted human history and their interaction with nature in several ways. To assuage our collective anxiety of the distant future, we tend to consider species and ecosystems as heirlooms that must be protected for future generations. While *ex-situ* conservation as a concept may have started primarily with gene banks and preservation of the DNA of endangered species, which were more "dormant" in terms of their impact. In the past few decades, we have taken an active approach towards maintaining endangered wild fauna and flora *ex-situ* by creating our own "*live archives*", which some scientists also refer to as a shift from a "repository to a speculative investment" (Breithoff et al., 2020). Contemporary zoos have made great strides since the 1970s in the field of *ex-situ* conservation by saving several species from the brink of extinction. However, as pointed out by several experts (Alroy, 2015; Kaumanns et al., 2015), there are limits to the success that is expected from conservation breeding programmes. Snyder (1996) defined six limitations of conservation breeding programmes.

- Establishing self-sufficient captive populations.
- Poor success in reintroductions.
- High costs.
- Domestication.
- Pre-emption of other recovery techniques.
- Disease outbreaks.
- Maintaining administrative continuity.

1.3.3 Conservation breeding programmes at Indian zoos

Conservation breeding of endangered species has been one of the primary mandates of modern zoos. Most conservation breeding programmes are borne out of the now commonplace narrative of the ark. Wherein endangered animals threatened with habitat loss and other stochastic ecological or anthropological pressures can be brought into captivity and then bred to sustainable numbers to supplement the ailing natural fragmented populations. The growing number of conservation breeding programmes in the Indian zoological parks is intriguing. The surge in *ex-situ* CBP signals a growing concern for the future of the species resulting from a failure to conserve the species *in-situ*. It is also interesting to note the dearth of welfare research published from the findings of the aforementioned CBPs. This could indicate that the managers of these CBPs are already following best practices and have nothing new to contribute to science, or it could point towards a lack of scientific inquiry and data-driven decision-making at these CBPs. Presently Indian zoos manage the *ex-situ* conservation breeding programmes for more than 29 indigenous species of mammals, reptiles and birds.

The National Wildlife Action Plan of 1983 and its current version (2016) emphasize the role of zoos as centres for conservation breeding of endangered wild fauna and their rehabilitation in the wild as per the IUCN guidelines for reintroductions. The Government of India framed and endorsed the National Zoo Policy in 1998, which stated that zoos should complement and strengthen national efforts to conserve the country's unique biodiversity. As per the Recognition of Zoo Rules, zoos are constantly exhorted to house their stock of animals in a naturalistic environment that provides adequate opportunities for the expression of species-typical behaviour. Zoo policy in India mandates the welfare of all captive animals, irrespective of their conservation status. Today India has more than 145 recognized zoos, with 29 ongoing conservation breeding programmes. Some of the conservation breeding programmes like that of the Asiatic lions (*Panthera leo persica*) and Red Panda (*Ailurus fulgens fulgens*)has been operational for over 25 years. Yet there is a significant dearth of research on the welfare of animals housed at the programmes. I chose the Asiatic lion conservation breeding programme to ask some questions about the welfare status of the species in captivity and to understand gaps in incumbent management practices.

1.4 Asiatic lion conservation

Since the early Palaeolithic depictions of the "lowenmensch" by the Aurignacians (Chauvet et al., 1996; Kind et al., 2014), lions have been heralded across several cultures as emblems of man's relationship with nature (McCall, 1973). The Asiatic lion probably diverged from the African stock 50,000 years BP (Jhala et al., 2019) around the same time the "lion man" figurine was carved. Once ubiquitous across south-western Asia between Syria and parts of eastern India (Ball, 1880; Joslin, 1984; Sanyal, 1892), they were hunted to extinction from large parts of their range by the late 19th century (Jhala et al., 2019; MacKenzie, 2017; Storey, 1991). During the 1860s, the Asiatic lion population in Gir forests, India was struggling to survive. Timely conservation measures along with a blanket ban on sport hunting by the erstwhile nawabs of Junagadh, were instrumental to the recovery of the species. Sakkarbaug zoo, established in

Figure 1.1: Entrance of Sakkarbaug Zoological Garden.

1863, played a definitive role in the conservation of Asiatic lions by treating injured and diseased individuals and maintaining a viable captive stock for future repatriation. Subsequent affirmative conservation actions including an *ex-situ* conservation breeding programme (Smith, 1984) to the species revival from the edge of extinction. The fate of this species has been intertwined with the ruling classes across ages. Since time immemorial, Lions have been hunted by the royals to showcase their strength to rule over the masses. In the modern era, the cultural protectionism that once saved the species has turned into a political battle that has been detrimental to the natural range extension of the species (Jhala et al., 2019).

1.4.1 Published research on Asiatic lions

A significant corpus of work on the behavioural ecology of Asiatic lions in the wilderness exists (Chellam et al., 1993; Chellam et al., 1995; Jhala et al., 2009; Joslin, 1973; Saberwal et al., 1994; Sinha, 1987). The sociality and reproductive behaviour of Asiatic lions is another focal point for research highlighted by the works of Meena (Meena,

2009) and Chakrabarti (Chakrabarti et al., 2021; Chakrabarti et al., 2017). Banerjee et al. (2013) and others (Jhala et al., 2019; Jhala et al., 2018; Paulson, 1999; Saberwal et al., 1994)delved into the economics of co-existence and conflict between humans and lions in a landscape stretching up to 22,500 km^2. Ramanathan and colleagues (Ramanathan et al., 2007) also conducted seroepizootiological surveys on the captive Asiatic lion population in Sakkarbaug Zoological Garden.

Most experts agree that the future of the Asiatic lion lies in repatriation to lost ranges, along with securing a viable future for the species in its existing refuges (Jhala et al., 2019; Johnsingh et al., 2007; Meena et al., 2014). It is alarming that the *in-situ* research on Asiatic lions that emphatically discuss the future of the species, forget to mention anything about the future of the Asiatic lions housed at the conservation breeding programmes. In this thesis, I shall refrain from forecasting the future of the Asiatic lions. Instead, I will explore avenues for the improvement of the welfare of captive Asiatic lions. Few studies (Powell, 1995; Van Metter et al., 2008) on the enclosure enrichment of captive African lions have been conducted at various European zoos, little information exists for Asiatic lions, especially in the Indian context. The Asiatic lion Conservation breeding programme has been running for the past two decades and despite its boasts of great success (Srivastav et al., 2018). there have been no publications on the welfare status of the animals housed in the CBP.

In the past few years, the welfare of captive Asiatic lions has started receiving some attention, as evident from the trends in published research on the genetic status and welfare of the species in captivity (Atkinson et al., 2018; Pastorino et al., 2016; Pastorino et al., 2017; Suárez et al., 2017; Williams et al., 2021). Pastorino et al. (2016) evaluated the relationship between the role of personality on lion-keeper relationships (Pastorino et al., 2016)and later the role of personality on the repsonse to new enclosures (Pastorino et al., 2017). Suárez et al. (2017), also explored the effects of visitors on the welfare and behaviour of five feline species, including Asiatic lions. While each of the aforementioned studies addressed a particular aspect of Asiatic lion welfare, a holistic approach was missing. Both Pastorino and Suarez conducted their studies at on-display enclosures housing between two to five Asiatic lions. Hence existing literature on Asiatic lion welfare at European zoos cannot fulfil the research gaps in the welfare status of Asiatic lions housed at the Indian conservation breeding programme. Given that the range nation is home to over 193 individuals of the species spread among various zoological parks and CBPs, further study on the species' husbandry, management, and welfare is expected. Unfortunately, captive-raised Asiatic lions have not piqued the interest of conservation biologists and historians in the same way that their wild counterparts have.

We have not yet addressed or audited the welfare status of Asiatic lions at Indian *ex-situ* facilities, even though India is the only range nation for the species with over 600 wild animals and about 200 captive individuals, the largest captive population for the species, and the longest-running conservation breeding programme. . My thesis aims to address this crucial research gap to drive novel approaches to the improvement of welfare.

1.4.2 Status of the Asiatic lion conservation breeding programme

On December 2, 1957, Asiatic lions were released at Chnadraprabha Wildlife Sanctuary in the state of Vindhya (now Uttar Pradesh) (Rangarajan, 2013). The reintroduction programme was successful and the animals started breeding in the area and the numbers increased to eleven individuals but all the animals disappeared mysteriously by 1965. The fate of these lions was a serious blow to the future of carnivore reintroduction programmes in India.

In 1993, a Population Habitat Viability Analysis (PHVA) was conducted in Baroda, India, to understand the scope of measures required to proliferate the Asiatic lions in the nation (Ashraf et al., 1995; Walker, 2007). The PHVA discussed several environmental, demographic, genetic and social factors that could thwart the long-term survival of the species. During this PHVA two crucial points were discussed, first, the obvious one was the repatriation of Asiatic lions to old habitats across the nation. Second, the committee recommended that a captive management programme be established to create a genetically pure and robust captive population of 400 to 600 animals, which led to the establishment of the Asiatic lion conservation breeding programme in 1995.

The modern conservation breeding programme for Asiatic lions was initiated in 1995 by the Chief Wildlife Warden (CWLW) of Gujarat Forest Department (GFD). These were the salient objectives of the breeding programme as defined by the report of the PHVA workshop (Ashraf et al., 1995).

- The breeding of the species will ensure that all founders contribute equally to the population.
- provisions were made to ensure that new founders could be brought into the captive population to maintain its genetic diversity.
- The aim was to create a genetically viable population of 400-600 individuals with three-generational breeding.
- fourteen animals (Male = 6 and Female = 8) were selected for the first phase of this breeding programme.
- Chennai zoo, Ahmedabad zoo, Kanpur zoo and van vihar national park, Bhopal were initially short-listed to be participant zoos for the programme.

Sakkarbaug Zoological Garden (SZG) is the coordinating zoo for the conservation breeding programme of Asiatic Lions. SZG is the most important *ex-situ* institution that has been intimately involved with the conservation and proliferation of species for over 150 years. Therefore the welfare status of Asiatic lions housed in the CBP must be examined and ways of improving existing housing and management techniques explored.

1.4.3 Overview of the Asiatic lion CBP

The Asiatic lion conservation breeding programme has been running for a quarter of a century. As masters of their fate, and sole caregivers, it is our responsibility to ensure that these Asiatic lions, regardless of their origin or worthiness in the eyes of conservationists, are housed under welfare-oriented management practices that improve their quality of life and increase their chances of post-repatriation survival. My primary objective in this thesis is to improve the welfare status of Asiatic lions and other felids housed in CBPs. Welfare can be improved by removing stressful stimuli or introducing pleasurable ones. Welfare can also be improved by allowing animals the opportunity to experience positive affective states. Asiatic lions if housed in good conditions of welfare and under appropriate husbandry and management practices can retain the repertoire of species-typical behaviour important for survival. This study aims to make the CBP a better place to house Asiatic lions so that we can conserve the behaviour diversity of these captive animals and make them ideal candidates for repatriation and hope that the conservation agencies deem them fit for repatriation to the wild.

1.4.4 Welfare of felids in captivity

Daigle and associates (2015) linked reproductive success with the temperament of captive African lions.

Bashaw et al. (2007) studied the effect of visual barriers on the pacing behaviour of three African lions and two Sumatran tigers and reported significant changes in the levels of stereotypic behaviours across enclosure types. They found significant differences in the behaviour responses between African lions and Sumatran tigers. This adds on to other similar research which suggests that different members of the Felidae family may have different welfare requirements. Suárez et al. (2017) conducted a study on the effects of visitors on multiple captive felids including two Asiatic lions. Here also the researchers found that different species reacted differently to visitor presence. They also reported that the captive felids were mostly affected by the presence of visitors and visitor densities were not correlated with stereotypic behaviours.

Pastorino et al. (2016) studied the effect of personality on the feline-keeper relationships in African lions housed at London zoo. Pastorino et al. (2017) also reported the effect of personality on the response to novel enclosures in four Asiatic lions housed at London Zoo.

(Quirke et al., 2011) reported an improvement of cognitive skills of captive African lions due to enclosure enrichment interventions. Enrichment studies conducted on other species Such as bears (Carlstead et al., 1994; McGowan et al., 2010) suggest that environmental enrichment can improve the reproductive potential and therefore contribute to the Species Survival Plan. Empirical studies have effectively shown that enclosure enrichment help improves the welfare of captive animals. Enclosure enrichment provides animals with the ability to manipulate their captive environment and (Ross, 2006). Skibiel et al. (2007) showed that as a result of enrichment that gave food rewards, Felids tend to decrease pacing and other stereotypical behaviours and showed more natural exploratory behaviours. Young animals growing in conservation breeding centres should be able to learn and hone skills to survive in the wilderness, enrichment devices that help the expression of species-typical behaviours should be a part of the husbandry practices at lion conservation breeding centres (Greenwald et al., 2003). Until now, most enrichment research at zoos focus on the welfare at the species level. Few studies concentrate on the importance of animal personality in husbandry guidelines (Rouff et al., 2005). Personality-based welfare measures can lead to husbandry and enrichment guidelines that account for individual differences in welfare requirements in captive wild animals (Mehta et al., 2008). Research has also shown that personality differences can also affect the health status of different animals. Preliminary studies have been conducted that explore the possibility of how individuals with different personalities access enrichments (Franks et al., 1992). Since lions are social animals, the personality research of these felids is vital and should be a part of husbandry practices to understand the physical and psychological requirements of each species in a captive condition. The research by Watters and Powell (2012) indicate that animal personality research is a vital avenue that needs to be explored for endangered species management in zoos. However such personality-based welfare evaluations have not been reported from Indian zoos. Herborn and colleagues (2010) further suggest that personality studies of animals in captivity may be a good way to study the personality of wild animals, this study will help understand the entire gamut of personality differences in lions. India is home to more than 145 zoos housing more than 50,000 animals and over 23 conservation breeding programmes for endemic and highly endangered species. Indian zoos are yet to adopt data-centric scientific management practices and zoo evaluations continue to be based on the subjective knowledge of evaluators.

1.4.5 Enrichment design for Asiatic lions

Enclosure enrichment should be a part of modern enclosure design and husbandry practices at all zoos and for all captive animals. Enclosure enrichments provide animals with the necessary stimulus to display instinctive behaviours which can help in vast improvements in animal welfare (Kry et al., 2007; Quirke et al., 2011). Quirke and O'Riordan (Quirke et al., 2011) opine that environmental enrichment can be used not only to improve species-typical behaviours but also to break the monotony of exhibits and bring in novelty. (Quirke et al., 2011) has conducted a few enrichment experiments with captive African lions with positive results. Similarly, (Van Metter et al., 2008) suggest that simple enclosure enrichment devices such as Frozen blood balls, ungulate dung, and scented vegetables can introduce sufficient novelty in the enclosure to encourage species-typical behaviour patterns among captive big cats. Enclosure evaluation based on behaviour repertoire of animals in Indian zoos was also conducted by Mallapur and colleagues for leopards (*Panthera pardus fusca*) (Mallapur et al., 2007) and Lion-tailed macaques (*Macaca silenus*) at Chennai zoo (2005). Powell studied the effects of enrichment interventions on the welfare parameters of African lions (1995). Mellen suggests an integrated enrichment intervention is necessary to maintain the novelty of the enclosure environment (1997). Similarly, Gartner et al (2016) reported that the personality measures had a significant relationship with subjective wellbeing in captive African lions, snow leopard and jungle cats. In the last few decades, a large number of zoo-based research has focused on the effect of enclosure enrichment on animal welfare. Unfortunately, similar studies are few and far apart in the Indian context. Indian zoos account for the largest number of tiger, leopard and Asiatic lions housed in captive facilities and yet there is little empirical research reported on the effect of these enrichment interventions on the welfare of these animals.

1.5 Justification of the study

In this thesis, I have asked some simple questions about the welfare status of Asiatic lions, the external and internal factors that may influence them and explored ways to improve incumbent management practices. Indian *ex-situ* institutions are currently involved in the CBP of more than 25 endangered endemic species. The metric for the success of a CBP continues to be surrogates of genetic diversity and the demographic parameters of the captive population. There is an urgent need to turn our attention to the importance of welfare assessments and individual-centric management practices at CBPs (Kaumanns et al., 2015). I hope that my findings can help start the conversation about the welfare status of animals housed at conservation breeding programmes.

- What is the effect of visitor presence and enclosure complexity on the welfare of Asiatic lions?

- Does the personality trait and rearing-history play a role in determining the welfare of Asiatic lions?

- Can enrichment interventions improve welfare and encourage positive affective states in Asiatic lions?

I framed the questions with the hope that their answers, would be species-agnostic, and maybe able to highlight some focus areas for animal management practices at CBP. Animal welfare is a growing point of concern for most *ex-situ* institutions managing wild animals. Especially since, modern zoos have a mandate to maintain wild animals in captivity to further the cause of biodiversity conservation through research, visitor education and actual *ex-situ* conservation breeding practices. Since the 1950s, there has been a surge in the amount of research on the welfare of captive animals in the livestock industry and on-display enclosures at zoos. However, CBPs are different from a traditional zoo enclosure, since animals are not exposed to visitors in such institutions.

Compared to on-display enclosures, conservation breeding centres are built on a different set of principles and require special attention in terms of welfare-based management practices. This thesis is primarily concerned with the welfare of Asiatic lions at Indian *ex-situ* CBP.

1.6 Organization of the thesis

This thesis is organised into five chapters.

- Chapter 1: I have discussed a chronology of animal welfare science and its relationship with zoo biology. I provide a conceptual framework for this thesis and justify the importance of the questions asked in the thesis.
- Chapter 2: I discuss in depth the differences in enclosure attributes between the different enclosure types of SZG and their effects on the welfare of Asiatic lions. I also explore the effect of visitor presence on the activity budget and welfare measures of Asiatic lions.
- Chapter 3: I explore the effect of inter-individual differences on the behavioural and physiological welfare of Asiatic lions. Intraspecific variations in welfare indices as a result of individuality is discussed in detail (Goswami et al., 2020).
- Chapter 4: I present the result of a combined enrichment intervention on the welfare of Asiatic lions housed in the conservation breeding programme (Goswami et al., 2021).
- Chapter 5: I discuss the conservation implications of my findings in the context of *ex-situ* conservation breeding programmes and their incumbent management practices.

2

Role of enclosure complexity and visitor presence on Asiatic lion welfare

2.1 Introduction

Animal welfare should be one of the primary priorities at *ex-situ* institutions like zoos and conservation breeding programmes (CBP) (Rabin, 2003). Since enclosures act as the surrogate for a naturalistic environment for captive animals, their complexity and the ability to provide a welfare-optimized habitat to housed animals must be carefully evaluated (Healy et al., 2000). Enclosure design and husbandry practices are arguably the strongest determinants of the welfare of captive animals (Marshall et al., 2016; Melfi, 2009). Ross et al. (2009) found that animal behaviour and enclosure use can be a good basis for designing species-appropriate enclosures. However, enclosure design and husbandry practices are seldom modified to cater to the needs of individuals and are often standardized based on the group of animals housed in them. Most zoos have similar husbandry practices for all species of large felids, irrespective of their species-specific and individual welfare requirement. On the other hand, some zoos house multiple individuals of the same species in enclosures with largely varying levels of complexity and visitor intrusions that often lead to differential welfare outcomes. Most studies confirm that designing an ideal captive environment for long-ranging carnivores poses unique challenges (Mallapur, Sinha, et al., 2005; Marinath et al., 2019; Veasey, 2020). Existing literature unanimously agrees that a complex enclosure is a key to ensuring optimum welfare and cognitive functioning of captive animals (Fàbregas et al., 2012; Marshall et al., 2016).

Curiously, although the effect of visitors on the behaviour and welfare of zoo-housed animals has been explored extensively and yet opinions are divided whether visitor presence act as an enrichment or a stressor (Table 2.1). Despite the large corpus of work on the subject, findings and opinions seem to differ with most claiming visitor presence as a type of a stressor (Glatston et al., 1984; Mallapur, Sinha, et al., 2005; Sekar et al., 2008) ,some claiming that visitor presence does not affect animal welfare (Farrand et al., 2014; O'Donovan et al., 1993), while others proclaiming it to be a form of novel enrichment (Fernandez et al., 2021a; Hosey, 2000; Marinath et al., 2019). Most studies I reviewed claim visitor presence as the single most stressful characteristic of the life of zoo animals (Davey, 2007; Mallapur, Sinha, et al., 2005). Chamove et al. (1988) reported that the presence of visitors excites captive primates (in a negative manner), which led to an increase in aggression and attention to visitors, and often resulted in stereotyped behaviour, this was confirmed by later captive primate studies (Hosey et al., 2016; Mallapur, Sinha, et al., 2005). ()found that construction near the paddock area of felids enclosures led to a significant increase in pacing behaviours. Some other studies advise us to tread cautiously about our perceptions of visitor presence by tinting their conclusions with a fair bit of ambivalence (Birke, 2002; Farrand, 2007). At the other end of the spectrum, I also found certain studies that promoted public feeding and forced visitor interaction of elephants as a form of enrichment (Fernandez et al., 2021a). I found it concerning that zoos (Fernandez et al., 2021a)by exalting public feeding as enriching might be inadvertently sending a wrong conservation message leading to a rise in human-wildlife conflict. The literature seems to suggest that some species are more likely to be negatively affected by visitor presence. Suárez et al. conducted a study on the visitor effect on multiple felid species including two Asiatic lions, where they found that visitor presence led to a reduction in a usable enclosure space in all study subjects, along with a reduction in complex species-typical behaviours. Interestingly, they found no correlation between visitor density and animal activity. I also found some studies that made an initial attempt to link visitor levels with enclosure complexity. Bashaw and associates (Bashaw et al., 2007) conducted a study to understand the effects of enclosure complexity and the effects of visual barriers on the stereotypy of Sumatran tigers and African lions. They reported that lions showed less stereotypy in a large and complex on-display enclosure as compared to a smaller less complex off-display enclosure.

Mallapur and colleagues approached the effects of visitors presence and enclosure complexity in the context of the welfare of Indian leopards and Lion-tailed macaques at Indian zoos (2002; 2005). However, Mallapur kept enclosure complexity (2002) separate from visitor presence (2005) and did not explore the synergistic effect of both features on the welfare outcomes of the study species. Most researchers agree that a complex stimulating environment that encourages the expression of species-typical behaviours is ideal for housing most species. Bashaw et al. (2007) found that the activity patterns of the same animal varied as they were moved to a different enclosure. Recent studies have also started to recommend enrichment as a way to bring novelty to the captive environment to supplement welfare requirements (Lyons et al., 1997; Mallapur & Chellam, 2002; Mellen et al., 1997). Based on the literature survey (Table 2.1), I surmised that the relationship between enclosure complexity and visitor effect has not been addressed in its entirety. First, most studies on the subject dealt with small sample sizes, which is usually the case with most zoo-based studies. Second, many of the studies focused primarily on the negative indicators of welfare such as stereotypy and faecal corticosterone levels, but none mentioned the effects of visitors on positive indicators like behaviour diversity. Third, existing studies did not address enclosure complexity when comparing between on-display and off-display paddock areas (Bashaw et al., 2007).

Here, I try to contextualize the question of whether visitor presence is truly harmful to the welfare of felids in captivity? Since most studies on the effects of enclosure complexity and visitor -presence have primarily focused on the negative indicators of welfare viz., aggression, self-harm, and stereotypy. I have provided a more holistic view of the situation by also highlighting the effects of the visitors and enclosure on the behaviour diversity and enclosure usage patterns.

Vaz et al. (2017) reported the effect of visitor presence and other enclosure attributes on the physiological and behavioural status of tigers and leopards across several Indian zoos. The behavioural attribute of choice in the most welfare-related paper focuses on stereotypic behaviour, which along with faecal corticosteroid metabolites are negative indicators of welfare and therefore should be complemented with other positive welfare indicators like behaviour diversity, and enclosure space usage patterns (Schildkraut, 2016). In this chapter, I hope to address the effects of enclosure attributes and visitor presence on the behavioural welfare indicators of Asiatic lions housed in three enclosure types. Our results will probably restart the conversation about the importance of enclosure complexity and species-specific design for the welfare of housed animals.

Sakkarbaug Zoological Garden (SZG) has two on-display enclosures, in which animals are confined in small and sterile enclosures for public viewing. There are four naturalistic zoo safari enclosures, where the lions are kept in vast naturalistic enclosures with little visitor disturbance. Finally, there are 17 off-display CBC enclosures, which are vast naturalistic enclosures completely free of interference. In this chapter, I demonstrate the differences in activity patterns and welfare indices of captive Asiatic lions exposed to three levels of enclosure complexity and visitor exposure (high, low, none).

In this chapter, I explore the effects of visitors and enclosure complexity as external determinants of welfare.

Since Asiatic lions are housed at several zoos and *ex-situ* institutions across the world, our findings might help zoo managers find the optimum levels of enclosure complexity and visitor presence for the best welfare outcomes for the species.

2.2 Objectives

I tested the following hypothesis on the test subjects

1. Does the behavioural welfare indices of Asiatic lions vary based on enclosure complexity?

2. Does the welfare indices and activity budgets of lions vary based on visitor presence?

Table 2.1: Literature review on the welfare outcomes of visitor presence on captive animals

Title	Species	Welfare effect	Visitor effect	Authors
The influence of the zoo environment on social behavior of groups of cotton-topped tamarins, Saguinus oedipus oedipus	Cotton-top tamarins	Decrease in affiliative beahviours, increase in stereotypy, aggression	Negative effect	Glatston et al., 1984
The influence of zoo visitors on the behaviour of captive primates	Primates	activity levels increased, visitor interaction increased	Positive	Hosey & Druck, 1987
Visitors excite primates in zoos	Primates	Increased activity, attention to visitors and stereotypy	Stressful	Chamove et al., 1988
Effects of visitors and cage changes on the behaviours of mangabeys	Mangabeys	Increased locomotion and aggression	Negative effect	Herring et al., 1991
Effect of visitors on the behaviour of female cheetahs Acinonyx jubutus and cubs	Cheetahs	No effects	No effect	O'Donovan et al., 1993
Effects of browse, human visitors and noise on the behaviour of captive orangutans	Orangutans	Stereotypy	Stressful	Birke, 2002
Influence of visitor presence on the behaviour of captive lion-tailed macaques (Macaca silenus) housed in Indian zoos	Lion-tailed macaque	Increase in stereotypy	Stressful	Mallapur et al., 2005
Environmental effects on the behavior of zoo-housed lions and tigers, with a case study on the effects of a visual barrier on pacing	Felids	Positive effect	Unknown	Bashaw et al., 2007
Impact of zoo visitors on the fecal cortisol levels and behavior of an endangered species: Indian blackbuck (Antelope cervicapra L.	Indian blackbuck	Avoidance	Stressful	Rajagopal et al., 2011
Behavioural and physiological responses in felids to exhibit construction	Felids	Increase in stereotypy, Sustained increase in faecal glucocorticoid metabolite levels	Stressful	Chosy et al., 2014

Table 2.1: Literature review on the welfare outcomes of visitor presence on captive animals *(continued)*

Title	Species	Welfare effect	Visitor effect	Authors
The visitor effect in petting zoo-housed animals: Aversive or enriching?	Goats and Llama	avoidance, aggression	No effect	Farrand et al., 2014
Zoo visitor effect on mammal behaviour: Does noise matter?	Different mammal species	Increase in vigilance, negative social behaviour	Stressful	Quadros et al., 2014
Is wounding aggression in zoo-housed chimpanzees and ring-tailed lemurs related to zoo visitor numbers?	Ring-tailed lemur & Chimpanzee	Aggression	Stressful	Hosey et al., 2016
A comparison of zoo animal behavior in the presence of familiar and unfamiliar people	African elephant, Rothschild's Giraffes, Brazilian Tapir, Slender-tailed meerkat	Avoidance	No detrimental effect of unfamiliar keeper	Martin and Melfi, 2016
Behaviour and welfare: the visitor effect in captive felids	Asiatic lion, Bobcat, ocelot, Jaguar	Reduction in sterotypy	Negative effect	Suarez et al., 2017
Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos	Bengal tigers and Indian leopards	Increase in stereotypy and FGM levels	Negative effect	Vaz etal., 2017
Effect of Covering a Visitor Viewing Area Window on the Behaviour of Zoo-Housed Little Penguins (*Eudyptula minor*)	Penguins	Fear-provoking, avoidance	Stressful	Chiew et al., 2020
Can Zoos Ever Be Big Enough for Large Wild Animals? A Review Using an Expert Panel Assessment of the Psychological Priorities of the Amur Tiger (Panthera tigris altaica) as a Model Species	Amur tiger	Stereotypy	Negative effect	Veasey et al., 2020
Effects of the presence of zoo visitors on zoo-housed little penguins ('*Eudyptula minor*')	Penguins	Fear-provoking, avoidance	Negative	Chiew et al., 2021
Environmental influences on stereotypy and the activity budget of Indian leopards (Panthera pardus) in four zoos in Southern India	Leopard (Panthera pardus)	Stereotypy	Stressful	Mallapur et al., 2005

Table 2.1: Literature review on the welfare outcomes of visitor presence on captive animals *(continued)*

Title	Species	Welfare effect	Visitor effect	Authors
Drivers of stereotypic behaviour and physiological stress among captive jungle cat (Felis chaus Schreber, 1777) in India	Jungle cat	Increase in inactive behaviour. stereotype, heightened FGM levels	Stressful	Marinath et al., 2019
Public Feeding Interactions as Enrichment for Three Zoo-Housed Elephants	Asian elephants and African elephants	Reduction in sterotypy	Positive outcome	Fernandez et al., 2021

2.3 Methodology

My objective was to understand the impacts of enclosure complexity and visitor disturbance on the activity patterns and welfare status of Asiatic lions. I had access to 23 different enclosures housing all the study subjects. Since some of the enclosures were on display and most were part of the conservation breeding programme the amount of complexity were likely to vary across different enclosures. I devised a method to score the enclosures based on the species-appropriate complexity levels in their designs. I modified a pre-existing enclosure complexity scoring rubric to measure the species-appropriateness of the Asiatic lion enclosures (Goswami et al., 2016). In the next step, I conducted animal behaviour recordings at all enclosures to understand the welfare status of lions housed at those enclosures.

2.3.1 Study area

Sakkarbaug zoological garden (SZG) has three types of Asiatic lion enclosures that represent three levels of visitor presence, first the on-display enclosures(N = 2), which are open to the public six days of the week. Second, the zoo-safari enclosures (N = 4) are accessible to visitors who pay for the safari bus ride. In this enclosure, visitor presence is tempered by the fact that visitors are not allowed to disembark from the vehicle and the bus stops at each enclosure for approximately three minutes. Finally, the off-display conservation breeding enclosures (N = 17), which do not expose Asiatic lions to any visitor presence. Given the distinct differences in visitor exposure levels, and a large number of subjects, which is a rarity in most zoo-based studies, this facility provided us with the ideal opportunity to test our hypotheses.

2.3.2 Subjects and housing

I observed the behavioural responses of 41 Asiatic lions to three types of enclosures in terms of complexity during visitor-presence and visitor-absent conditions. Six lions (M = 3, F = 3) were housed at two "on-display" enclosures while 10 lions (M = 4, F = 6) were housed at four large naturalistic "zoo-safari" enclosures, and 25 (M= 10, F= 15)

Figure 2.1: Moated enclosure at the zoo safari area

Figure 2.2: On-display barred enclosure at SZG

Figure 2.3: Off-display chain-link enclosure at SZG

lions were housed in chain-linked "Off-display" enclosures at the same off-display area. The animals housed in the off-display area were a part of the conservation breeding programme for the species. . All subjects were housed in Sakkarbaug zoological park, Junagadh, India. 35 subjects were housed in the conservation breeding centre of SZG.

On-display enclosures

Sakkarbaug zoo has two on-display enclosures for Asiatic lions adjacent to one another (Figure 2.2). These enclosures along with two tiger and two leopard enclosures constitute the large cat complex for the zoo. These enclosures are virtually unchanged since the zoo was built under the patronage of the erstwhile nawabs of Junagadh in 1863. These enclosures conform to the menagerie style of zoo-keeping and are extremely limiting in terms of space for a large animal like the Asiatic lions. Each enclosure at these complexes, houses three individuals each, with only a pair allowed in the paddock area daily. Measuring at 100 m^2, these enclosures are insufficient to house a single lion and certainly does not provide for the welfare requirement of a pair of Asiatic lions.

Off-display enclosure CBC

Sakkarbaug zoo has a conservation breeding programme for Asiatic lions situated at a off-display site contiguous to the main zoo (Figure 2.3). There are 17 off-display enclosures, that house 25 Asiatic lions at these off-display enclosures. These off-display enclosures range from 1000 m^2 to 5600 m^2 in size and provide ample space for Asiatic lions to display species-typical behaviours. Moreover, these enclosures are completely off-display, therefore the lions housed in this facility are not exposed to the visitors.

Zoo-safari enclosures

There are four enclosures at the zoo safari area that house 10 Asiatic lions (M=4, F=6) (Figure 2.1). At the CBC 4 enclosures falls on the access road of the zoo safari and can be viewed by the visitors from the confines of the safari bus. Visitors are not allowed to get down from the bus and the bus usually spends two to three minutes act each enclosure before it leaves. Visitors are actively discouraged from harassing the animals, during the visit. The zoo-safari tour starts at 11 am and continues till 4 pm every day of the week except Wednesday when the zoo is closed for all visitors. The enclosures are large and naturalistic, ranging between 1000 m^2 to 1500 m^2 in size for a pair or group of Asiatic lions. These enclosures have a moated design as compared to the chain-link fence barriers found at the off-display enclosures. The Lions housed at the zoo safari enclosures are exposed to a certain level of visitor presence, which is imperceptible when compared to that of the levels experienced at the on-display enclosures.

2.3.3 Study design

The enclosure complexity scoring system was based on the species-appropriate requirement for Asiatic lions (Goswami et al., 2016). The complexity scoring system (Table 2.2) was broadly divided into three sections viz., Enclosure features and husbandry regimen. Enclosure specific attributes that contribute to the welfare of animals viz., withdrawal space, vegetation feature, size of the enclosure, type of barrier were evaluated. Husbandry regimen included questions about daily practices of the keepers and management that were likely to affect the welfare of animals viz., social grouping, feeding practices, enrichment schedule. Keepers and zoo managers were interviewed to understand the species-appropriateness of the measures and based on the interview, two interviewers rated each enclosure on a scale of 1-5 on every attribute. I only accepted entries where both interviewers provided the same rating.

For behaviour data collection, I used pre-existing ethograms for felids (Powell, 1995; Stanton et al., 2015) and modified them to include unique behaviours displayed by subjects (Table 2.3). At the onset of the study, I conducted *ad-libitum* sampling to create a detailed ethogram, which was used to standardize the data collection protocols between multiple observers (Table 2.3). We recorded 46 unique behavioural states that were grouped into three broad classes, viz., rest behaviours, typical behaviours (active), and aberrant repetitive behaviours (Table 2.3). We categorized observation days into "visitor days" and "no-visitor" days. The zoo is closed on Wednesday, hence I had more visitor days (N = 70 days) compared to non-visitor days (N = 14 days). We employed a double observer method to validate the behaviour recordings at the beginning of the study to reduce observer bias. Due to a large number of enclosures and only two observers, we relied on gathering some observations through video recordings To minimize inter-observer bias, behaviour recording was commenced after inter-observer reliability reached satisfactory levels from the same group of animals (Cronbach's alpha >0.9) (Caro et al., 1979; Gliem et al., 2003). I recorded ten, one hour-long behaviour observation sessions (for four subjects) and found one-minute instantaneous scans (Altmann (1974); Martin et al. (1993); Amato et al. (2013)) were comparable to focal animal behaviour observation data (Altmann, 1974; Amato et al., 2013; Gilby et al., 2010) in recording behavioural states and events for multiple subjects. We used instantaneous scan sampling at 1-minute intervals to record the behaviour of Asiatic lions from on-display (N = 6 lions, housed in two enclosures), Zoo-safari (N = 10 lions, housed in four enclosures) and off-display (N = 35 animals housed in 17 enclosures) enclosures between March to June 2016. Behavioural data were collected during three-hour blocks between 0700-1000, 1000-1300, 1300-1600, 1600-1900, and 1500-1700 hours. 15 minutes of rest was provisioned to mitigate observer fatigue after every 45 minutes of behaviour recording. This 15 minutes (one hour per observation block) of data was recovered from video footage. During each scan, we recorded the behavioural state of the subject and its location in the enclosure. All occurrences of behaviour events were recorded separately. I used the frequencies of behavioural states and events to measure the behaviour diversity of each subject during an observation session (one hour).

Enclosure complexity

I designed a enclosure complexity rating system based on Goswami et al. (2016). The rating system included four broad categories with subcategories that included enclosure features pertaining to welfare in animal husbandry and enclosure design that could be scored 1-5 (5 being for features that are most species-appropriate). Table 2.2 lists all the enclosure attributes that were used to evaluate the enclosures (N = 23). The enclosures at the conservation breeding centre were distinctly different compared to the on-display disclosures that were based on a menagerie design dating back to the late nineteenth century when the Sakkarbaug zoo was founded in 1863. There are predominantly three types of enclosures for Asiatic lions at Sakkarbaug zoo.

Table 2.2: Complexity rating rubric for Asiatic lion enclosures

Sl no	Attributes	Description
Enclosure features		
1	Barrier safety	Does the enclosure barrier pose a safety hazard to the animals?
2	Area for animal actvity	Does the enclosure provide enough space for the animal to exhibit species-typical behaviour patterns?
3	Substrate type	Does the enclosure substrate provide natural materials that promote natural behaviours?
4	Terrain complexity	Does the enclosure have a complex terrain structure or landscaping that includes, viz., caves and vantage points that allow animals to exhibit natural behaviour patterns?
5	Shelter	Does the enclosure provide shelter to the animals from inclement weather?
6	Aspect	Does the enclosure provide animals equitable opportunities to thermoregulate?
7	Withdrawal spaces	Are there sufficient withdrawal zones within the enclosure to counter visitor disturbance in all animals?
8	Vegetation	Does the enclosure have adequate species-appropriate vegetation that can promote species-typical behaviours in housed animals?

Table 2.2: Complexity rating rubric for Asiatic lion enclosures *(continued)*

Sl no	Attributes	Description
9	Enrichment	Does the enclosure provide species-appropriate enrichment for all animals with the aim of encouraging species-typical behaviours?
10	Furniture	Does the enclosure provide furniture such as vantage points, water pool, that appropriate for the species and provide opportunity for natural behaviour repertoire
Social features		
11	Inter-individual distance	Does the enclosure provide enough space for all animals to coexist?
12	Social grouping	Is the social grouping appropriate or unnatural due to iso-sexual pairing of unrelated individuals?
13	View beyond enclosure	Do the animals have the ability to view beyond the enclosure through vantage points?
Feeding practices		
14	Feed availability	Is feed provided consistently and periodically to all subjects based on their body weight and biological requirement?
15	Feeding practice	Does the husbandry practices incorporate species appropriate feeding practices that encourage natural behaviour?
16	Availability of drinking water	Does the enclosure provide animals with safe clean drinking water throughout the day?
Retiring area		
17	Ventilation	Does the Retiring area provide natural ventillation to the species
18	Illumination	Is the retiring area deisgned to receive natural illumination at all times of the day?
19	Enrichment	Does the retiring area provide animals with enrichment devices to promote natural behaviours

Table 2.3: Ethogram of captive Asiatic lions

Sl no	Behaviour name	Category	Description
1	Sphinx	Rest	The animal sits like a sphinx
2	Lying down	Rest	The animal lies on its side
3	Belly up	Rest	The animal resting in its back with legs pointing upwards
4	Sit	Rest	The animal sits on its haunches
5	Stand	Rest	Animal standing and doing nothing else
6	fixate	Typical	Looking at an object and staring at it with intent and reacts as soon as the object moves
7	Eating	Typical	Eating food material and ingesting the same
8	Drink	Typical	Drinking water
9	Clearing hide	Typical	Clearing the fur from the hide of food item
10	Chew	Typical	Chewing Bones
11	Walk	Typical	Animal walking straight with head at slight upward angle to the rest of the body
12	Stalk	Typical	Animal crouching or walking with slow calculated steps, head at a lower angle than the body. Note down the subject stalked
13	Charge	Typical	Animal shows a sudden burst in pace and runs upto a short distance to a certain object, individual, with tail swaying above the body line, note down the subject
14	Chase	Typical	Animal chasing object, another animal in a fast paced manner, here also note down the subject chased
15	Solicit Play	Typical	Animal runs or walks up to another animal and tries to play by rolling in front of them or pawing their
16	Nuzzling	Typical	Rub nose and head with a conspecific
17	Play	Typical	Animal showing different types of playful behaviour
18	Mount	Typical	Animal mounting a conspecific
19	Neckbite	Typical	Male bites the scruff of the female while copulating
20	Roll over	Typical	Female rolls on the ground, an act preceded by copulation
21	Yawn	Typical	Animal yawns
22	Scentmark	Typical	1. Scentmark a tree or a vertical object with perianal glands 2. scent mark on ground and then scratch with hind legs.
23	Rubbing mane	Typical	Animal rubs mane or body against a scent-marked area or against a conspecific
24	Sniff	Typical	Animal sniffing object, conspecific, air
25	Flehmen	Typical	Wrinkled face, upper lip curled in a grimace
26	Cough	Typical	Animal coughs

Table 2.3: Ethogram of captive Asiatic lions *(continued)*

Sl no	Behaviour name	Category	Description
27	Aggression	Typical	Animal shows aggression towards enclosure mates, keepers, and other lions visible through enclosure barriers, charges up a short distance growls, with tail swaying from side to side. The recipient if in physical contact with the aggressor either fights back or lies on its back and bares teeth. Usual injuries to haunches, face, shoulders in recipients.
28	Dig	Typical	Trying to dig ground, leaves, enclosure substrate
29	Allogrooming	Typical	Animal licking the head and body of conspecifics
30	Autogrooming	Typical	Animal licking its paws and geinitals
31	paw	Typical	Animal inspecting an object or conspecific with jabs of the front paw
32	Scratch	Typical	Animal scratching post or tree
33	Bite	Typical	Animal mouthing an object
34	Chew	Typical	Animal intently and purposefully chewing a twig or a foreign object
35	Suffocate	Typical	Animal grabs any long object like a branch or enrichment device and holds it down with the paws and bites on it as if to suffocate it.
36	Neckbite	Typical	Animal Playfully bites the neck of the conspecific and tries to bring them down
37	Paw-swipe	Typical	Animal playfully swipes the hind leg of the conspecific
38	Initiate-call	Typical	Animal starts to vocalize
39	Response_vocalization	Typical	Animal responds to the vocalization of another conspecific
40	Growl	Typical	Animal makes a low rumbling noise lasting for a short duration
41	Aggressive growl	Typical	Loud growl followed by an immediate charge
42	Copulation growl	Typical	Vocalization made by males and females while copulating
43	Pacing	ARB	Animal walks up and down a fixed path occasionally raising its head up and fixed points
44	Rubbing nose	ARB	Animal rubs its nose against a wall or a chainlink barrier that leaves a distinct black mark on the snout
45	Swaying	ARB	Animal stands facing a wall or the barrier and shifts its weight from one leg to the other
46	Body rub	ARB	Animal rubs body at the same area that leaves a mark on the wall or enclosure barrier. Often results in hair loss in certain parts of the body.

Behaviour diversity

Behaviour diversity is indicative of the scope of novelty, and complexity afforded to animals in captivity (Haskell et al., 2018; Wemelsfelder et al., 2000). Maintaining behaviour diversity of captive animals housed at breeding programmes is essential for the preservation of essential learned behaviours required for post-release survival (Rabin, 2003). Complex and cognitively enriching enclosures have been shown to stimulate captive animals to display a diverse behaviour repertoire (Spiezio et al., 2018). I used the Shannon-Weiner diversity index (SWI) to measure species-typical behaviour diversity as this approach considers both richness and evenness of species-typical behaviours in the data set (Clark et al., 2012; Razal et al., 2016; Spiezio et al., 2018).

$$Behaviour - diversity = \sum p_i * \log p_i \}(2.1)$$

p_i = Proportion of time spent in each species-typical behaviour

I compiled an ethogram of all behaviour states and events observed from all subjects during the study period (Table 2.3). I pooled all behaviour observations of each subject to calculate behaviour diversity. I excluded aberrant repetitive behaviours from the calculations since they did not qualify as species-typical behaviours.

Enclosure use

Enclosure usage patterns can indicate the species-appropriateness of the captive environment (Suárez et al., 2017; Williams et al., 2021). I divided every enclosure area into nine equal zones with three broad zones proximal, median and distal zone that were subdivided into left, middle and right zones (Figure 2.4). A tenth zone was added which was the area next to the entrance to the retiring cells. During each instantaneous scan, I recorded the location of each animal in the enclosure. At all enclosures, observers and visitors only had access (visual) to the animals from the proximal zones, while the keepers could access the enclosures through the median and distal zones when needed. I used a modified SPI calculation as proposed by Plowman (2003).

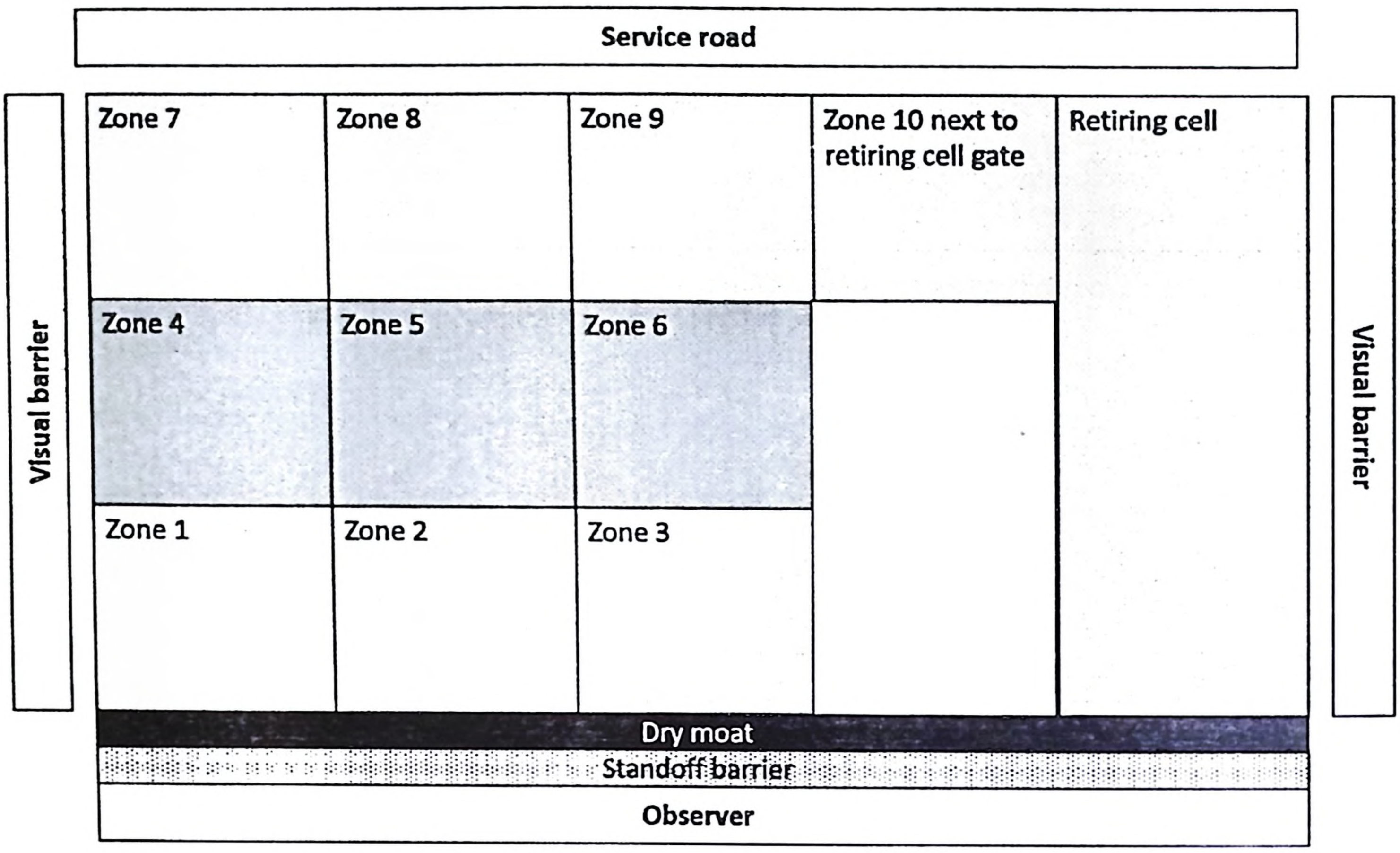

Figure 2.4: Enclosure zonation for usage assessment

$$SPI = \frac{\sum |f_o - f_e|}{2(N - f_{emin})} \tag{2.2}$$

In this equation f_o is the observed frequency of observations at a given zone. f_e the expected frequency of observations at that enclosure zone. N is the total number of observations and f_{emin} is the expected frequency of observation at the smallest zone. This is a departure from the previous formula for SPI calculation which expects equal-sized zones.

Aberrant repetitive behaviours

Aberrant repetitive behaviours (ARB) are reliable measures of poor welfare conditions (Dawkins, 2004; Kroshko et al., 2016; Watters et al., 2009) as they are precursors of cognitive dysfunction and neurophysiological changes (Muehlmann et al., 2012). I measured the proportion of scans spent by each subject performing ARBs as an indicator of poor welfare (Mason et al., 2004). ARBs mostly included, stereotypic swaying, pacing, and nose rubbing behaviours (Table 2.3). I did not record anticipatory displacement behaviours, for example, pacing before feeding or during interaction with conspecifics as ARB. I considered behaviours persisting over five consecutive scans and without an observer-discernible cause as ARB. Therefore, displacement behaviours performed before feeding time, or in response to keeper activities were not considered as aberrant repetitive behaviours.

2.3.4 Data analysis

Behaviour observations collected from all subjects were collated and then grouped based on their housing conditions (i.e. enclosure type, visitor presence), an hour of observation, period of observation, date of observation, and animal details (i.e., ID, sex, age-class).

I measured the welfare indices (viz., behaviour diversity, SPI, ARB) of test subjects during each observation period (viz.,0700-0900, 0900-1100, 1100-1300, 1300-1500, 1500-1700, and 1700-1900 hours) across multiple observation days (45 $\pm$ 4.4 days per subject) which led to the accumulation of 383,132 scans of behaviour data.

I used Kruskal-Wallis analysis of variance (non-parametric) to measure the differences in activity budget and other welfare indices (behaviour diversity and enclosure usage) across enclosure types and visitor levels. If I found significant differences at an alpha of 95%, I conducted a pairwise Dunn's test on the welfare indices and activity budget data. I used R ver 4.3 for statistical analysis and Rstudio as the integrated development environment(IDE) (Team, 2020). I primarily used the tidyverse packages for data wrangling (Wickham et al., 2017).

2.4 Results

2.4.1 Enclosure complexity

Enclosure attributes

On-display enclosures had the lowest complexity scores on all complexity parameters as compared to the off-display and zoo-safari enclosures. While the off-display and zoo safari enclosures scored close 2.57 on enclosure features, the on-display enclosures scored one (Figure 2.5). Feeding practices were similar across all enclosure types and therefore all enclosures scored around three on these parameters. The on-display enclosures did not provide any access today kraals and were limited in their ventilation and illumination and sanitation and hence scored the lowest. The off-display enclosures had well-designed retiring cells ubiquitous across modern Indian zoos, which although better than the on-display enclosures, still lacked species-appropriate features and hence scored approximately 2.5 on these parameters. In terms of social grouping, all enclosures housed lions in either paired or group-housed configurations. However, the inter-individual space was close to 50 m^2/lion at the on-display enclosure as opposed to 750 m^2/animal at the off-display and zoo-safari enclosures. Therefore the off-display enclosures scored (3.59-3.82) on these parameters. The low score of the on-display enclosures could be attributed to the lack of space, old enclosure design that was unchanged since the inception of the zoo in 1863. On the other hand, the off-display and zoo-safari enclosures were of a modern design, provided enough space for the expression of species-typical activity patterns and allowed for pair and group-housed Asiatic lions without overcrowding the enclosures.

A detailed comparison across the enclosure features is given below.

Enclosure barrier:

Enclosure barriers should be specifically designed to impart naturalism to the paddock area and reduce the chances of human imprinting. A good enclosure barrier should effectively limit any chance of physical interaction between animal and man and thereby create a safe withdrawal space for both. In a conservation breeding centre, it is more important to create this disconnect so that animals have a safe withdrawal distance from their human caregivers and may have the opportunity to display instinctive behaviours. The On-display enclosures were of the barred type, with a stand-off barrier on the visitor-side. The distance between the stand-off barrier and the enclosure barrier was less than 5ft which allowed visitors to reach out to the animals. This is an unsafe enclosure barrier design and must be changed. I rated both the on-display enclosures the

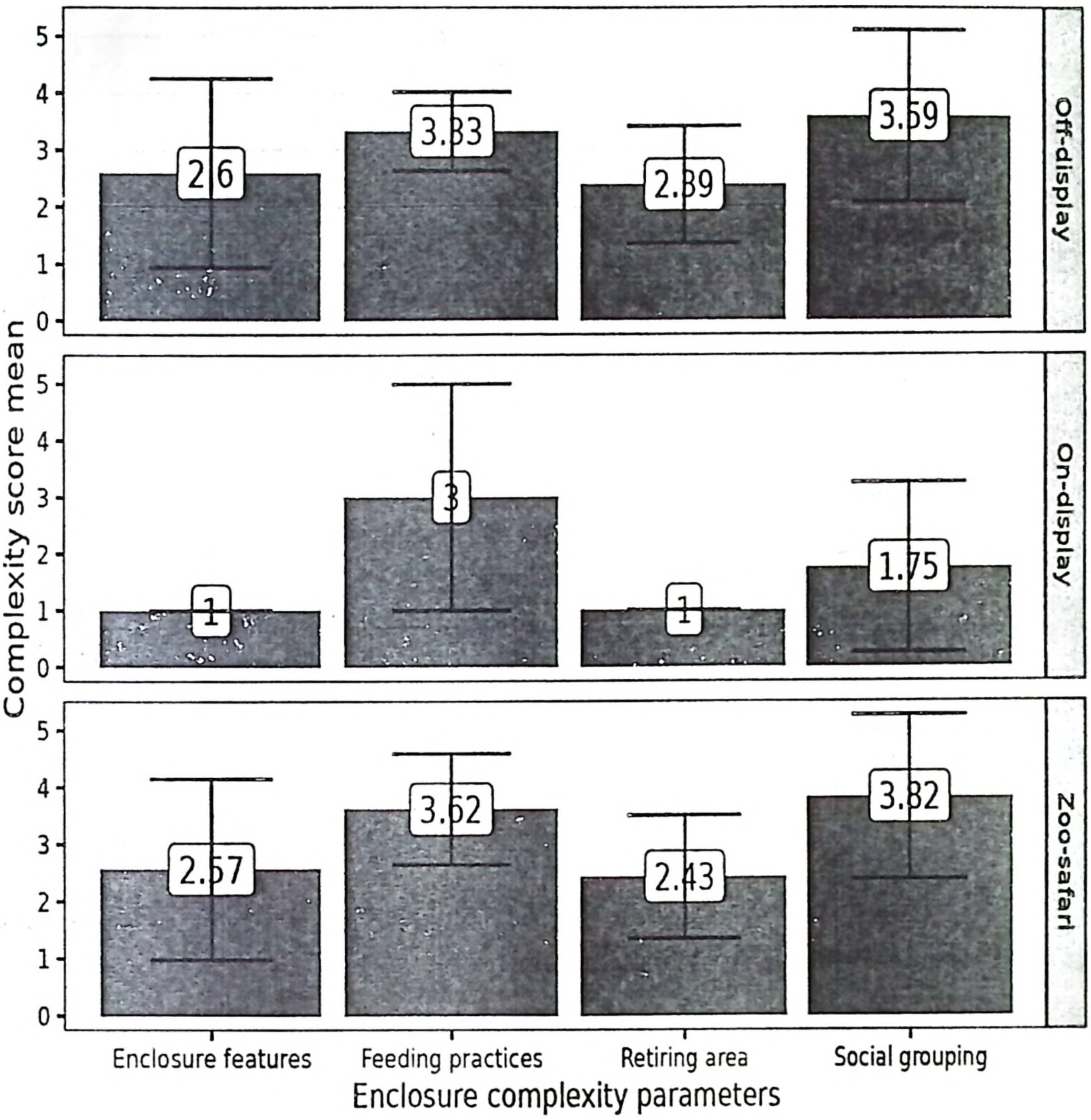

Figure 2.5: Complexity rating comparison of Asiatic lion enclosures

lowest score of 1 on enclosure barrier safety. The zoo-safari enclosures had a dry moat (v-shaped) primary barrier, which was augmented by a stand-off barrier and a hedge and a parapet wall, which is ideal for felids as per the CZA guidelines (Gupta, 2008). I gave a score of four to the Zoo-safari enclosures. Most off-display enclosures used chain-link fences as the only barrier. There were no stand-off barriers or visual barriers from the keeper's gallery, which created chances of imprinting. More importantly, at some parts of the CBC, the keeper gallery was sandwiched on both sides with a chain-link fence of two adjacent enclosures, which is a safety hazard.

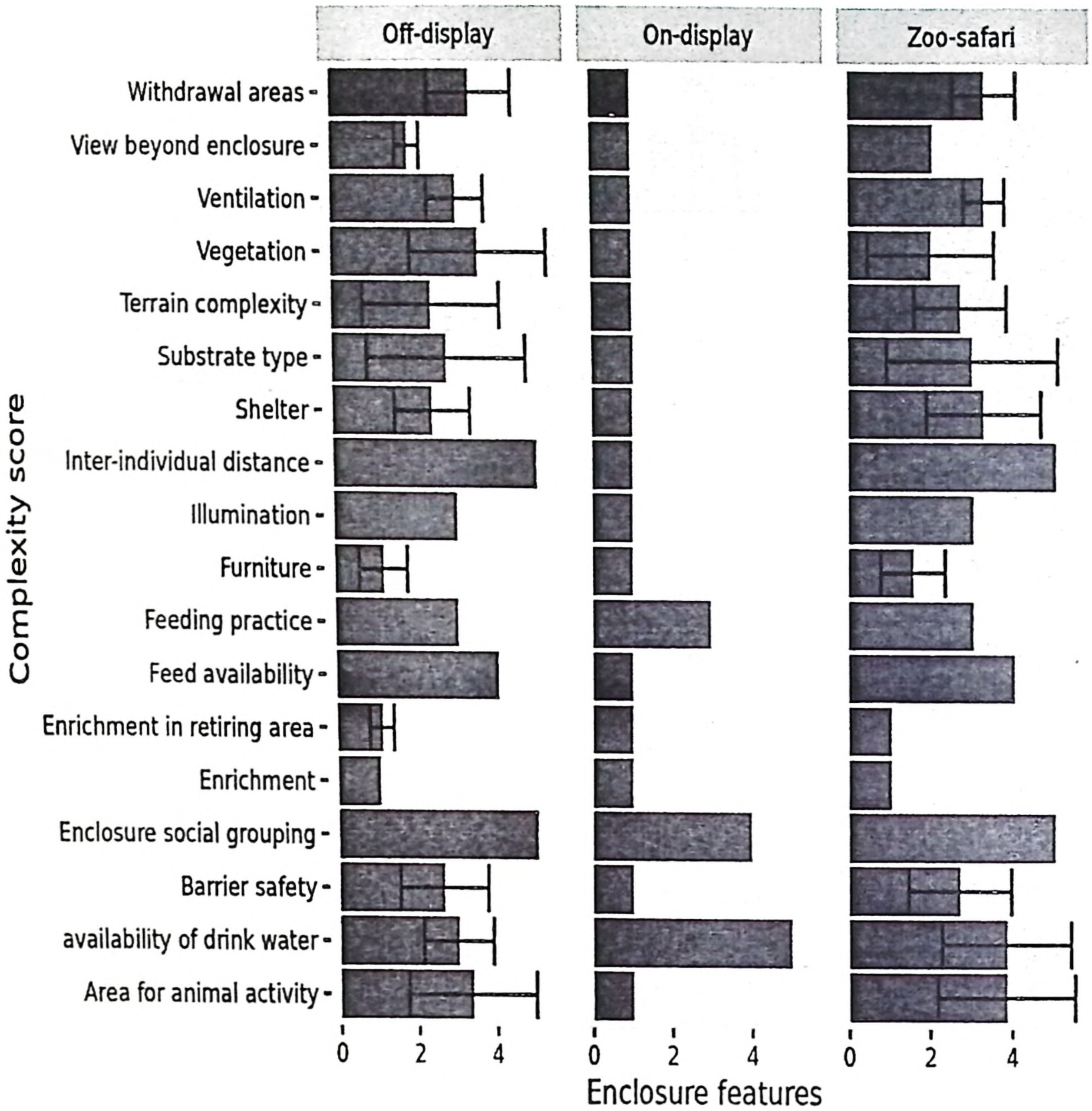

Figure 2.6: Enclosure complexity comparison across three enclosure types a. Off-display, b. On-display, c. Zoo-safari

Area for animal activity:

The on-display enclosures provided less than 100 m^2 space for a pair of animals. On the other hand, all the CBC enclosures including the ones included in the zoo safari provide at least 1000 m^2 space for a pair of animals and often exceeded that measure. The on-display enclosures at the on-display enclosures prohibit the performance of most species-typical behaviours and violate global and Indian guidelines for housing felids in captivity. Juxtaposed with the large naturalistic enclosures at the off-display and safari enclosures, one cannot help but notice the gross inequities in management practices and the neglect towards the welfare of the on-display animals.

Substrate type:

Natural and diverse substrate is important for all captive animals. While the off-display enclosure provided the lions with plenty of natural substrates to exhibit natural behaviours, there was a distinct dearth of the same at the off-display enclosures. The substrate of the off-display enclosures was primarily sandy and did not offer enough traction that could elicit species-typical behaviours like ground scratching. Therefore the on-display enclosures scored lower than the off-display and zoo-safari enclosures on this measure as well.

Terrain complexity:

The on-display enclosure was homogeneous in landscaping and terrain complexity and did not allow for animals to get to vantage points and exert some control over their captive environments. The on-display enclosures were small and did not have any opportunities that the lions could use to express species-typical behaviours. The zoo-safari and the off-display enclosures provided for mounds and a certain extent of terrain complexity that allowed lions to get a vantage point and also get out of the line of sight of visitors.

Shelter:

The on-display enclosure provided only one place for shelter to the lions as compared to multiple shelter areas that were provided to the lions at the off-display enclosures. The off-display enclosures, also provided elevated mounds conveniently placed under a tree that the lions could use to rest during rain showers. The availability of multiple shelter locations allowed all animals equitable opportunities to protect themselves from inclement weather while maintaining a safe distance from no-friendly conspecifics or visitors. Such opportunities were unavailable to the lions housed at the on-display enclosures.

Withdrawal spaces:

Withdrawal areas are important because of two reasons, first, it provides animals with a way to maintain a critical distance from conspecifics housed in the same exhibit. Second, the withdrawal areas provide sufficient flight distance to the lions that can reduce the chances of sustained aggressive interactions between conspecifics. While the off-display enclosures and the zoo-safari enclosures had adequate sizes, which allowed all housed Asiatic lions sufficient flight distance and critical distance, the same cannot be said about the on-display enclosures. The on-display enclosures provided drinking water next to the visitor barrier, which forced lions to come close to the visitors for the basic need of drinking water. Such design flaws in the on-display enclosure are problematic and expose zoo-housed animals to chronic stress, which violates the basic tenets of all husbandry guidelines for any species.

Vegetation:

All the off-display enclosures had diverse canopy structures that allowed lions to find places of shade and thermoregulate. On the other hand, the on-display enclosures had all but one tree at the centre of the enclosure, which provided enough shade to the lions housed in them. However, apart from a single tree, there was no other vegetation inside the enclosure, including grass or shrubs that could be used by the lions as a source of diverse sensory stimuli. Vegetation plays an important role for all animal enclosures and helps promote species-typical behaviours. The dearth of a diverse array of vegetation inside the on-display enclosure was limited to the performance of species-typical behaviours by the housed animals.

Enrichment practices:

None of the enclosures at SZG had an active enrichment programme. Enrichment programmes bring novelty to a sterile captive environment and create a positive relationship between the animal and the paddock area. Enrichment devices also create opportunities for animals to display species-typical behaviour patterns. Enrichment interventions although ubiquitously accepted as beneficial for positive animal welfare outcomes, have not found widespread application at Indian *ex-situ* institutions (Szokalski et al., 2012). Therefore, all enclosures scored low on this measure (score = 1) (Figure 2.5).

Furniture:

Furniture is an important feature inside the enclosure like water pool, caves, logs, platforms that add functionality to different areas. Elevated log platforms were the only form of furniture inside all three enclosure types. The lack of diverse furniture types prevented animals from forming functional relationships with enclosure zones and therefore all enclosures scored low on this attribute (Figure 2.5).

Social features:

All enclosures housed lions in either pairs or groups. Lions were housed in iso-sexual pairs for related animals and heterosexual for mating pairs. The on-display enclosures did not have sufficient space (100 m^2) and were crowded for housing a pair of Asiatic lions. The off-display enclosures housed lions in paired or group (M = 1, F = 2) formats in large enclosures (1000 m^2). The on-display enclosures scored much lower (M = 1.75) on this attribute as compared to the off-display (3.59) and zoo-safari (M = 3.82) enclosures.

Feeding practices

The feeding protocol was identical across all enclosure types. The daily ration was provisioned based on the weight and health of each individual in their retiring cubicles between 1700-1800 hours. The lions would be brought into the retiring cubicles and then the keepers would drop chunks of meat and bones through a feeding window. The meat was cut into small pieces due to the small size of the feeding window, which lowered the food processing time and did not allow for the lions to display species-typical feeding behaviours. Adult lions are fed approximately 6-7 kg of lean buffalo meat each day, with a fast on Sundays. Since the provisioned meat does not have much fat and the animals are not provided with organs, the food is supplemented with multivitamins. Due to the lower food processing time and the method of food provisioning, all enclosures scored close to three on this attribute (Figure 2.5).

Retiring Area

The retiring cells were similar across all three enclosure types. All enclosures had separate retiring cubicles for each animal. However, the retiring cubicles were not used to house animals for extended periods. Once feeding was completed, the lions were allowed to roam in the paddock area. All enclosures had access to day kraals that provided some amount of freedom to lions undergoing treatment. The retiring cubicles were sterilized on a bi-weekly schedule with a blow torch and were regularly sanitized of leftover food and faecal matter with disinfectants. Keeper safety was accounted for at the off-display and zoo-safari enclosures, which allowed keepers to ensure that the lions were outside the retiring cubicles before entering them themselves for cleaning tasks. The on-display retiring cubicles had an old design that prevented keepers from having a good view of the cubicle before entering themselves. Therefore two keepers needed to be present at all times to ensure that all the animals were safely out of the retiring cells before the cleaning team could enter. Moreover, the retiring cubicles at the On-display enclosures did not have appropriate ventilation or illumination and hence posed a risk of disease or injury to the animals. The CBC is situated at the foothills of the Girnar range, and hence often receives heavy showers during July and August, which leads to increased moisture in the retiring cells across all enclosure types. The retiring cells had concrete floors that although hygienic, which became slippery for lions and posed a risk of injury to the animals. The retiring cells provided water to the animals in a small concrete trough, All enclosures lacked enrichment devices or species-appropriate furniture at the retiring areas or the day kraals (Figure 2.5).

I evaluated the complexity scores of all enclosures in the study area (Figure 2.5). Subjectively, the enclosures were of three distinct types, first the on-display enclosure at the zoo (N = 2, which had the lowest enclosure complexity score ($M_{complexity}$ = 1.47, N = 2). Second and third, the enclosures inside the zoo-safari ($M_{complexity}$ = 2.98, N =

Table 2.4: Activity budget of Asiatic lions at different enclosure types at from 0700-1900 hours

Enclosure_Type	Behaviour_class	7-10	10-13	13-16	16-19
Off-display	ARB	18.65	8.51	8.99	14.65
Off-display	Rest	73.97	87.88	83.60	73.53
Off-display	Species-typical active	7.37	3.62	7.42	11.82
On-display	ARB	46.52	32.86	40.80	45.59
On-display	Rest	46.44	62.56	53.47	48.38
On-display	Species-typical active	7.04	4.58	5.73	6.03
Zoo-safari	ARB	26.41	8.57	10.58	20.96
Zoo-safari	Rest	59.83	85.08	84.69	67.99
Zoo-safari	Species-typical active	13.76	6.35	4.74	11.04

4) , and those within the conservation breeding center ($M_{complexity} = 2.89$, N = 17). The low complexity score of the on-display enclosures can be attributed to the old design concepts dating back to the early twentieth century when the menagerie style was still predominant. The on-display enclosures were overcrowded and provided insufficient space for all animals to perform species-typical activity patterns.

We found that the off-display and zoo-safari enclosures had the most species-appropriate, including, sufficient space for animal activity, availability of withdrawal spaces, vegetation and artificial terrain complexity, which was starkly absent at the on-display enclosures. However, the lack of enrichment protocols at all the enclosures is concerning. All enclosure types scored their lowest enclosure complexity scores in the retiring area sections ($M_{off\text{-}display} = 2.39$, $M_{zoo\text{-}safari} = 2.43$, $M_{on\text{-}display} = 1$) , this was primarily due to the limited space available to the animals in the cramped retiring cubicles. The lack of enrichment and furnishing was a matter of concern in the off-display areas. On the other hand, the on-display enclosures had poorly designed retiring cells that lacked proper ventilation and illumination.

2.4.2 Activity budget

The activity budget variations across enclosure types revealed some interesting patterns. I found that the lions housed in the zoo-safari and the off-display enclosures showed an

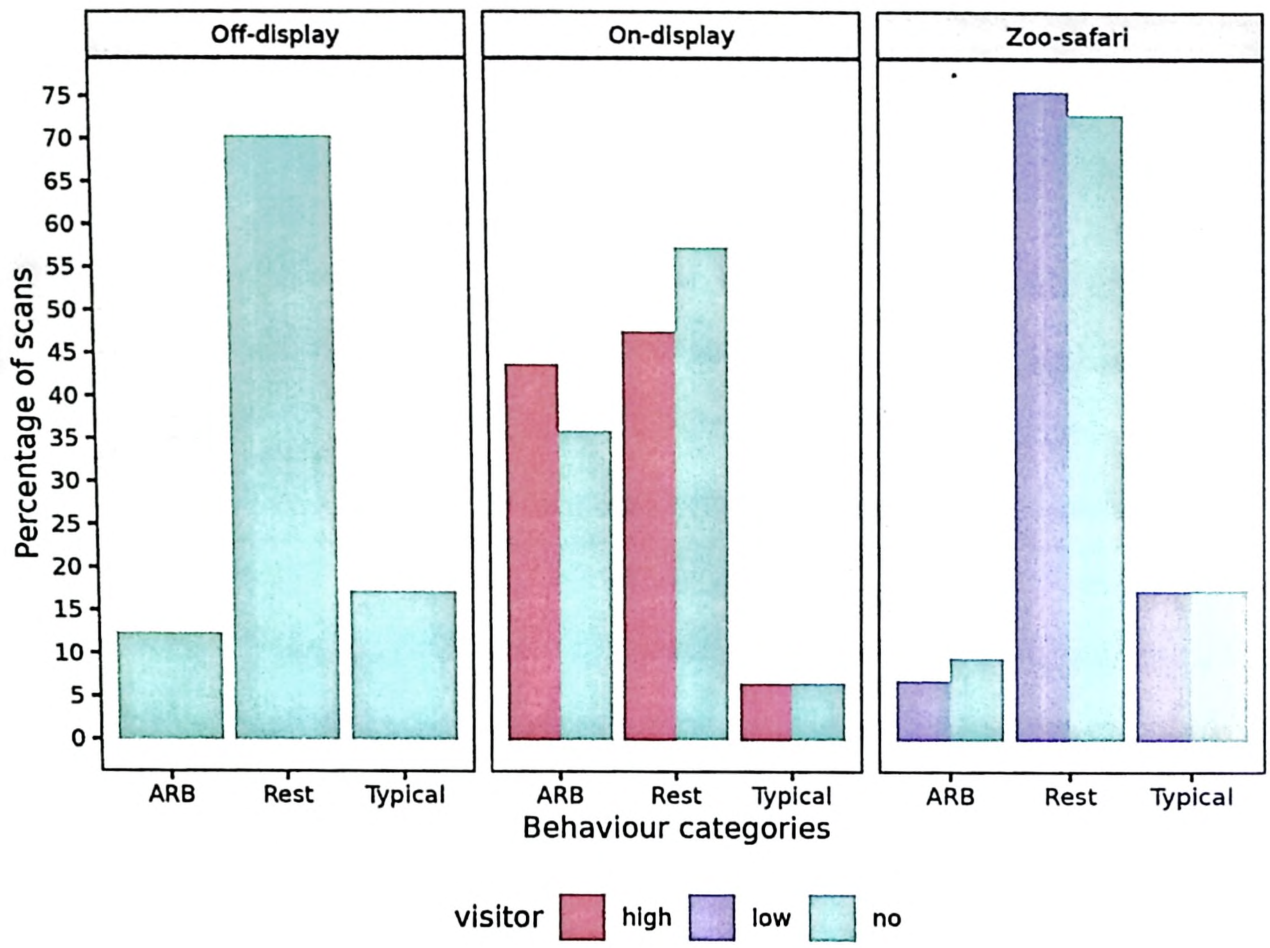

Figure 2.7: Activity budget of Asiatic lions across enclosure types and levels of visitor presence (N = 383,132 scans)

Table 2.5: Activity budget of Asiatic lions across enclosure types and visitor presence

Enclosure	Visitor	ARB	Typical	Rest	BD	SPI
Off-display	no	11.12± 6.04	16.54±8.12	72.34±6.13	0.97±0.3	0.55±0.13
On-display	high	43.8± 8.22	6.57±2.47	47.65±11.46	0.32±0.09	0.93±0.06
On-display	no	36±3.39	6.57±2.47	57.43±4.43	0.54±0.13	0.72±0.13
On-display	total	39.9±7.25	6.57±2.35	52.54±9.73	0.43±0.16	0.82±0.15
Zoo-safari	low	10.69±6.73	17.85±7.91	71.46±4.87	1.12±0.41	0.49±0.49
Zoo-safari	no	9.56±7.23	19.3±9.29	71.14±5.54	1.14±0.46	0.58±0.2
Zoo-safari	total	8.47±5.71	17.44±6.74	74.08±5.51	1.16±0.36	0.54±0.16

Note:
ARB = Aberrant reptitive behaviours (Mean and SD) , Typical = Species-typical active behaviours, Rest= Rest behaviours, BD = Behaviour diversity, SPI= Enclosure usage

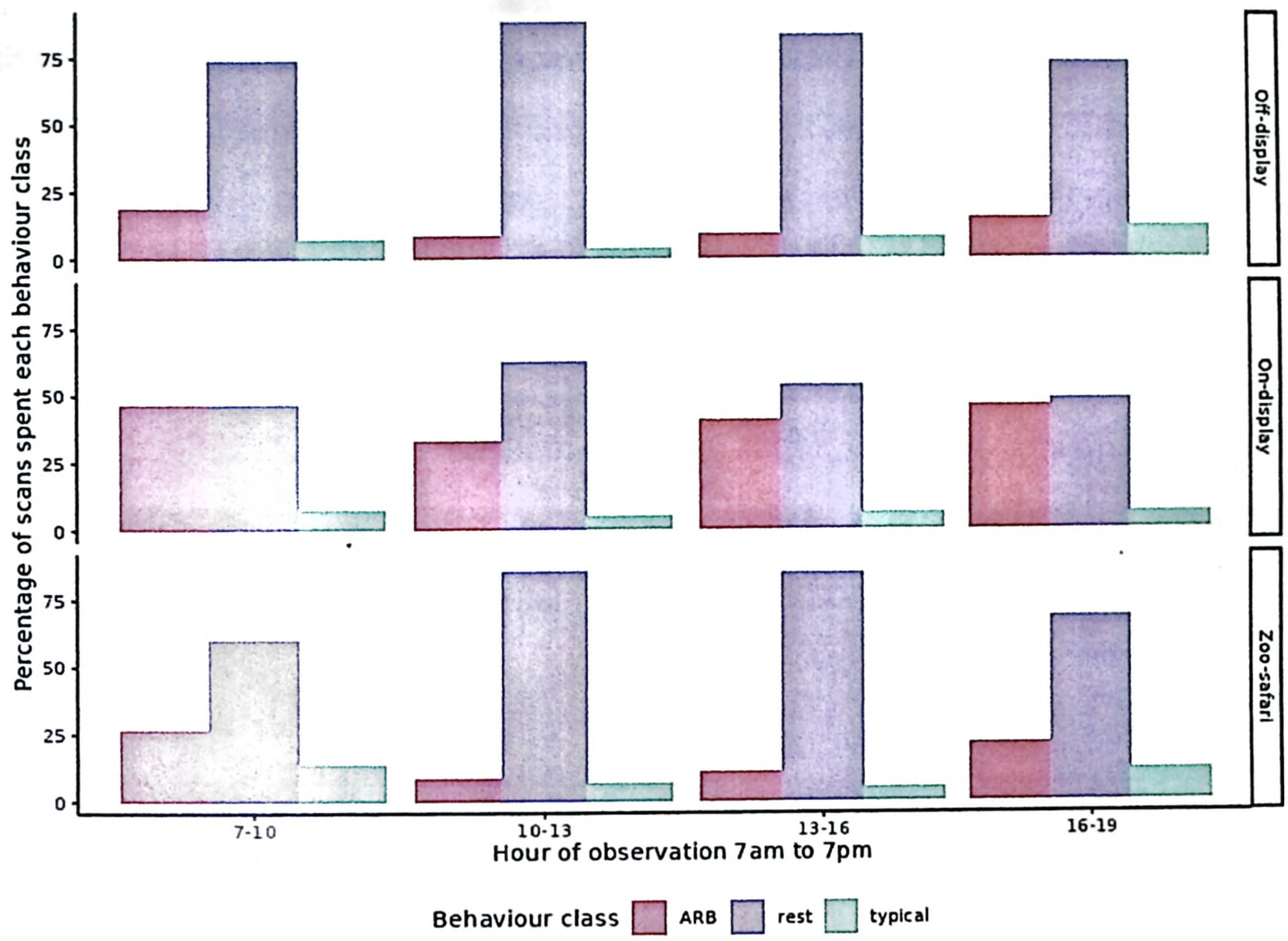

Figure 2.8: Hourly percentages of behaviours spent in different behaviour classes (N = 383,132 scans)

identical activity pattern across most times of the day irrespective of visitor presence (Figure 2.8). The activity patterns of the lions housed at the on-display enclosures differed significantly from the other two enclosures across both visitor-presence and absence days (Figure 2.7, Table 2.6). When the visitors were absent, the enclosure usage pattern of all the enclosures was largely similar (Figure 2.12, Table 2.5). In Table 2.4 and Figure 2.8, I have shown the changes in the activity budget of the Asiatic lions at three types of enclosures from 0700 hours to 1900 hours. ARB levels at the off-display and the zoo safari peaked in the early morning and the evening. Rest behaviours peaked during the midday and afternoon between 1000 and 1600 hours at all three enclosures. Species-typical active behaviours peaked in the early morning (7.37%) and evening periods (11.82%) at off-display enclosures. A similar pattern was shown by the lions at the zoo-safari enclosures, wherein the species-typical active behaviours peaked in the early morning (13.76%) and evening (11.04%). The on-display enclosures showed a distinctly different pattern, wherein the ARB levels stayed constant throughout the day and lowered during the mid-day when most animals started resting. Curiously the lions housed at the on-display enclosures rested significantly less and performed less species-typical behaviours compared to the off-display enclosures.

Table 2.6: Pairwise comparison of welfare indices across enclosure types using Dunn's test

Comparison	Z	P.unadj	P.adj
ARB			
Off-display - On-display	-4.82	0.00	0.00
Off-display - Zoo-safari	2.17	0.03	0.03
On-display - Zoo-safari	5.92	0.00	0.00
Typical-active			
Off-display - On-display	3.98	0.00	0.00
Off-display - Zoo-safari	-0.38	0.70	0.70
On-display - Zoo-safari	-3.90	0.00	0.00
Rest			
Off-display - On-display	4.63	0.00	0.00
Off-display - Zoo-safari	-2.14	0.03	0.03
On-display - Zoo-safari	-5.72	0.00	0.00
Enclosure-usage (SPI)			
Off-display - On-display	-3.50	0.00	0.00
Off-display - Zoo-safari	1.41	0.16	0.16
On-display - Zoo-safari	4.18	0.00	0.00
Behaviour diversity (BD)			
Off-display - On-display	3.89	0.00	0.00
Off-display - Zoo-safari	-2.02	0.04	0.04
On-display - Zoo-safari	-4.96	0.00	0.00

Table 2.7: Pairwise comparison (Dunn's test) of behaviours (scan percentages) and welfare indices across combination of enclosure complexity and visitor levels

Comparison	Z	P.unadj	P.adj
ARB			
Off-display_no - On-display_high	-3.78	0.00	0.00
Off-display_no - On-display_no	-3.40	0.00	0.00
On-display_high - On-display_no	0.28	0.78	0.78
Off-display_no - Zoo-safari_low	2.07	0.04	0.15
On-display_high - Zoo-safari_low	4.54	0.00	0.00
On-display_no - Zoo-safari_low	4.23	0.00	0.00
Off-display_no - Zoo-safari_no	1.39	0.17	0.50
On-display_high - Zoo-safari_no	4.22	0.00	0.00
On-display_no - Zoo-safari_no	3.88	0.00	0.00
Zoo-safari_low - Zoo-safari_no	-0.76	0.45	0.90
Typical-active			
Off-display_no - On-display_high	2.96	0.00	0.03
Off-display_no - On-display_no	2.96	0.00	0.03

Table 2.7: Pairwise comparison (Dunn's-test) of behaviours (scan percentages) and welfare indices across combination of enclosure complexity and visitor levels *(continued)*

Comparison	Z	P.unadj	P.adj
On-display_high - On-display_no	0.00	1.00	1.00
Off-display_no - Zoo-safari_low	-0.35	0.72	1.00
On-display_high - Zoo-safari_low	-2.71	0.01	0.04
On-display_no - Zoo-safari_low	-2.71	0.01	0.03
Off-display_no - Zoo-safari_no	-0.25	0.80	1.00
On-display_high - Zoo-safari_no	-2.79	0.01	0.04
On-display_no - Zoo-safari_no	-2.79	0.01	0.04
Zoo-safari_low - Zoo-safari_no	0.12	0.90	1.00
Rest			
Off-display_no - On-display_high	3.66	0.00	0.00
Off-display_no - On-display_no	3.22	0.00	0.01
On-display_high - On-display_no	-0.33	0.74	0.74
Off-display_no - Zoo-safari_low	-2.20	0.03	0.11
On-display_high - Zoo-safari_low	-4.54	0.00	0.00
On-display_no - Zoo-safari_low	-4.17	0.00	0.00
Off-display_no - Zoo-safari_no	-1.24	0.22	0.65
On-display_high - Zoo-safari_no	-4.02	0.00	0.00
On-display_no - Zoo-safari_no	-3.63	0.00	0.00
Zoo-safari_low - Zoo-safari_no	0.98	0.33	0.66
Enclosure-usage (SPI)			
Off-display_no - On-display_high	-3.59	0.00	0.00
Off-display_no - On-display_no	-1.61	0.11	0.43
On-display_high - On-display_no	1.48	0.14	0.42
Off-display_no - Zoo-safari_low	1.64	0.10	0.50
On-display_high - Zoo-safari_low	4.10	0.00	0.00
On-display_no - Zoo-safari_low	2.44	0.01	0.10
Off-display_no - Zoo-safari_no	0.67	0.50	0.50
On-display_high - Zoo-safari_no	3.62	0.00	0.00
On-display_no - Zoo-safari_no	1.85	0.06	0.39
Zoo-safari_low - Zoo-safari_no	-0.91	0.36	0.73
Behaviour diversity (BD)			
Off-display_no - On-display_high	3.41	0.00	0.01
Off-display_no - On-display_no	2.37	0.02	0.09
On-display_high - On-display_no	-0.78	0.44	0.87
Off-display_no - Zoo-safari_low	-1.49	0.14	0.41
On-display_high - Zoo-safari_low	-3.85	0.00	0.00
On-display_no - Zoo-safari_low	-2.97	0.00	0.02
Off-display_no - Zoo-safari_no	-1.64	0.10	0.41
On-display_high - Zoo-safari_no	-4.04	0.00	0.00
On-display_no - Zoo-safari_no	-3.11	0.00	0.01
Zoo-safari_low - Zoo-safari_no	0.08	0.93	0.93

Note:
The comparisons are between different combinations of enclosure complexity and visitor presence. "On-display_no" means that the enclosure is On-display and there were no visitors present

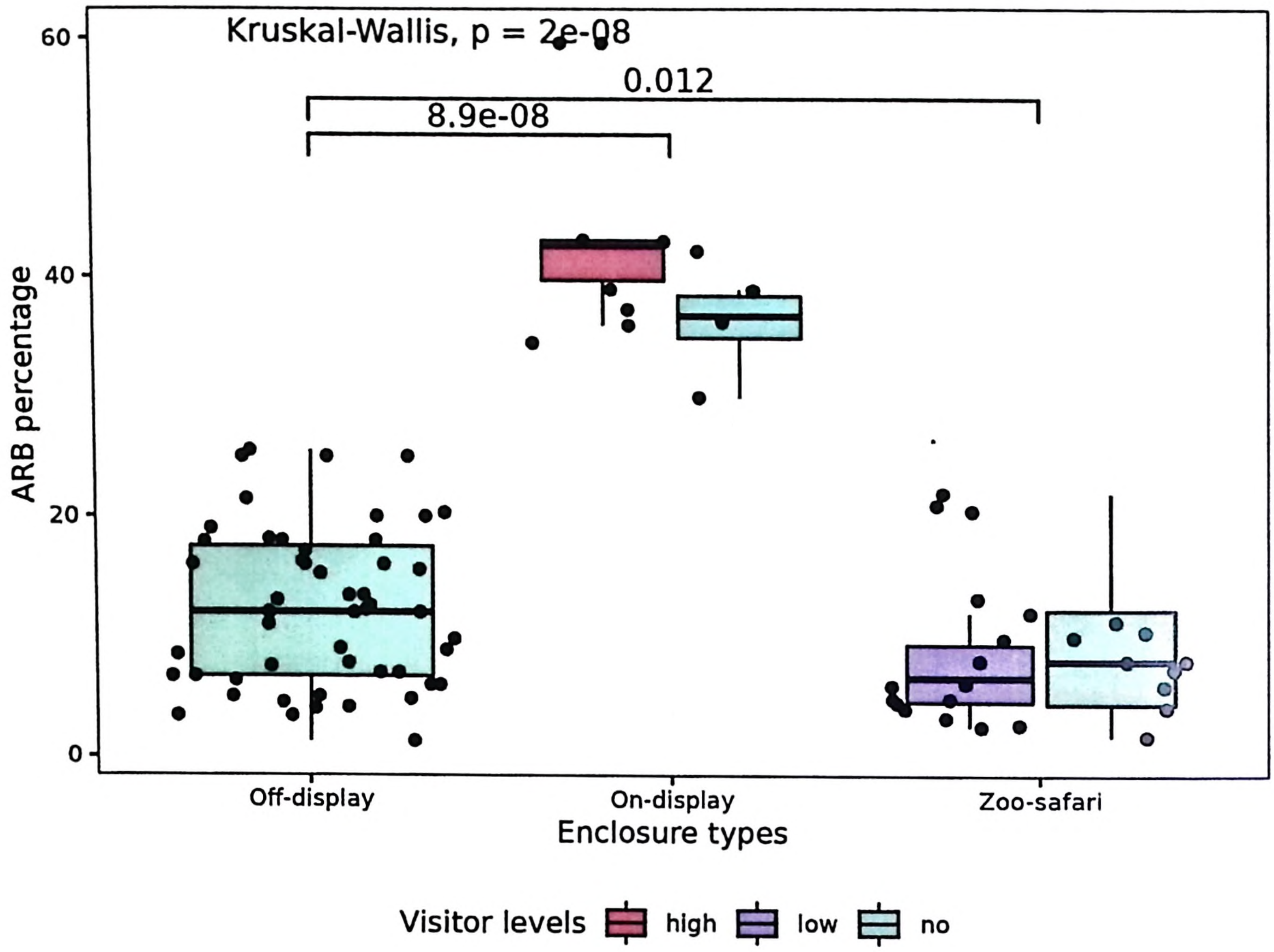

Figure 2.9: Aberrant repetitive behaviours displayed by Asiatic lions across enclosure types and levels of visitor presence (N = 383,132 scans)

2.4.3 Aberrant repetitve behaviours (ARBs)

ARB levels displayed by lions housed in different enclosures varied significantly (Table 2.6 and Figure 2.9). Lions at the off-display enclosures showed significantly lower levels of ARB ($M_{off\text{-}display}$= 11.12, $SD_{off\text{-}display}$ = 6.04) as compared to the zoo-safari ($M_{zoo\text{-}safari}$ = 8.47, $SD_{zoo\text{-}safari}$ = 5.71, $z = 2.17$, $p = 0.03$) and the on-display enclosures ($M_{on\text{-}display}$ = 39.9 , $SD_{on\text{-}display}$ = 7.25, $z = -4.82$, $p < 0.05$). The lions housed at the zoo-safari enclosures showed significantly lower levels of ARB as compared to those housed at the on-display enclosures ($z = 5.92$, $p < 0.05$) (Table 2.6).

ARB levels peaked at early morning and evening for lions housed at off-display enclosures and zoo-safari enclosures, while it remained constant at around at the on-display enclosures (Table 2.4 and Figure 2.8).

There was no effect of visitor presence or absence, on the ARB levels of lions housed at the zoo-safari enclosures (M_{Low}= 10.69, SD_{Low} = 6.73,M_{No} = 9.56, SD_{No} = 7.23 ,z = -0.76, p=0.9) and the on-display enclosures (M_{High}= 43.8, SD_{High} = 8.22,M_{No} = 36, SD_{No} = 3.39, z = 0.28, p = 0.78) (Table 2.7 and Figure 2.9). During the no visitor days, ARB levels of lions at the off-display enclosures was significantly higher than the lions housed at the zoo-safari (z = 3.88, $p < 0.05$) but significantly lower than the lions at the on-display enclosures (z = -3.4, $p < 0.05$) (Table 2.7). On the other hand, during the visitor presence days, the ARB levels of lions at zoo-safari enclosures was similar to the lions at off-display enclosures (z = 2.07, p = 0.15). Presence visitors did not impact the ARB levels of lions housed at the zoo safari or the on-display enclosures. It is curious that on the no visitor days, the lions at the zoo-safari enclosures showed significantly lower ARBs than those housed at off-display sites.

2.4.4 Species-typical active behaviours

Species-typical active behaviours displayed by lions housed in different enclosures varied significantly (Table 2.5 and Figure 2.10). The off-display enclosures showed similar levels of typical active behaviours ($M_{off\text{-}display}$= 16.54, $SD_{off\text{-}display}$= 8.12) as compared to the zoo-safari ($M_{zoo\text{-}safari}$ = 17.44, $SD_{zoo\text{-}safari}$= 6.74, z = -0.38, p = 0.7) enclosures (Table 2.6). The lions at the off-display (z = 3.98, $p < 0.05$) and zoo-safari (z = -3.9, $p < 0.05$) enclosures showed a significanly higher level of species-typical behaviours than those housed at the on-display enclosures ($M_{on\text{-}display}$= 39.9 , $SD_{on\text{-}display}$ = 7.25) (Table 2.6). Species-typical active behaviours peaked at early morning and evening for lions housed at off-display enclosures and zoo-safari enclosures, while it remained constant at around at the on-display enclosures (Table 2.4, Figure 2.10). Interestingly, species-typical behaviour levels were slightly higher in the zoo-safari enclosures as compared to the lions housed at the off-display facilities (Table 2.5).

There was no effect of visitor presence or absence, on the species-typical behaviours of lions housed at the zoo-safari enclosures (M_{Low} = 17.85, SD_{Low} = 7.91 ,M_{No}= 19.3, SD_{No} = 9.29 ,z = 0.9, p = 1) and the on-display enclosures (M_{High} = 6.57, SD_{High} = 2.47 ,M_{No}= 6.57, SD_{No} = 2.47 ,z = 0, p = 1) (Table 2.7). During the no visitor days,

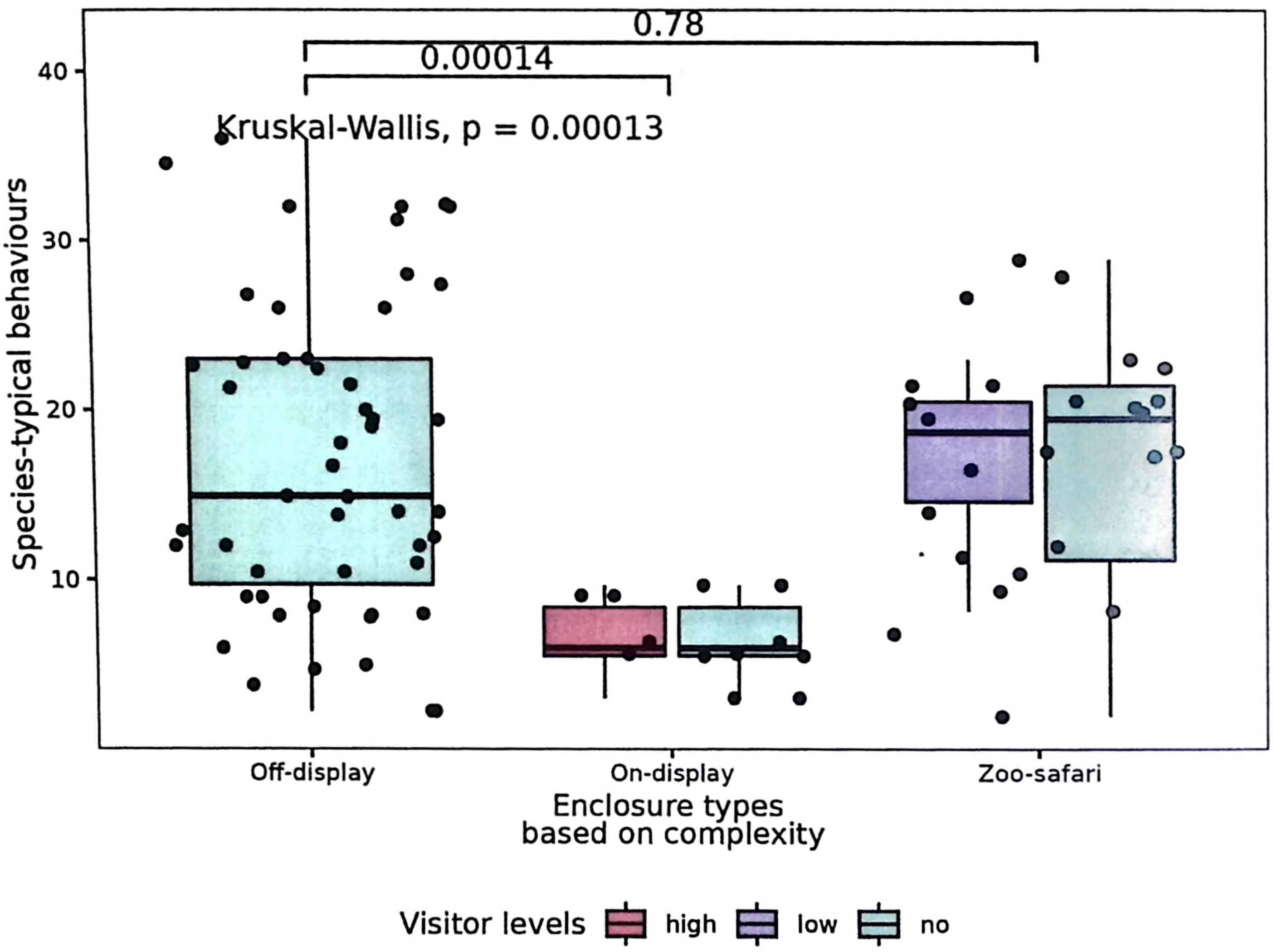

Figure 2.10: Species-typical active behaviours across enlcoure types and visitor levels(N = 383,132 scans)

species-typical behaviour levels of lions at the off-display enclosures was significantly higher than the lions housed at the zoo-safari ($z = 2.79$, $p = 0.04$) and the on-display enclosures ($z = 2.96$, $p = 0.03$) (Table 2.7, Figure 2.10). Therefore presence or absence of visitors affected did not lead to a significant increase in species-typical behaviour levels within the zoo-safari and on-display enclosures.

2.4.5 Rest

Resting behaviours displayed by lions housed in different enclosures varied significantly (Figure 2.11, Table 2.5).

Lions at the off-display enclosures rested significantly less ($M_{off\text{-}display} = 72.34$, $SD_{off\text{-}display} = 6.13$) compared to the zoo-safari ($M_{zoo\text{-}safari} = 74.08$, $SD_{zoo\text{-}safari} = 5.51$, $z = -2.14$, $p = 0.03$) and significantly more than the lions at on-display enclosures

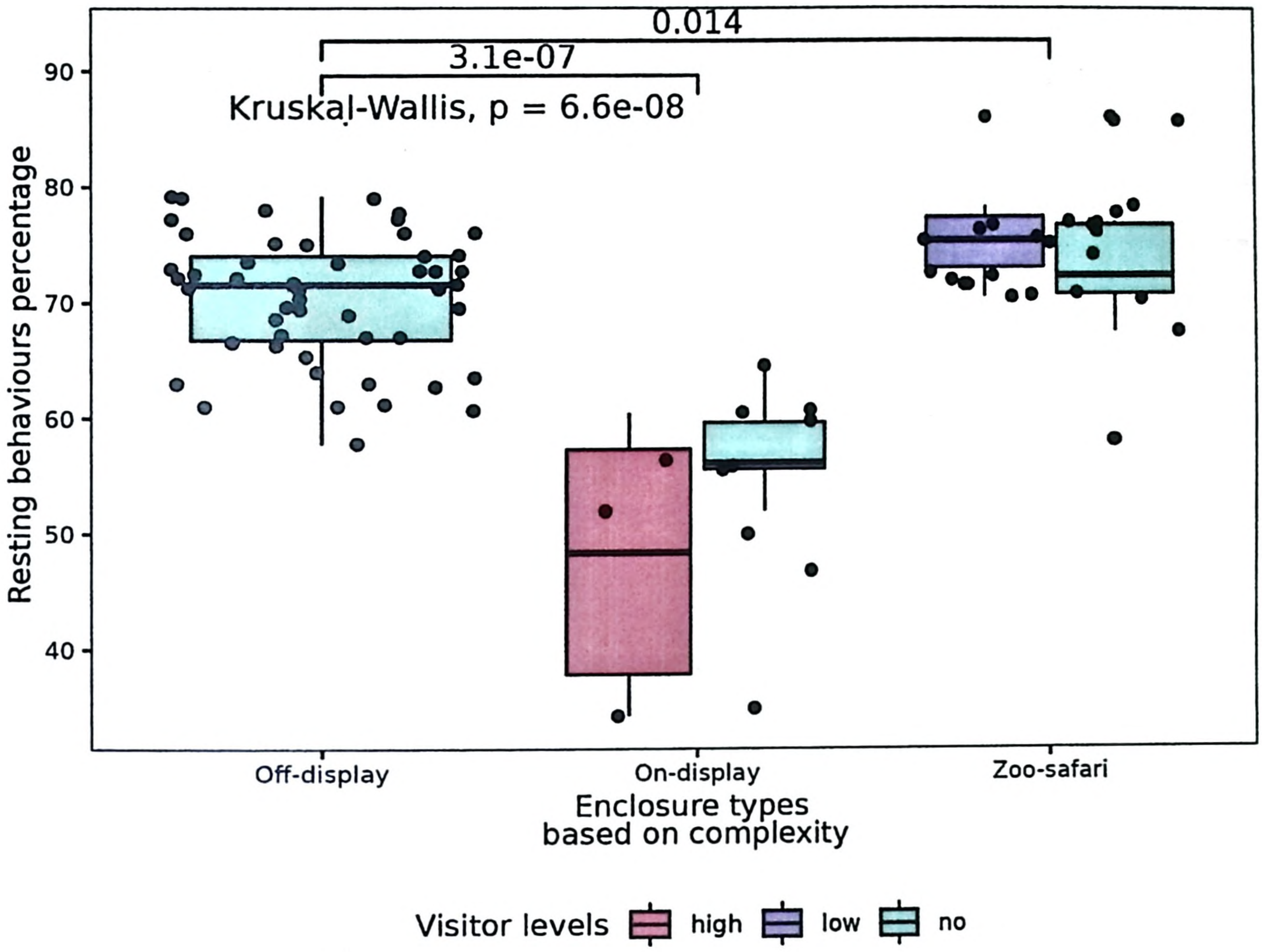

Figure 2.11: Resting behaviour levels across enclosure types and visitor levels (N = 383,132 scans)

($M_{on\text{-}display}$ = 52.54, $SD_{on\text{-}display}$ = 9.73, z = 4.63, $p < 0.05$) (Table 2.6 and Figure 2.11). The lions housed at the zoo-safari enclosures rested significantly more than the lions at on-display enclosures (z = 5.72, $p < 0.05$) (Table 2.6).

Resting levels peaked at midday for lions across all enclosures (Table 2.4, Figure 2.11).

There was no effect of visitor presence or absence, on the resting levels of lions housed at the zoo-safari enclosures (M_{Low}= 71.46, SD_{Low} = 4.87, M_{No} = 71.14, SD_{No} = 5.54, z = 0.9876, p=0.66) and the on-display enclosures (M_{High}= 47.56, SD_{High} = 11.46, M_{No} = 57.43, SD_{No} = 4.43, z = -1.61, p = 0.43) (Table 2.7 and Figure 2.11).

During the no visitor days, lions at the off-display enclosures rested for the same amount of time as the lions at the zoo-safari (z = -1.24, p = 0.65) but significantly more than the lions at the on-display enclosures (z = 3.22, $p < 0.05$). On the other hand,

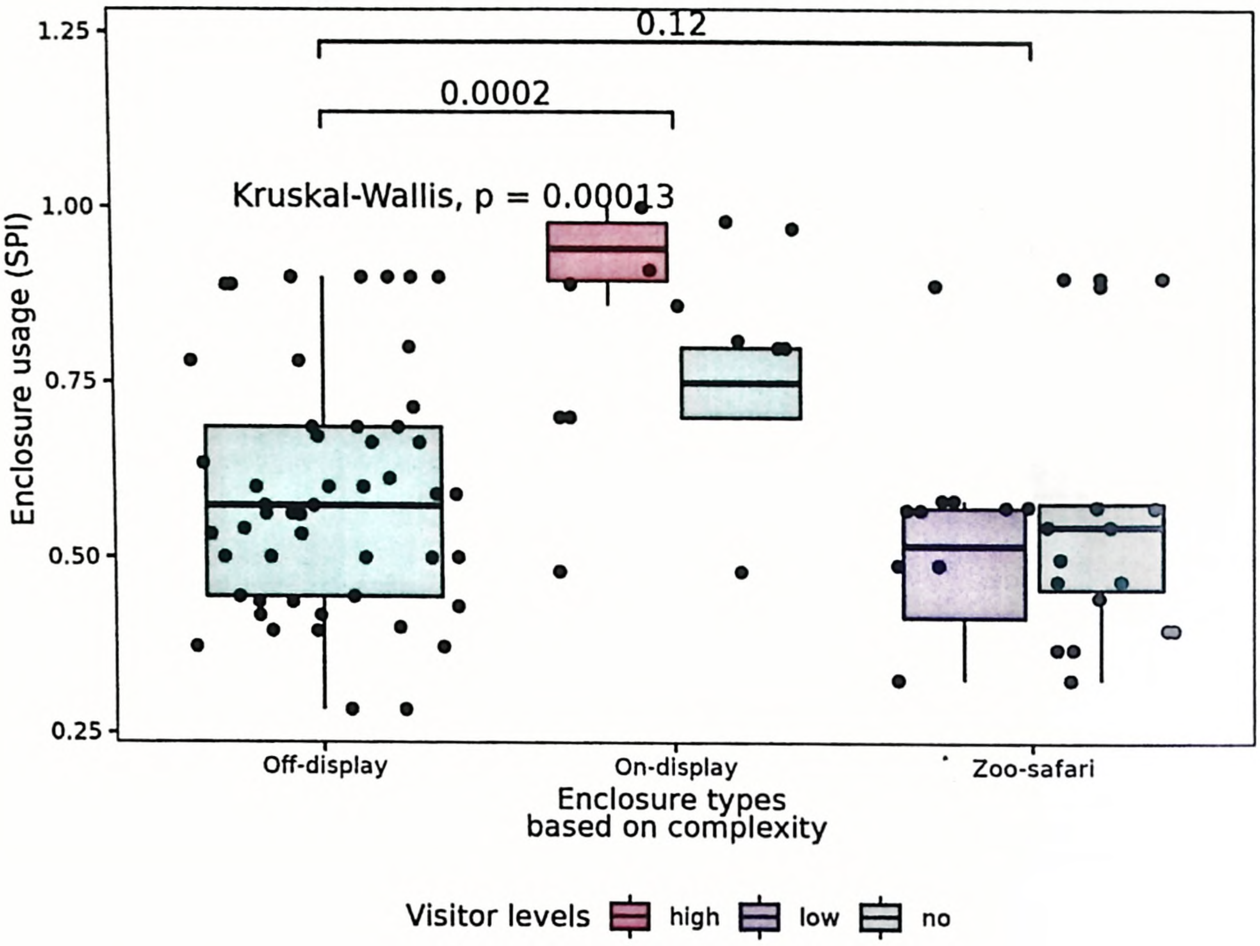

Figure 2.12: Enclosure usage across enclosure types and visitor levels (N = 383,132 scans)

during the visitor presence days, the ARB levels of lions at zoo-safari enclosures was similar to the lions at off-display enclosures ($z = 2.07$, $p = 0.15$) (Table 2.7). Presence visitors did not impact the resting patterns of lions housed at the zoo safari or the on-display enclosures (Table 2.7 and Figure 2.11). Enclosure type was the only factor that affected the patterns of resting behaviours of Asiatic lions.

2.4.6 Enclosure use

Lions housed at off-display enclosures showed significantly homogenous enclosure usage patterns ($M_{off\text{-}display} = 0.55$, $SD_{off\text{-}display} = 0.13$) as compared to the lions housed at the on-display enclosures ($M_{on\text{-}display} = 0.82$, $SD_{on\text{-}display} = 0.15$) , $z = -3.5$, $p < 0.05$) (Figure 2.12 and Table 2.5). There was no difference in enclosure usage between the lions at zoo-safari enclosures ($M_{zoo\text{-}safari} = 0.54$, $SD_{zoo\text{-}safari} = 0.16$) (Figure 2.12 and Table 2.5) and off display enclosures ($z = 1.41$, $p = 0.16$). The enclosure usage of lions at the on-display enclosures was significantly lower than those at the zoo-safari enclosures ($z = 4.18$, $p < 0.05$)

Interestingly, the enclosure usage of lions at on-display enclosures remained similar across high and no visitor days at the barred on-display enclosure ($M_{High} = 0.93$, SD= 0.06, $M_{No} = 0.72$, $SD_{No} = 0.13$, $z = 1.48$, $p = 0.42$). Similarly, the enclosure usage pattern of lions at zoo-safari enclosures remained similar across low-visitor ($M_{Low} = 0.58$, $SD_{Low} = 0.2$) and no-visitor days ($M_{No} = 0.58$, $SD_{No} = 0.2$, $z = -0.91$, $p = 0.73$) (Table 2.5 and 2.6). On the other hand, the visitor absence did not play a major role in determining enclosure usage at the zoo safari enclosures ($M_{Low} = 0.49$, $SD_{Low} = 0.49$, $M_{No} = 0.57$, $SD = 0.19$) or the off-display CBC enclosures. The enclosure usage levels were similar across both zoo-safari and off-display enclosures. Enclosure usage patterns differed significantly between animals housed at the off-display enclosures and the on-display enclosures (Table 2.6). Regardless of visitor presence, the animals at the zoo safari enclosures had similar enclosure usage patterns compared to the lions housed at the off-display enclosures(Table 2.5 and Table 2.7). Therefore we can suggest that the zoo-safari enclosures, by being complex and large were offsetting the effects of visitor presence and there was no discernible effect of visitor presence or absence at these enclosures.

2.4.7 Behaviour diversity

Lions housed at the off-display enclosures had a significantly higher behaviour diversity ($M_{off-display} = 0.97$, $SD_{off-display} = 0.3$) as compared to the lions at on-display enclosures ($M_{on-display} = 0.43$, $SD_{on-display} = 0.16$, $z = 3.89$, $p < 0.05$) (Table 2.6 and Figure 2.13).

Lions housed at zoo-safari enclosures had significantly higher behaviour diversity ($M_{zoo-safari} = 1.16$, $SD_{zoo-safari} = 0.36$) as compared to the lions at the on-display enclosures ($z = 4.96$, $p < 0.05$) (Table 2.5 and Figure 2.13) and off-display enclosures ($z = 2.02$, $p = 0.04$).

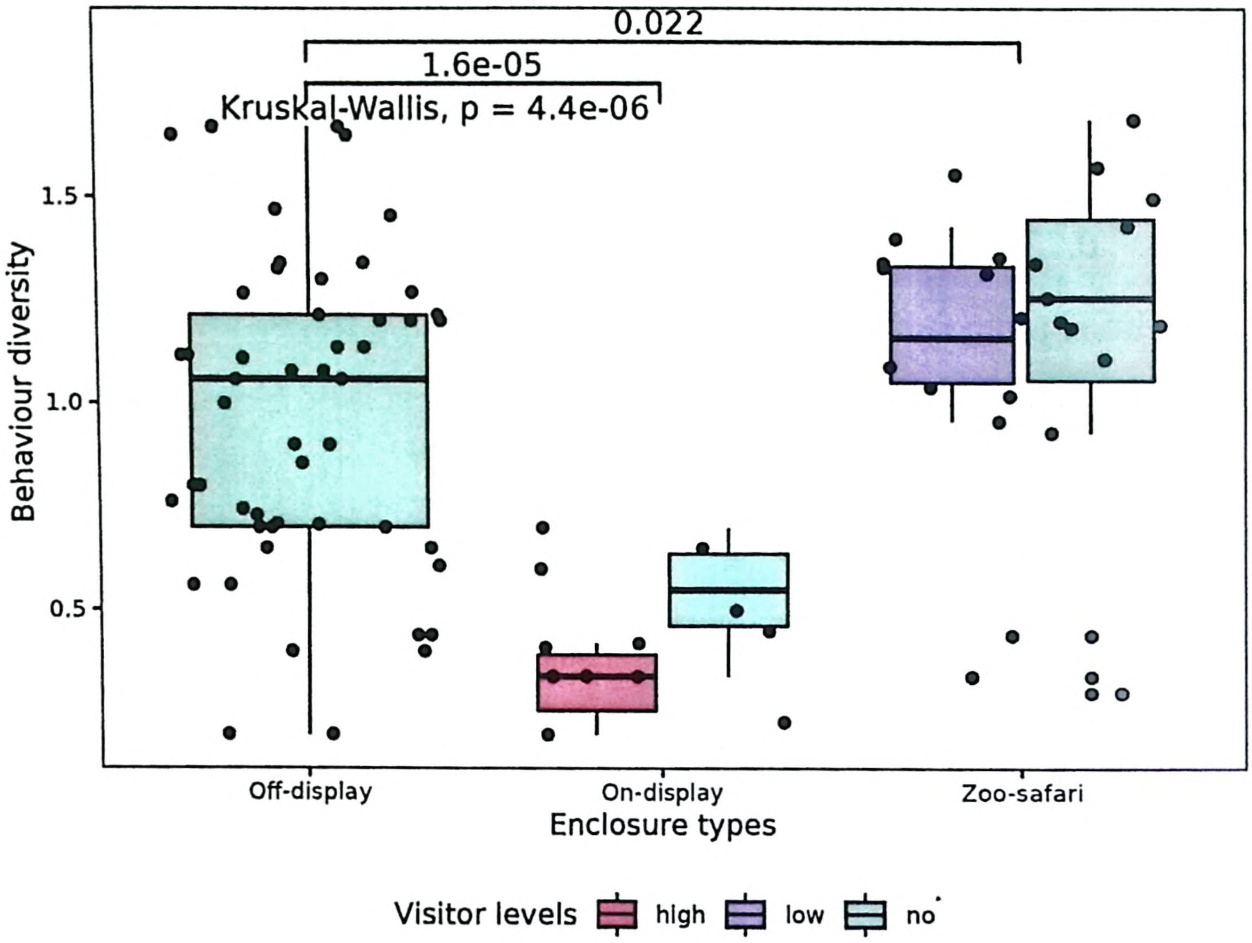

Figure 2.13: Behaviour diversity across enclosure types and visitor levels (N = 383,132 scans)

Lions at zoo-safari enclosures had significantly higher behaviour diversity than the lions at zoo-safari enclosures ($M_{zoo\text{-}safari} = 1.16$, $SD_{zoo\text{-}safari} = 0.36$) (Table 2.5 and Figure 2.13) and off display enclosures ($z = 1.41$, $p = 0.16$).

The behaviour diversity of lions at on-display ($M_{High} = 0.32$, $SD_{High} = 0.09$, $M_{No} = 0.54$, $SD_{No} = 0.13$, $z = -0.78$, $p = 0.87$) and zoo-safari enclosures ($M_{Low} = 1.12$, $SD_{Low} = 0.41$) and no-visitor days ($M_{No} = 1.14$, $SD_{No} = 0.46$, $z = -0.91$, $p = 0.73$) remained similar across visitor present and absent days (Table 2.7).

Interestingly, we found that the behaviour diversity of lions at zoo-safari enclosures was comparable to the off-display lions on visitor days ($z = 1.49$, $p = 0.41$). Results suggest that the behaviour diversity of the Asiatic lions in this study was more impacted by the complexity and the type of the enclosure than the presence or absence of visitors.

2.5 Discussion

These findings highlight several glaring issues pertaining to the welfare of captive felids at Indian zoos. The biggest of them being the welfare inequity within the same zoological institution, where lions designated for display areas do not have access to the complex, feature-rich, large enclosures like their conspecifics housed at the CBP. The lack of enrichment protocols and species-appropriate furnishing at most enclosures is a significant issue that often gets overlooked. Although the CBC enclosures benefited from large enclosure space and natural vegetation, the lack of an active enrichment program inter-linked with husbandry practices stands in stark contrast to the incumbent welfare practices of most modern zoos. Modern zoos need to have a tangible enrichment plan integrated with the husbandry guidelines for each species under their care. Indian zoos and conservation breeding programmes need to shift to a more proactive approach regarding the welfare management of the animals under their care. This study highlights the need to integrate good enclosure design principles with an effective enrichment plan at most zoos. I found that the Asiatic lions housed at the on-display enclosures showed more ARB, had lower behaviour diversity and had a highly biased enclosure zone usage pattern as compared to subjects housed in larger and more complex enclosures. I found that the lions housed at the on-display enclosures used a fraction of the limited 100 m^2 enclosure space on visitor days and started using the space more homogenously on non-visitor days. However, there was no discernible effect of visitors on the behaviour diversity and ARB levels of the subjects at any of the enclosures. This leads me to posit that enclosure complexity can offset the effects of visitor presence at zoo-safari enclosures. But the lack of enclosure space, and complexity at the on-display enclosures is exacerbated by the presence of visitors, which leads to significant behavioural artefacts in the subjects housed at these enclosures. At off-display enclosures, stereotypy although present was comparatively lower than the animals at the on-display enclosures. The causes of stereotypy for lions at off-display enclosures could be attributed to other factors such as the lack of an active enrichment protocol and novel features in the enclosures.

Our results are in line with the findings of Glatston and associates (1984), who found that the cotton-top tamarins (*Sanguinus oedipus*) housed in off-display enclosures showed more affiliative behaviours and less aggression as compared to conspecifics exposed to visitor presence.

Effects of enclosure complexity on the welfare of tigers and leopards have been studied at Indian zoos by Mallapur and colleagues (2002) and more recently by Vaz (2017). The study conducted on captive jungle cats at Indian zoos by Marinath and colleagues (2019) also reported that enclosure characteristics like availability of natural substrate, withdrawal areas and species-appropriate husbandry practices led to a lower proportion of inactive and aberrant behaviours. Our results show that enclosure complexity and its ability to provide natural withdrawal areas to animals is particularly essential in mitigating the stressful stimulus associated with visitor disturbance. When animals are housed in small enclosures and forced to come close to visitors, it may lead to chronic stress, the effects of which do not subside on non-visitor days. The moated enclosures which had exposure to visitors through safari buses were not particularly affected by the visitor disturbance and there were no discernible differences in activity patterns from the off-display enclosures. As suggested by our results, I did observe a variable amount of stereotypy even in enclosures that were complex and free from visitor disturbance. This leads me to believe that under similar housing and husbandry practices welfare outcomes may vary as a function of inter-individual differences (Bashaw et al., 2007).

Broom (1996) defined animal welfare as the "attempts of an animal as it copes with its environment", he also emphasized the need for addressing their subjective emotional well-being. Subsequent studies conducted on the subject have more or less agreed that positive affective states are crucial to good welfare in captive animals (Ahloy-Dallaire et al., 2018; Clegg, 2018; Crump et al., 2018; de Oliveira et al., 2018; Mellor, 2019; Squibb et al., 2018). Several studies have shown that high visitor presence is detrimental to the welfare of captive animals. Our study is the first to show that visitor effects can be mitigated with enclosure complexity by providing captive animals with the choice to escape a stressful stimulus i.e., visitor presence. Our study adds to the evidence that a complex enclosure environment can be effective in reducing the deleterious impacts of visitor effect as suggested by previous studies (Jensvold et al., 2001; Marinath et al., 2019). Gareth Davey in his (2007) review asked an important question, what is the actual effect of visitor presence on the welfare of zoo-housed animals? He recommended that visitor effects should be measured across several animal groupings and with reliable measures of stress. In this study, I attempted to showcase the joint effects of enclosure complexity and visitor presence on a wide range of welfare indices for Asiatic lions. I could not find any immediate effect of visitor presence or absence on the changes in activity budget or welfare indices of Asiatic lions across three different types of enclosures. I did find a significant effect of enclosure type on the activity budget and welfare indices of Asiatic lions. Lions housed at off-display enclosures with no visitor presence and zoo-safari enclosures with a low amount of visitors had similar activity budgets and welfare outcomes. Most probably the feature-richness of the off-display and the zoo-safari enclosures were responsible for better welfare outcomes than on-display enclosures. Interestingly, the lions housed at the zoo-safari enclosures did not appear to be affected by visitor presence and had similar welfare outcomes as their conspecifics at the off-display enclosures.

Zoos should be designed to keep wild animals in captivity at optimal welfare. However, welfare often takes a back seat to visitor entertainment which has led to a welfare crisis at Indian zoos. The results of this study resonate with the findings of few other studies which establish the poor state of welfare at Indian zoos. Results of this study clearly show that the animals housed at the same zoological institution are exposed to different welfare standards which sadly is a common occurrence across several Indian zoos. Zoo managers and regulators should take cognizance of these results and immediately shift wild captive felids from low-complexity enclosures to more species-appropriate enclosures that limit or offset the harmful impacts of visitor crowding.

3

Role of personality and rearing-history on Asiatic lion welfare

3.1 Introduction

Conservation breeding programmes should conduct periodic welfare evaluations for the improvement of incumbent housing and husbandry practices, and realign with conservation goals (Broom, 2011; Engel, 1980; Koolhaas et al., 1999; Moorhouse et al., 2015). In practice, *ex-situ* institutions continue to rely on unidimensional measures such as keeper ratings, physiological, and behavioural measures without accounting for individuality (Boissy et al., 2014; Mason et al., 2007; Sih et al., 2004). Intraspecific variations originating from personality (Locurto, 2006) and early-life experiences (Gartner et al., 2014; Watters et al., 2012) determine the umwelt of individuals (Loehlin, 1992; Loehlin et al., 1998; Stamps et al., 2010), affective states (Boissy et al., 2014; Harding et al., 2004), and ultimately welfare (Carere et al., 2011; Izzo et al., 2011). Inter-individual differences in bold/shy personality traits (Gartner et al., 2016; Gartner et al., 2014; Gosling et al., 1999; Mills, 1998; Powell et al., 2011) are associated with differential decision-making abilities (Carter et al., 2013), cognition (Griffin et al., 2015; Morton et al., 2013) and coping responses (Wilson et al., 1993) to welfare deprivation (Franks et al., 2014; Goold et al., 2017; Koolhaas et al., 1999; Moneta et al., 2009), and ultimately have a bearing on post-release fitness (Bremner-Harrison et al., 2004) . Early-life experiences can also have a bearing on the personality development of animals (Ainsworth et al., 1991; Frost et al., 2007; Higley et al., 1991; Loehlin, 1992; Stamps et al., 2010; Watters et al., 2012). Therefore, addressing early-life experiences and personality traits in welfare evaluation protocols can be vital to the success of *ex-situ* conservation breeding programmes (Rabin, 2003; Wemelsfelder, 1997). Unfortunately, parameters of individuality are seldom addressed while designing housing and husbandry protocols for wild animals at conservation breeding programmes. Focused studies are required to understand how personality and early life experiences (rearing-history) may be associated with welfare measures. Using captive Asiatic lions as a study system, I tried to understand the relationship between individual variations (viz., bold-shy traits, rearing history, sex, social grouping) with behavioural and physiological welfare measures.

It is vital to standardize the welfare evaluation practices for this species to meet its long-term conservation goals. Since Indian *ex-situ* facilities account for more than 60 per cent of the global captive Asiatic lion population (Srivastav et al., 2018), holistic welfare assessments at these sites can have a tangible impact on the conservation goals for the species. I aimed to understand if rearing-history and personality (Allport et al., 1921) are important factors associated with intraspecific variations in behavioural welfare indices (Broom, 1986; Fraser, 2009). I categorized these subjects based on their rearing histories (wild-rescued and captive-raised), sex, social grouping (pair-housed and group-housed) and personality traits (bold and shy) (Wilson et al., 1993). I measured species-typical behaviour diversity (Clark et al., 2012; Owen et al., 2017; Powell, 1995; Rabin, 2003; Razal et al., 2016; Skrzypczak, 2016; Watanabe, 2007; Wemelsfelder et al., 2000), space usage patterns (Kessel et al., 1996; Ross et al., 2016), latency to novel objects (Meehan et al., 2002; Murphy, 1977; Sneddon et al., 2003), proportion of aberrant behaviours (Japyassú et al., 2014; Kroshko et al., 2016; Mason, 2006; Tan et al., 2013) to understand the relationship between individual variations and behavioural welfare measures. Physiological parameters are important to the understanding of animal welfare since affective states have a strong physical connection as shown by Schildkraut (2016). I also measured the faecal corticosterone metabolites(FGM) of the Asiatic lions to understand their relationship with inter-individual variations and other behavioural welfare measures. The combination of different welfare indices is important to have a holistic understanding of the welfare of Asiatic lions (Watters et al., 2012). I believe that this study will address knowledge gaps in animal welfare evaluation procedures, leading to the adoption of individual-focused husbandry and management practices at *ex-situ* endangered species conservation programmes.

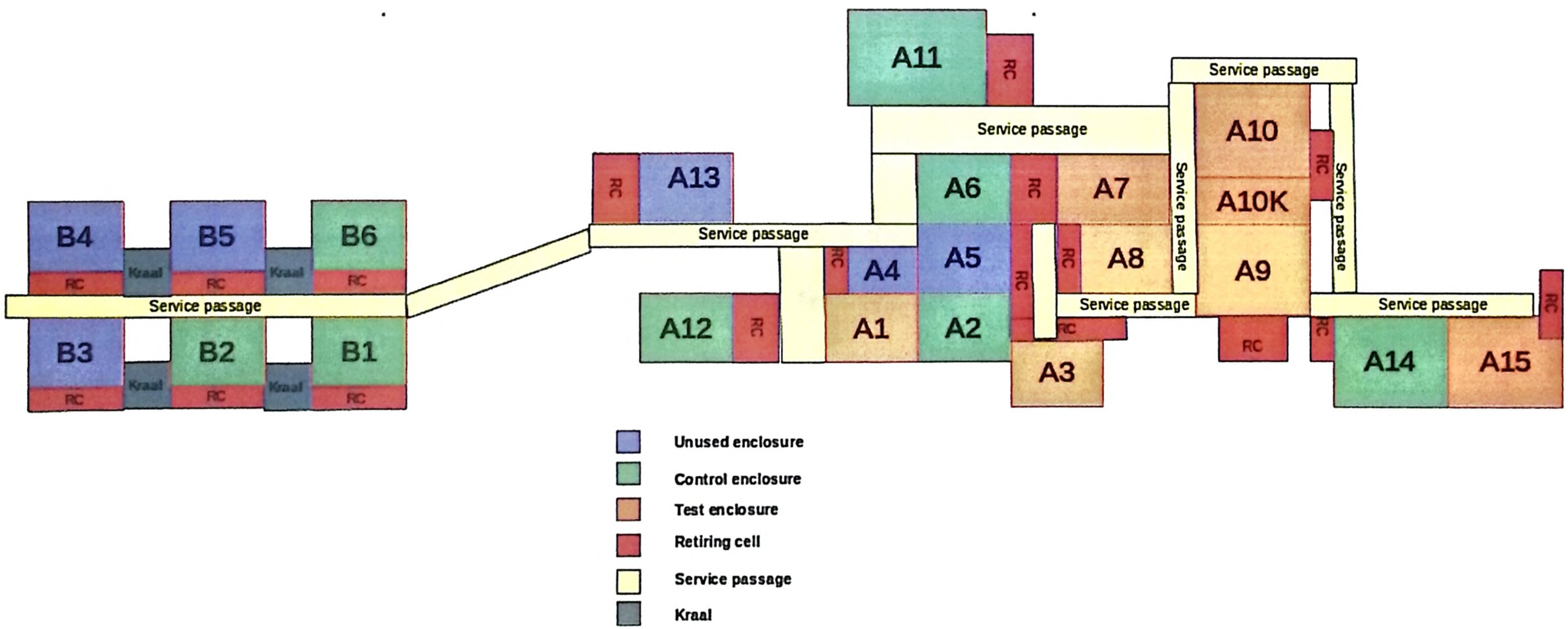

Figure 3.1: Enclosure layout at the conservation breeding center

3.2 Methodology

3.2.1 Subjects and housing

This study was conducted at the off-display conservation breeding centre of SZG. A map (unscaled) of the off-display conservation breeding enclosures of SZG is provided in Figure 3.1. I collected data from 38 (Male = 15, Female = 23) healthy Asiatic lions housed in the conservation breeding facility of SZG. During the study I removed three individuals (Male = 1, Female = 2) due to ongoing veterinary treatments, reducing our sample size to 35 individuals (Male = 14, Female = 21) (Table 3.1). The study subjects were either born in captivity (N = 16; M = 3, Female = 13) or rescued from wild (N = 19; Male = 11, Female = 8). Individuals born in the zoo (N = 14) and rescued as cubs (N = 2) were categorized as captive-raised since they have similar life experiences. Lions that were rescued at a young age and spent most of their lives in captivity cannot have similar life experiences as adult wild-rescued lions and hence were grouped in the captive-raised category. Most wild-rescued lions were rehabilitated as adults for the treatment of injuries incurred due to infighting, and after making full recovery were assimilated into the conservation breeding programme. Some wild-rescued animals (N = 3) were rescued to ameliorate conflict caused by livestock depredation. All subjects were either housed in pairs (N = 17) or housed in a sex ratio of 1:2 (N = 18). All subjects (including the wild-rescued lions) were in socially cohesive groups and were housed in the same enclosure (with the same enclosure mates) for at least a year before the commencement of the study. This facility provided us with a unique opportunity to study the behaviour of wild-rescued and captive-raised lions under similar housing conditions. Subjects were housed in 15 naturalistic enclosures spread across an 8-ha area resembling the habitat of Asiatic lions. All enclosures were similar in size, devoid of enrichment devices, evenly populated with leafy trees (for shade and cover) and provided similar enclosure space and complexity (Figure) per animal (400 m^2), ensuring uniformity of housing conditions for all subjects. Due to the absence of complexity and an active enrichment intervention programme, all enclosures were deemed functionally barren to the subjects. The enclosure sizes ranged from 1100-6542 m^2, with an average size of (M = 1970 m^2, SD = 1685.24 m^2).

Table 3.1: Details of subjects included in the study, viz., house name, sex (M = Male, F = Female) , origin (C= Captive, W = Wild) , sex ratio for housing, Enclosure size, age in days, age class, and personality profiles

Sl no	Subject name	Sex	Origin	Sex ratio (M:F)	Enclosure size (m2)	Age in days	Space/animal	Age class	personality
1	A1	M	W	paired	1300	3414.00	650.00	old	shy
2	Aftab	M	W	paired	1200	3500.00	600.00	old	bold
3	Amal	M	C	group	1576	2214.74	788.00	prime	bold
4	Ambica	F	C	paired	1123	3984.56	561.50	old	bold
5	Amiya	F	C	paired	1123	2037.56	561.50	prime	bold
6	Ani	F	W	paired	1600	1946.00	800.00	prime	shy
7	Bahadur	M	C	group	1600	653.33	533.33	sub	bold
8	Bigtwin	F	W	group	1600	800.33	533.33	sub	shy
9	Dharicub	M	W	group	1100	619.50	366.60	sub	bold
10	Dheer	M	W	paired	1600	5389.08	800.00	old	bold
11	Gina	F	C	paired	1100	1316.90	550.00	prime	shy
12	Girm	M	W	paired	1123	3337.92	562.50	old	bold
13	Hemal	M	W	group	1700	2029.00	566.60	prime	shy
14	Hemali	F	W	group	1271	2149.00	635.50	prime	shy
15	Jenifer	F	C	paired	1700	1491.33	850.00	prime	bold
16	Jesal	M	W	group	1300	5722.18	433.30	old	bold
17	Maheswari	F	C	group	1300	3411.26	433.30	old	bold
18	Mariyam	F	C	group	1300	3423.35	433.30	old	bold
19	Maytri	F	C	group	1123	3072.90	562.50	old	bold
20	Nagraj	M	W	group	6542	3227.00	2180.00	old	shy
21	Patvad	F	W	paired	1200	3729.00	600.00	old	shy
22	Patvadm	M	W	paired	1271	1537.00	635.50	prime	shy
23	Radha	F	C	group	1576	1291.30	525.30	prime	bold
24	Rani	F	C	group	1576	1291.30	525.30	prime	bold
25	Ranita	F	C	group	6542	2119.00	2180.00	prime	bold
26	Ranshi	F	W	group	6542	5109.00	2180.00	old	shy
27	Rudi	F	W	paired	1600	4700.00	800.00	old	shy

Table 3.1: Details of subjects included in the study, viz., house name, sex (M = Male, F = Female) , origin (C= Captive, W = Wild) , sex ratio for housing, Enclosure size, age in days, age class, and personality profiles *(continued)*

Sl no	Subject name	Sex	Origin	Sex ratio (M:F)	Enclosure size (m2)	Age in days	Space/animal	Age class	personality
28	Smt	F	W	group	1600	800.33	533.30	sub	shy
29	Subhi	F	C	paired	1700	2240.33	850.00	prime	shy
30	Sujan	F	W	paired	1300	2790.80	650.00	prime	shy
31	Taukir	M	C	paired	1303	2448.00	651.50	prime	bold
32	Teeta	F	C	group	1700	2166.93	566.60	prime	bold
33	Tejaswini	F	C	group	1700	4320.93	566.60	old	bold
34	Trakuda	M	W	group	1700	3956.93	566.60	old	bold
35	Veer	M	W	paired	1600	5387.30	800.00	old	bold

Only one enclosure was 6542 m^2 in size, and most other enclosures were similar in sizes (M = 1424 m^2, SD = 224 m^2). All enclosures included outdoor (paddocks) and indoor (retiring/feeding cells) areas (3m x 3m x 2m dimensions) with continuous access to drinking water. Enclosure barriers consisted of v-shaped dry moats with walls at the proximal side and chain-linked fences with dual overhangs (4m high) on the other three sides. Adjacent enclosures were separated by visual barriers in the form of dense bamboo thickets. Subjects were confined to feeding cubicles only during feeding time and had free access to all enclosure areas (including feeding cubicles) for the rest of the day. Subjects were fed separately at the indoor cubicles between 1700-1900 hours six days a week, with a fast on Sundays. Subjects were fed in-house slaughtered and quality-inspected buffalo meat. The average meat consumption was 3.5 kg (SD = 0.5 kg) for females (N = 21) and 4.9 kg (SD = 1.4 kg) for males (N = 14). Most subjects were group-housed (1:2) (N = 18) or pair-housed (1:1) (N = 20). Four subjects were iso-sexually paired which included two male lions (2:0) and a mother-daughter dyad (0:2). A group of animal keepers carried out all husbandry work for the subjects on a rotational basis, which meant that all subjects were accustomed to the same group of keepers. Since the conservation breeding area is off-display and restricts access to unauthorized personnel, subjects' interactions with humans were limited to the keepers. The animal-keepers had trained the subjects to respond to their house names and vocal instructions for moving in and out of the feeding cubicles.

3.2.2 Study design

I tried to answer two broad research questions in this study:

a) How differences in bold/shy personality traits, rearing history, sex, and social grouping are associated with variations in behavioural and physiological welfare outcomes in a group of captive Asiatic lions?

b) How are the behavioural and physiological welfare indices (viz., enclosure usage, behaviour diversity, aberrant repetitive behaviours, and faecal corticosterone levels) interlinked?

Table 3.2: Personality traits adapted from Wielebnowski et al., 1999, Chadiwck et al., 2014 and Pastorino et al., 2016)

Trait	Bold/Shy	Definition
Active	Bold	Moves around enclosure (e.g. paces, runs, stalks)
Curious	Bold	Approaches and explores changes in the environment
Eccentric	Shy	Shows stereotypic or unusual behaviours
Excitable	Bold	Overreacts to changes in the environment
Friendly to conspecifics	Bold	Initiates and seems to seek proximity of other lions
Friendly to keepers	Bold	Initiates proximity with keepers: approaches fence readily and in a friendly manner (e.g. purrs, rubs on fence)
Fearful of conspecifics	Shy	Retreats and hides from other lions
Fearful of familiar people	Shy	Retreats and hides from familiar keepers and staff members
Fearful of unfamiliar people	Shy	Retreats and hides from unfamiliar staff and members of the public
Insecure	Shy	Seems scared easily; "jumpy" and fearful in general
Playful	Bold	Initiates and engages in play behaviour (seemingly meaningless, non-aggressive behaviour) with objects and/or other lions
Self-assured	Bold	Moves in a seemingly confident, well-coordinated and relaxed manner
Smart	Bold	Learns quickly to associate certain events and appears to remember for a long time
Solitary	Bold	Spends time alone; avoids company
Tense	Shy	Shows restraint in movement and posture

To answer these questions, I categorized subjects and recorded the outcomes of welfare measures. The detailed design for the study is given below.

Personality assessment

I used a combination of keeper-rating and behaviour-coding techniques to reliably assess personality traits (Funder, 1995; Funder et al., 1988; Gartner et al., 2012; Gosling et al., 2002; Highfill et al., 2010). I separately interviewed three animal keepers with at least ten years of work experience to rate 38 subjects (15:23) on a scale of 1-9 (1-very low and 9- very high) for pre-selected bold (N = 10) and shy traits (N = 10) (Table 3.2). All keepers agreed on their ratings for subjects (high inter-rater reliability, Cronbach's alpha >0.8). I averaged all keeper ratings (N = 3) subject-wise for all personality traits. Afterwards, I calculated the average rating on bold (N = 10) and shy (N = 10) traits for each subject. Subjects that received an average score above

Table 3.3: Bold and shy behaviours used for novel object tests

Behaviour	Description
Bold behaviours	
Approach	Subject approaches novel object confidently, or in a stalking stance
Play	Play with or solicit play with unknown conspecific
Sniff	Sniff the novel object cautiously
Interaction	Physical interaction with novel object (including destruction of the same) or showing the urge to interact physically with unknown person by rubbing body by the cage.
Shy behaviours	
Cowl or avoid	Limit oneself at the corner of the cell to maximize distance from novel object.
Run away	Sprint away from novel object
Vocalize	Growl at novel object without interacting with it
Aggression	Charging towards novel object combined with growling
Displacement	Perform autogrooming behaviours and avoid the novel object

seven on bold traits were categorized as bold, whereas an average rating above seven on shy traits was categorized as shy. To validate keeper ratings for personality traits of subjects (bold/shy), I conducted novel-object tests in day kraals for ten minutes using video recorders in the absence of keepers and observers (Highfill et al., 2010). Naive observers (N = 3) with no prior exposure to study subjects, recorded the latency of subjects to interact with novel objects (Frost et al., 2007) and calculated the percentage of bold vs shy behaviours (Corsetti et al., 2018; Powell et al., 2011; Powell et al., 2008) performed by the subjects during these tests (Table 3.3 and Table 3.4). During these tests, the subjects were exposed to the following novel stimuli:

a) Unknown conspecifics,

b) Unknown person and

c) Non-food novel objects (lion-sam ball and bungee cord).

All novel-object tests were conducted in an open-air day kraal adjacent to the paddock area of the enclosures. For the first test, I simultaneously released two subjects (same-sex but unknown to one another) at adjacent day kraals and recorded their reactions to encountering a same-sex unknown conspecific. The stopwatch was started as soon as both lions were released inside their respective day kraals. The watch was stopped when the animal approached and interacted with the novel object. For the second test, I released the subject inside the day kraal and had a volunteer (unknown to the lion and not wearing a keeper's uniform) approach the kraal, and stop at the median section facing the day kraal for ten minutes. The volunteer did not make any eye contact or vocal communication with the animal. The stopwatch was started as soon as the volunteer reached the day kraal. For the final test, I placed a novel object (lion-sam ball or bungee cord) at the centre of the enclosure, and then released the subject inside the day kraal. The stopwatch was started when the subject was released inside the day kraal. Observers used focal animal sampling (Altmann (1974); Martin et al. (1993)) to calculate the duration of all behavioural states and events performed by subjects during each of these tests. These focal observations were used to calculate the percentage of bold and shy behaviours performed by subjects (Table 3.4). I tested each subject separately to avoid confounding personality with dominance. I conducted the latency tests simultaneously for 12 individuals daily between 0900-1100 hours. Since I did not want to overwhelm the animals with multiple novel stimuli on a single day, three sessions of novel-object tests were conducted for each subject on consecutive days. The novel object tests were conducted for ten minutes, and if subjects failed to approach the novel object after five minutes, they were categorized as shy. I repeated the novel object tests with unknown humans and novel objects after a month to check for trait consistency and calculated the average latency values for each subject. In the first session, a lion-sam ball was used as the novel object, which was replaced in the second session with a hanging bungee cord. The order of the latency tests was kept the same for all subjects. Three Asiatic lions undergoing veterinary treatments for physical injuries (Male = 1, Female = 2) showed inconsistencies in trait measures across different sessions. I excluded these animals from the study, thus reducing the number of subjects to 35 individuals (Male = 14, Female = 21). I found that keepers (N = 3) and observers (N = 3) reliably agreed on the personality type (Cronbach's alpha > 0.9) of these 35 subjects. These subjects were further categorized based on rearing history (wild-caught = 19, captive-raised = 16) , sex (Male = 14, Female = 21) , and social grouping (pair-housed = 17, group-housed = 18).

Behavioural welfare indices

In this study, I used four behavioural welfare indices, viz.,

1. Latency measures
2. Enclosure usage
3. Diversity of species-typical behaviours
4. Aberrant repetitive behaviours

Latency scores were recorded as the number of seconds that the subject took to interact with the novel object after encountering it. Additionally, I also measured the species-typical behaviour diversity, enclosure-usage, and percentage of aberrant repetitive behaviours shown by the subjects, using the same methodology discussed in the last chapter.

Faecal corticosterone metabolites

We measured faecal corticosterone metabolites of all subjects to contrast the physiological impacts of the enrichment interventions on control and test subjects. Faecal corticosterone is a reliable physiological indicator of stress and compromised welfare in captive felids (Ruskell et al., 2015; Schildkraut, 2016; Vaz et al., 2017; Young et al., 2004). We collected two fresh faecal samples/week from each subject (both control and test) during the entire study period. We collected all fresh faecal samples using the dry sampling approach (Biswas et al., 2019) and stored them in -20°C freezer onsite and later transported them to the Wildlife Institute of India in dry ice. The samples were stored in the laboratory at -20°C freezer until further processing. To control for effects of moisture and diet, we pulverized the frozen samples before lyophilizing (#FD-5, Allied Frost, New Delhi, India) them for 72 hours before hormone extraction (Mondol

Table 3.4: Corticosterone FGM assay details

Assay method	Dilution	Slope (R2)	Inter-assay CV	Intra-assay CV	Cross-reactivity
EIA	1:60	0.92 (0.99)	9.0	9.8	100% with corticosterone, 12.30% with Desoxycorticosterone, 2.30% with Tetrahydrocorticosterone and <1% with Aldosterone, Cortisol, Progesterone, Dexamethasone, Corticosterone-21-Hemisuccinate, Cortisone and Estradiol

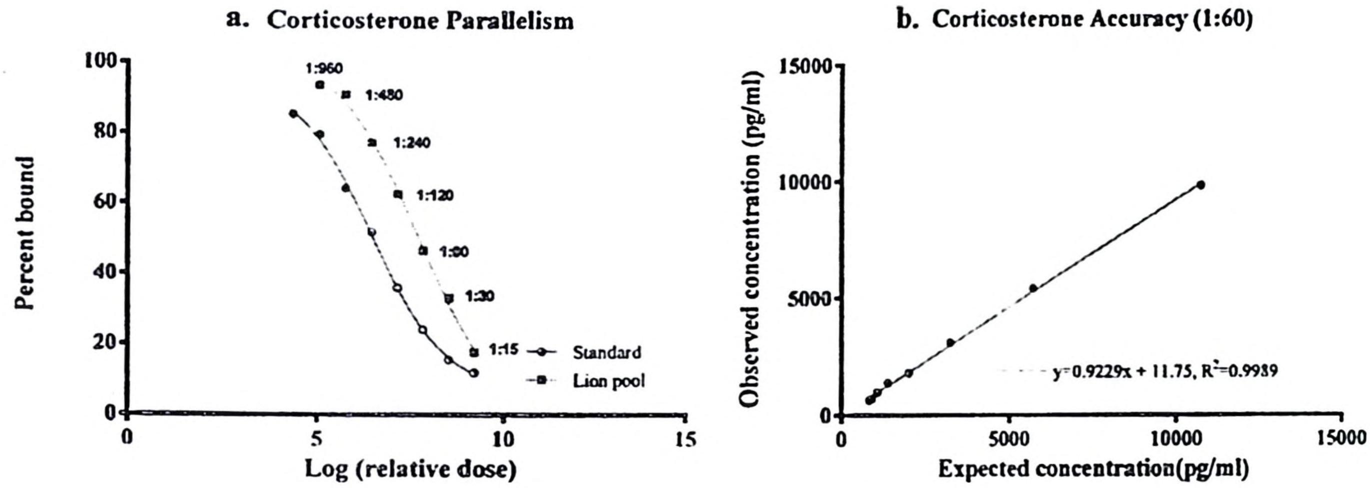

Figure 3.2: Parallelism and accuracy graphs for Corticosterone validation assay

et al., 2020). Subsequently, we sieved lyophilized samples through a 0.5 mm stainless steel mesh to obtain a homogenized faecal powder. We thoroughly mixed the dried faecal powder and extracted hormone by pulse-vortexing 0.1 grams of powder in 15 ml of 70% ethanol, followed by centrifugation at 2200 rpm for 20 min (Mondol et al., 2020; Wasser et al., 2010). The hormone extracts were stored in 2 ml cryochill vials (1:15 dilution) and stored in -20°C freezer till further analyses. We used a corticosterone EIA kit (#K014, Arbor Assays, MI, USA) for corticosterone metabolite estimation in faecal hormone extracts.

Sample extracts were air-dried inside an incubator and resuspended in assay buffer as per required dilutions. Each sample was assayed in duplicate using the kit protocol and the optical density was measured at 450 nm using an ELISA plate reader (#GMB-580, Genetix Biotech Asia Pvt. Ltd., New Delhi, India). Hormone metabolite concentration is interpolated using four parametric logistics (4PL) regression functions in GraphPad Prism software version 5 (GraphPad Software, California, USA). Cross-reactivities of the antibody are listed in Table 3.4. We tested for parallelism and accuracy to validate the corticosterone assay. We used dilutions of pooled extracts from a random combination of male and female samples (N = 20) to assess reliable quantification of corticosterone at different concentrations and find optimal dilutions for final assays (at 50% binding). The relative dose was plotted against per cent bound hormones for the pools and the standards and generated a best-fit curve using 4PL regression, where parallel slopes indicate similar immunoreactivity at different concentrations. For the accuracy test, I spiked corticosterone standards with equal volumes of diluted faecal extract of known concentration (dilution level close to 50% bound from parallelism test) and assayed with standards. We plotted the results as regression lines using observed and expected concentrations to show that faecal contaminants were not interfering with assay accuracy at the tested dilution. We calculated inter-and intra- assay coefficients of variation using repeated measures of same-pooled extract.

3.3 Data analysis

I tested the following hypotheses in this study:

1. There would be no variations in behaviour indices between male and female subjects and the measures would perform uniformly for both sexes.
2. The wild-rescued lions would display higher behaviour diversity than captive-raised animals.
3. Bold individuals would differ in behavioural welfare indices compared to shy individuals.
4. There would be no difference in behavioural welfare parameters between pair-housed and group-housed subjects.
5. There will be no significant prediction of behavioural diversity by the proportion of aberrant repetitive behaviour and the enclosure usage patterns of subjects.

I used R statistical software version 4.3 through RStudio (Team, 2020) using packages, "tidyverse" (Wickham et al., 2019), "ggstatsplot" (Patil et al., 2018), "effectsize" (Ben-Shachar et al., 2020) and "funModeling" (Casas, 2019) for all statistical analaysis and graphical outputs. For exploratory data analyses, I used the Shapiro-Wilk test and Levene's test to ascertain the normal distribution and homogeneity of variance in SPI, SWI, latency, ARB and FGM values, respectively. I conducted a bivariate Pearson's correlation to ascertain the strength of association between the four above-mentioned welfare indices.

I had four categorical predictor variables (personality trait, rearing history, sex, and social grouping), each with two levels (viz., bold and shy, wild-rescued and captive-raised, male and female and pair-housed and group-housed). I compared welfare indices across groups (categorical predictor variables) using independent samples t-tests (Delacre et al., 2019) for normally distributed dependent variables (enclosure usage, behaviour diversity, ARBs, and Faecal corticosterone metabolites). Later, I grouped animals in four categories viz., captivebold ($N = 14$) , captive-shy ($N = 2$) , wild-bold ($N = 7$) , wild-shy ($N = 12$) and compared welfare indices across these categories. We cannot make any reasonable assumptions about the captive-shy group since it is represented by only two animals. However, I have kept this group in all the subsequent figures and graphs since most *ex-situ* institutions are likely to have only captive-born animals across bold and shy personality traits. In the first step, I conducted a non-parametric Kruskal-Wallis test to find if there was any difference between the four groups of Asiatic lions. I used post-hoc pairwise comparisons using the Welch's t-test, Dunn's test and Games-Howell test to understand the differences in welfare indices. I primarily relied on non-parametric tests, since most of them rely on the equality of variances between the test groups. I used the non-parametric Kruskal-Wallis test for latency measures and the proportion of bold and shy behaviours performed by subjects during the novel object test. When comparing between means of two groups with different sample sizes, it is important to report effect sizes in addition to p-values to indicate the scale-independent degree of difference. I calculated effect sizes to quantify differences in welfare measures between groups (Cohen, 1992; Lakens, 2013).

Table 3.5: Comparative table for the proportion of bold and shy behaviours performed by subjects during novel object tests

Personality	N	Mean	Std. Dev	t(33)	Sig (2-tailed)
Shy lions	14	15.86	9.591	-10.574	0.001
Bold lions	21	87.24	8.746	NA	NA

Finally, I conducted multiple regression analysis to understand how the behaviour diversity of captive animals were predicted by their enclosure usage patterns and ARB levels. Before conducting a regression analysis, I checked for multicollinearity between independent variables using measures of VIF (variance inflation factor). Since enclosure usage and ARBs were not highly correlated, I used them as predictors for behaviour diversity in regression analysis.

3.4 Results

3.4.1 Validation of keeper ratings

The mean latency times for all subjects after averaging both trials was 47.76 seconds (SD = 46.85). Subjects categorized as bold by keepers (M = 11.13, SD = 3.65, N = 21) showed significantly lower latency values ($z = 2.89$, $p < 0.01$, Cohen's $d = 7.28$) compared to subjects categorized as shy (M = 102.71, SD = 17.4, N = 14) (Table 3.6, Figure 3.4).

Bold subjects also showed significantly higher percentage of bold behaviours (M = 87.24, SD = 8.74) than shy individuals (M = 15.86, SD = 9.5) ($t(33) = -10.57$, $p<0.01$) (Table 3.6). These results validate the keeper rating of subjects on the bold-shy scale.

3.4.2 Comparison of welfare measures across categorical independent variables

I will first discuss the effects of each trait of individual variation across the different welfare indices. Since it was apparent that personality and rearing-history are both instrumental in shaping the differential responses of subjects to similar husbandry practices. I combined personality trait features and rearing-history to create four category

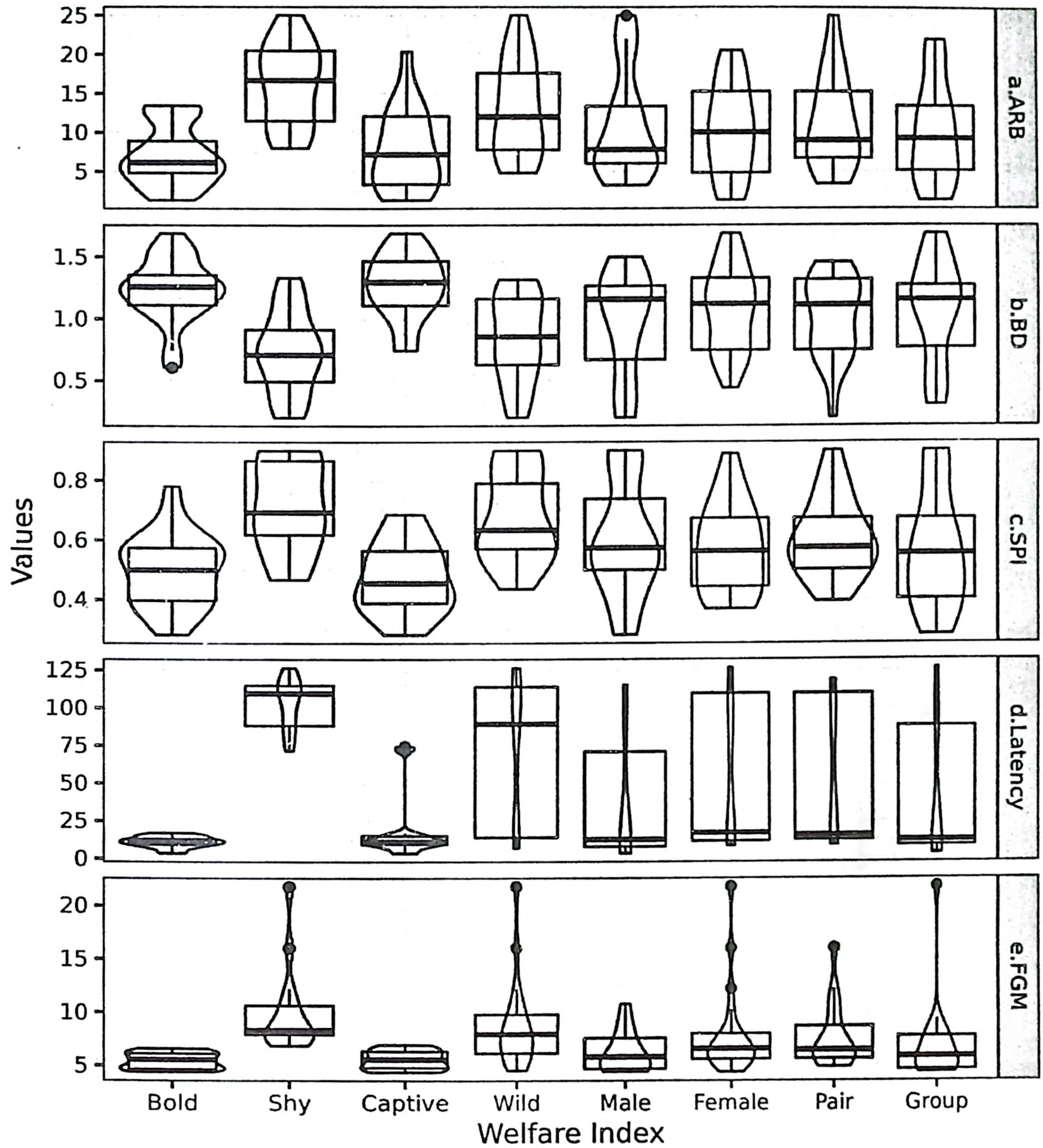

Figure 3.3: Comparison of welfare indices

of subjects, captive-bold (N = 14) , captive-shy (N = 2) , wild-bold (N = 7) , wild-shy (N = 12). In this section, I will also show how subjects belonging to each of these unique personality and rearing histories fare under similar husbandry conditions.

Latency to novel objects

Captive-raised individuals (M = 18.61, SD = 21.55, N = 16) displayed significantly (z = 1.86, p = 0.02, Cohen's *d* = 1.42) lower latency compared to wild-rescued individuals

Table 3.6: Pairwise comparisons for welfare indices in Asiatic lions with different lifehistory and personality traits

Comparison	Z	P.unadj	P.adj
Latency			
Bold_captive - Shy_wild	-4.695930	0	0.00
Bold_wild - Shy_wild	-3.620491	0	0.00
Enclosure usage (SPI)			
Bold_captive - Shy_wild	-4.050267	0	0.00
ARB			
Bold_captive - Shy_wild	-3.818894	0	0.00
Bold_wild - Shy_wild	-2.872927	0	0.02
Behaviour diversity			
Bold_captive - Shy_wild	4.069521	0	0.00
FGM level			
Bold_captive - Shy_wild	-4.713980	0	0.00
Bold_wild - Shy_wild	-3.591427	0	0.00

(M = 72.30, SD = 48.7, N = 19) (Table 3.7 and Figure 3.4). I found no difference in latency scores between male (M = 37.02, SD = 45, N = 14) and female (M = 54.9, SD = 47.8, N = 21) subjects (z = 0.89, p = 0.39, Cohen's *d* = 0.38) (Table 3.7). Latency values did not vary significantly between pair-housed (M = 55.14, SD = 48.2, N = 17) and group-housed (M = 40.78, SD = 45.81, N = 18) lions (z = 0.9, p = 0.31, Cohen's *d* = 0.3).

Latency measures varied significantly across the four groups of lions with different lifehistory and personality traits ($F_{welch}(3, 6.80) = 577.83$, p = 0). Wild-rescued shy lions (M = 107.65 , SD = 13.07, N = 12) and wild-rescued bold lions (M = 11.7 , SD = 3.45, N = 7) , were significantly different from one another in latency measures (z = -3.62, $p < 0.05$, Cohen's *d* = 8.96). On the other hand, the captive-raised bold lions (M = 10.84, SD = 3.85, N = 14), showed lower latency levels than captive-raised shy lions (M = 73.06, SD = 1.83, N = 2) (Cohen's *d* = 0.86) but the number of shy-captive animals were disproportionately lower to validate these findings (Figure 3.4).

Enclosure usage

The spread of participation index (SPI) for enclosure usage is an important welfare indicator that determines the functional role of the captive environment for the animals. Enclosure space usage patterns varied significantly between subjects with different

Table 3.7: Comparison of welfare indices (viz., enclosure usage, behaviour diversity, aberrant behaviours, and latency to novel objects) between Asiatic lions of different categories (captive-raised vs wild-rescued, bold vs shy, male vs female, and pair-housed vs group- housed)

Welfare indices	Group-A	Group-B	test statistic	p-value	Effect size (Cohen's d)
Personality type					
Personality	Bold(n = 21)	Shy(n = 14)			
Enclosure usage	0.5±0.12	0.71±0.15	-4.57	<0.000	1.54
Behaviour diversity	1.23±0.26	0.73±0.34	4.89	<0.000	1.64
Aberrant behaviours	7.01±3.9	16.13±5.4	-5.82	<0.000	1.94
Latency to novel object	11.13± 3.65	102.71±17.4	-4.95	<0.000	7.24
FGM	5.43±0.75	10.16±4.16	17.58	<0.000	1.76
Life-history type					
Lifehistory	Captive(n =16)	Wild(n = 19)			
Enclosure usage	0.47±0.12	0.67±0.15	4.28	<0.000	1.47
Behaviour diversity	1.26±0.3	0.83±0.35	-3.94	<0.00	1.35
Aberrant behaviours	7.74±5.3	13.12±6.25	2.71	0.1	0.92
Latency to novel object	18.61±21.55	72.30±48.7	2.89	0	1.42
FGM	5.57±4.23	8.8±4.23	10.51	0.004	1.01
Social grouping					
Social grouping	Pair housed (n =17)	Group housed (n =18)			
Enclosure usage	0.6±0.13	0.56±0.19	-0.69	0.49	0.22
Behaviour diversity	0.99±0.33	1.06±0.42	0.46	0.64	0.18
Aberrant behaviours	11.33±6.12	10.02±6.69	-0.6	0.55	0.2
Latency to novel object	55.14±48.2	40.78±45.81	0.95	0.31	0.3
FGM	7.72±3.02	6.94±4	0.42	0.51	0.22
Sex					
Sex	Male(n = 14)	Female(n = 21)			
Enclosure usage	0.61±0.2	0.57±0.15	5.28	0.6	0.17
Behaviour diversity	0.96±0.43	1.1±3.5	-0.85	0.4	0.28
Aberrant behaviours	11.04±7.05	10.41±6.02	0.28	0.78	0.09
Latency to novel object	37.02±45	54.91±47.8	-1.11	0.27	0.38
FGM	6.34±1.98	7.98 ± 4.18	2.39	0.13	0.47

Table 3.8: Effect of personality and rearing history on latency to novel objects

	n	Mean	Std.Dev	Median
Bold_captive	14	10.84	3.85	10.79
Bold_wild	7	11.70	3.45	12.53
Shy_captive	2	73.06	1.83	73.06
Shy_wild	12	107.65	13.07	111.50

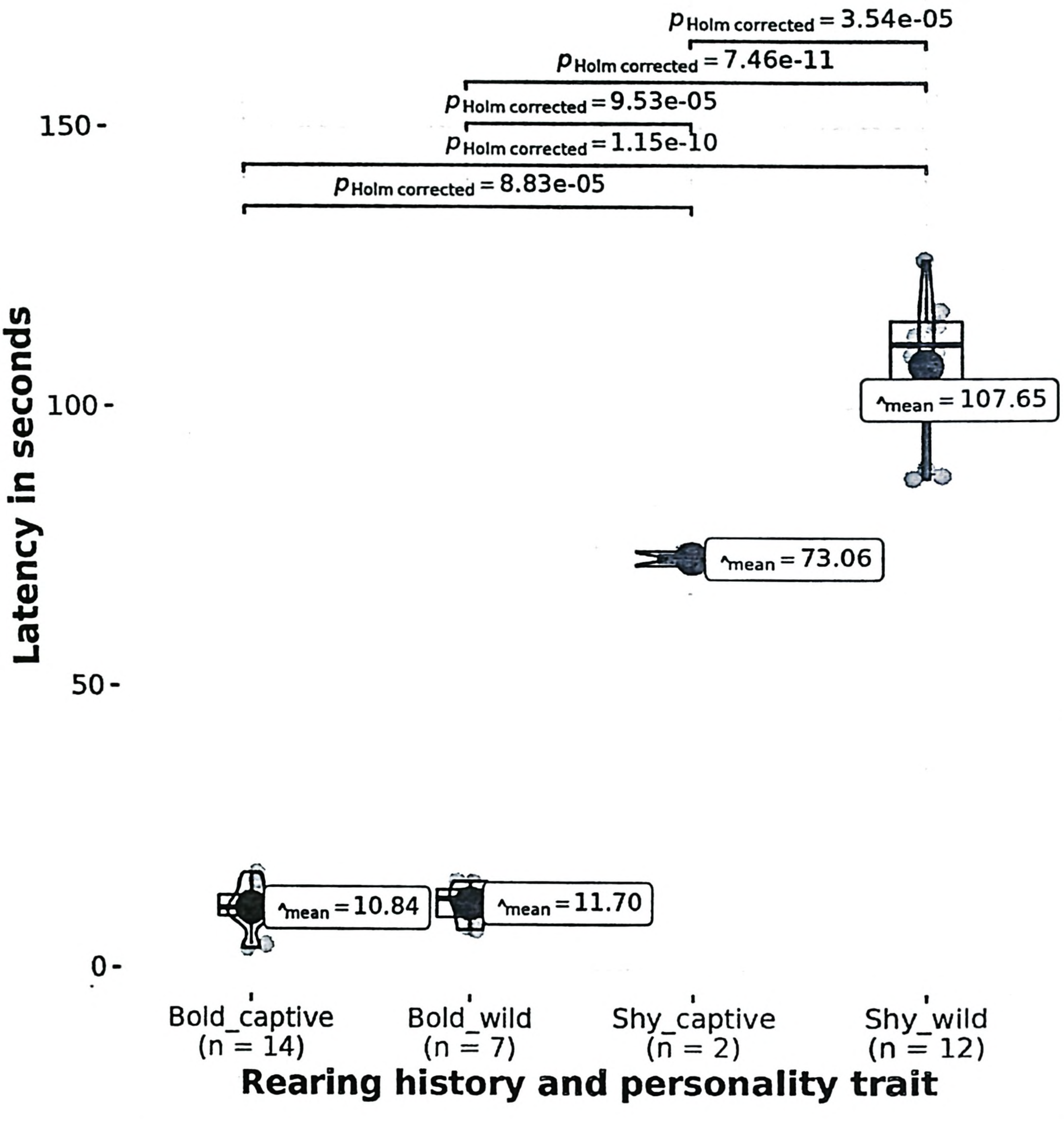

Figure 3.4: Latency measures across personality and rearing history categories

personality types and rearing histories. Wild-rescued individuals used enclosure space less homogeneously (M = 0.67, SD = 0.15, N = 19) than captive-raised individuals (M = 0.47, SD = 0.12, N = 16) (t (33) = 4.28, p<0.01, Cohen's *d* = 1.47) (Table 3.6 and Figure 3.5). Subjects with bold personality traits showed significantly less enclosure-zone bias (M = 0.5, SD = 0.12, N = 21) compared to individuals with shy traits (M = 0.71, SD = 0.15, N = 14) (t (33) = -4.572, p <0.01, Cohen's *d* = 1.54). Overall, the SPI value of males (M = 0.61, SD = 0.20, N = 14) was not significantly different from

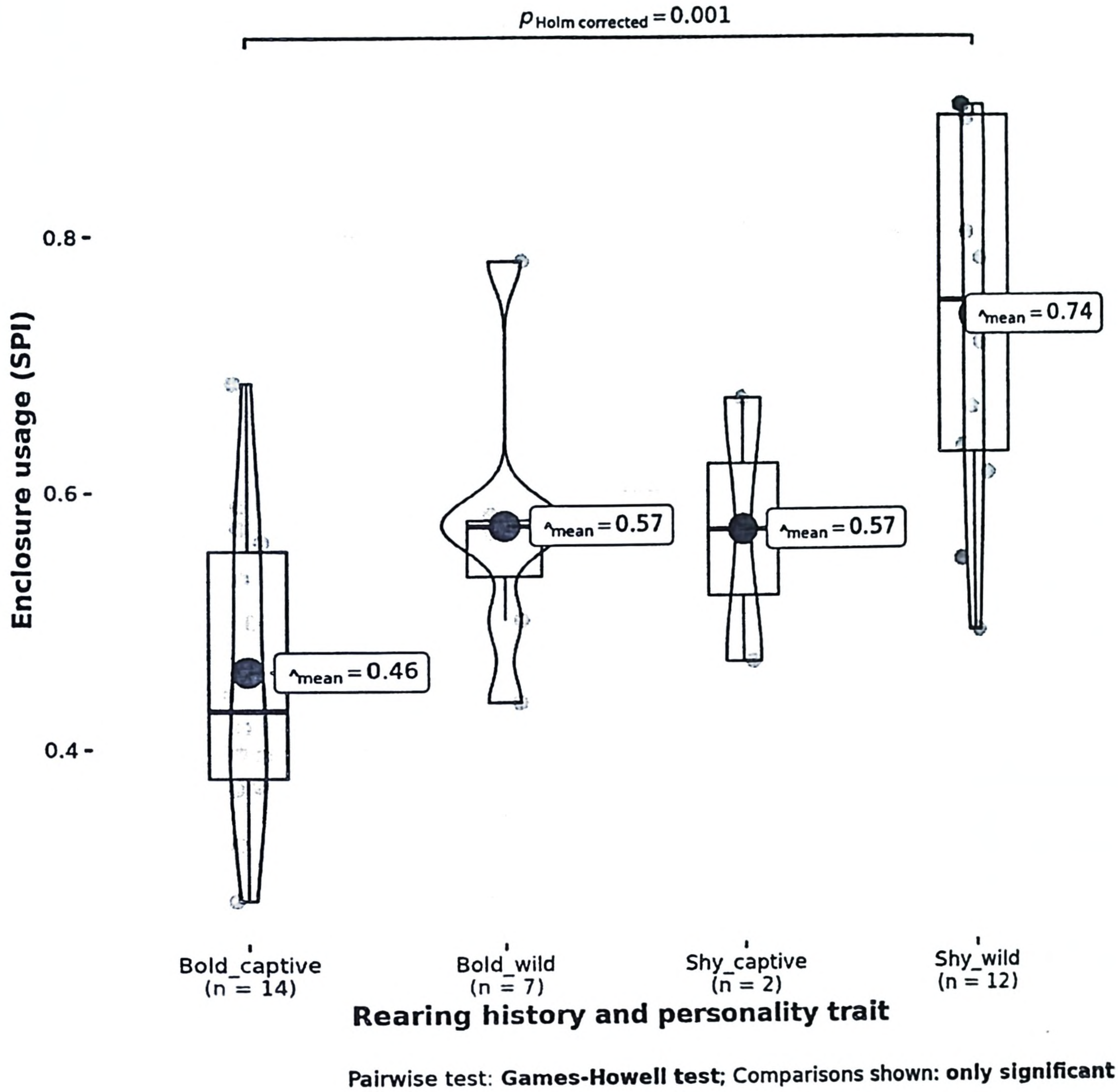

Figure 3.5: SPI measures across personality and rearing history categories (N = 120,540 scans)

Table 3.9: Effect of personality and rearing history on enclosure usage (SPI)

	n	Mean	Std.Dev	Median
Bold_captive	14	0.46	0.12	0.43
Bold_wild	7	0.57	0.11	0.57
Shy_captive	2	0.57	0.14	0.57
Shy_wild	12	0.74	0.15	0.75

female (M = 0.57, SD = 0.15, N = 21) lions (t (33) = 5.28, p = 0.6, Cohen's *d* = 0.17). The enclosure usage patterns of group-housed (M = 0.56, SD = 0.19, N = 18) and pair-housed subjects (M = 0.60, SD = 0.13, N = 17) (t(33) = -0.69, p = 0.49, Cohen's *d* = 0.22) were similar (Table 3.7 and Figure 3.5) .

Table 3.10: Effect of personality and rearing history on behaviour diversity (BD)

	n	Mean	Std.Dev	Median
Bold_captive	14	1.29	0.25	1.30
Bold_wild	7	1.10	0.25	1.20
Shy_captive	2	1.05	0.40	1.05
Shy_wild	12	0.68	0.32	0.70

We found that wild and shy lions had a higher (M = 0.74, SD = 0.15, N = 12) SPI compared to captive-raised bold animals (M = 0.46, SD = 0.12, N = 14). There were significant differences between different personality and rearing history groups for SPI measures ($F_{welch}(3, 4.60) = 7.14$, p = 0.0342). In termns of pair-wise comparisons, there was a stark difference in enclosure usage between captive-raised bold lions (M = 0.46, SD = 0.12) as compared to wild_rescued bold lions (M = 0.57, SD = 0.11, N = 7) (Table 3.3). Curiously we found that the captive-raised shy lions had a similar enclosure usage pattern to the bold, wild-rescued animals (M = 0.57, SD = 0.14, N = 2) (Table 3.7 and Figure 3.5).

Species-typical behaviour diversity

I found that species-typical behaviour diversity of captive-raised animals (M = 1.26, SD = 0.3, N = 16) was significantly higher than wild-rescued animals (M = 0.83, SD = 0.35, N = 19) (t (33) = -3.94, p <0.01, Cohen's *d* = 1.35) (Table 3.7 and Figure 3.6). Bold subjects displayed higher behaviour diversity (M = 1.23, SD = 0.26, N = 21) than shy individuals (M = 0.73, SD = 0.34, N = 14) (t (33) = 4.89, p<0.01, Cohen's *d* = 1.64) (Table 3.7. and Figure 3.3). Behaviour diversity levels were similar between male (M = 0.96, SD = 0.43, N = 14) and female (M = 1.1, SD = 0.35, n = 21) lions (t(33) = -0.85, p = 0.4). Group-housed (M = 1.06, SD = 0.42, n = 18) and pair housed subjects (M = 0.99, SD = 0.33, n = 17) showed similar levels of behaviour diversity (*t* (33) = 0.64, p = 0.64, Cohen's *d* = 0.18).

behaviour diversity levels differed significantly across personality and rearing history groups (F_{welch} (3, 4.48) = 7.53, p = 0.0327). Pairwise comparisons revealed that

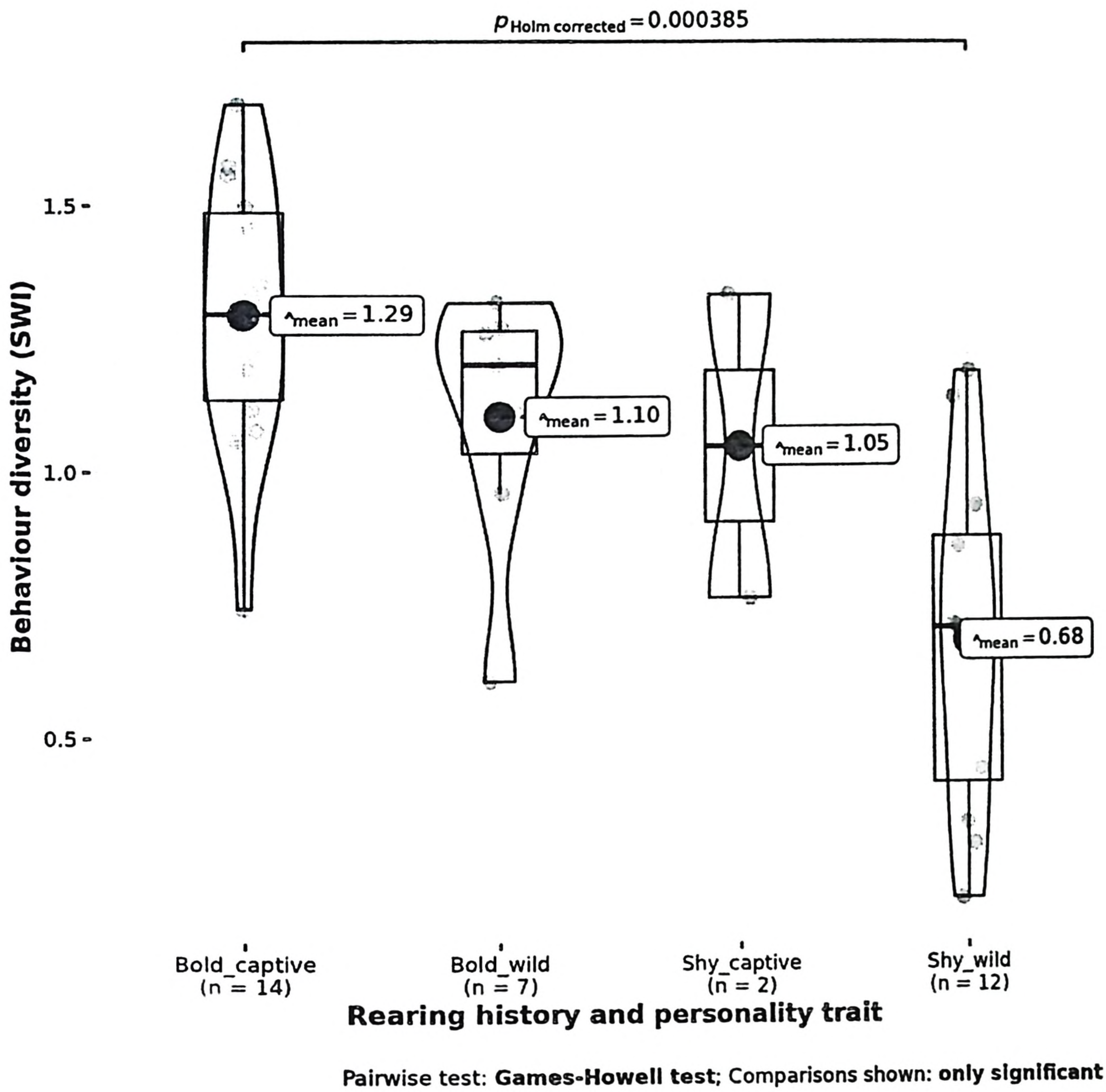

Figure 3.6: Behaviour diversity across personality and rearing history categories (N = 120,540 scans)

there were significant differences in behaviour diversity levels between the bold captive-raised lions (M = 1.29, SD = 0.25, N = 14) and the shy wild-rescued subjects (M = 0.68, Sd =0.32, N = 12) (z = -4.7, p <0.05, Cohen's *d* = 2.17). From Table 3.5 we can notice that the enclosure usage and behaviour diversity varied similarly across lions with different personality and rearing-histories.

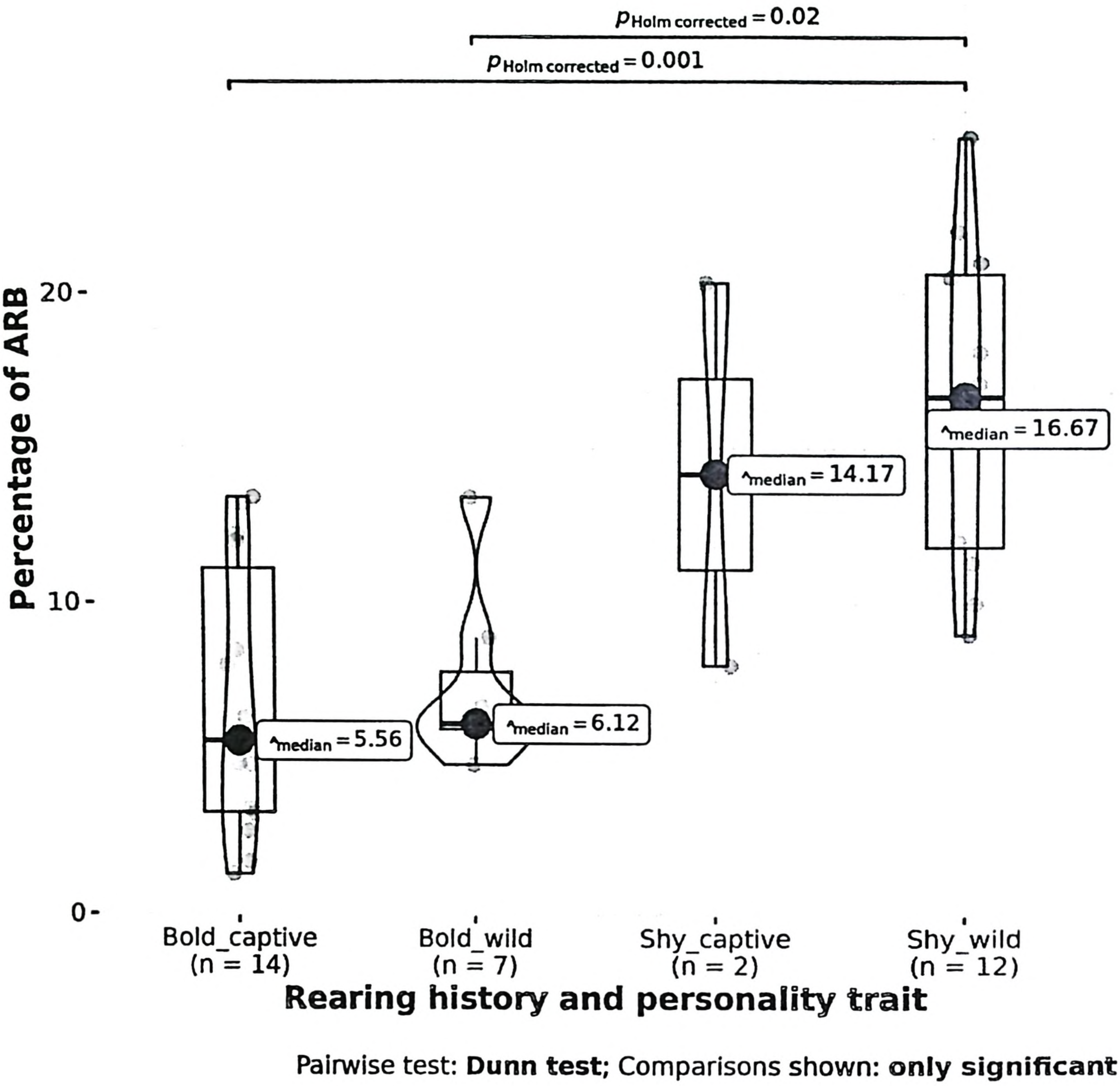

Figure 3.7: ARB measures across personality and rearing history categories (N = 120,540 scans)

Aberrant-repetitive behaviours (ARB)

Wild-rescued individuals (M = 13.12, SD = 6.25, N = 19) expressed higher proportions of ARBs than captive-raised individuals (M = 7.74, SD = 5.3, N = 16) (t (33) = 2.71, p = 0.01, Cohen's *d* = 0.92) (Table 3.7, Figure 3.7).

Bold individuals (N = 21) showed significantly lower levels of stereotypic behaviour such as pacing and swaying (M = 7.01, SD = 4, N = 21) compared to shy individuals (M = 16.13, SD = 5.4, N = 14) (t(33) = -5.82, p<0.01, Cohen's *d* = 1.94) (Table 3.7, Figure 3.7). I found no difference in the expression of ARBs between male (M = 11.04,

Table 3.11: Effect of personality and rearing history on aberrant repetitive behaviours (ARB)

	n	Mean	Std.Dev	Median
Bold_captive	14	6.83	4.40	5.56
Bold_wild	7	7.40	2.93	6.12
Shy_captive	2	14.17	8.73	14.17
Shy_wild	12	16.46	5.13	16.67

SD = 7.05, N = 14) and female (M = 10.41, SD = 6.02, N = 21) subjects (t (33) = 0.282, p = 0.78, Cohen's *d* = 0.09) , as well as between group-housed (M = 10.02, SD = 6.69, n = 17) and pair-housed subjects (M = 11.33, SD = 6.12, n = 17) (t(33) = -0.6, p = 0.55, Cohen's *d* = 0.2).

When I combined rearing-history and personality traits, lions belonging to different personality and rearing history groups varied significantly in their measures for aberrant repetitive behaviours (F_{welch} (3, 4.46) = 8.06, p = 0.03). Pairwise comparisons revealed that the bold and captive born lions (M = 6.83, SD = 4.4, N = 14) showed significantly lower amount of stereotypic behaviours as compared to the Shy and wild rescued animals (M = 16.46, SD = 5.13, N = 12) (z = -3.18, p<0.05, Cohen's *d* = 2.03). Bold and wild rescued lions (M = 7.4, SD = 2.93, N = 7) showed significantly lower levels of ARB as compared to Shy and wild rescued lions (z = -2.87, p < 0.05, cohen's d= 2.02). We had only two Shy-captive born lions (M = 14.17, SD = 8.73, N = 2), whose ARB levels were closer to that of the Shy and wild-rescued animals and much higher compared to Bold and captive-raised animals.

Faecal corticosterone metabolite levels (FGM)

Parallelism and accuracy tests for corticosterone metabolites showed reliable measures from lion faeces across different concentration ranges. Serial dilutions of faecal extracts paralleled the standard curves (Figure 3.2a). There were no differences between slopes of standard and pooled extract curves for corticosterone (F (1,10) = 2.06, p = 0.182) , and the curves were significantly different in their elevation (F (1,11) = 66.25, p = 0.0001). Accuracy tests produced slopes of 0.92 at working dilution of 1:60 (Figure

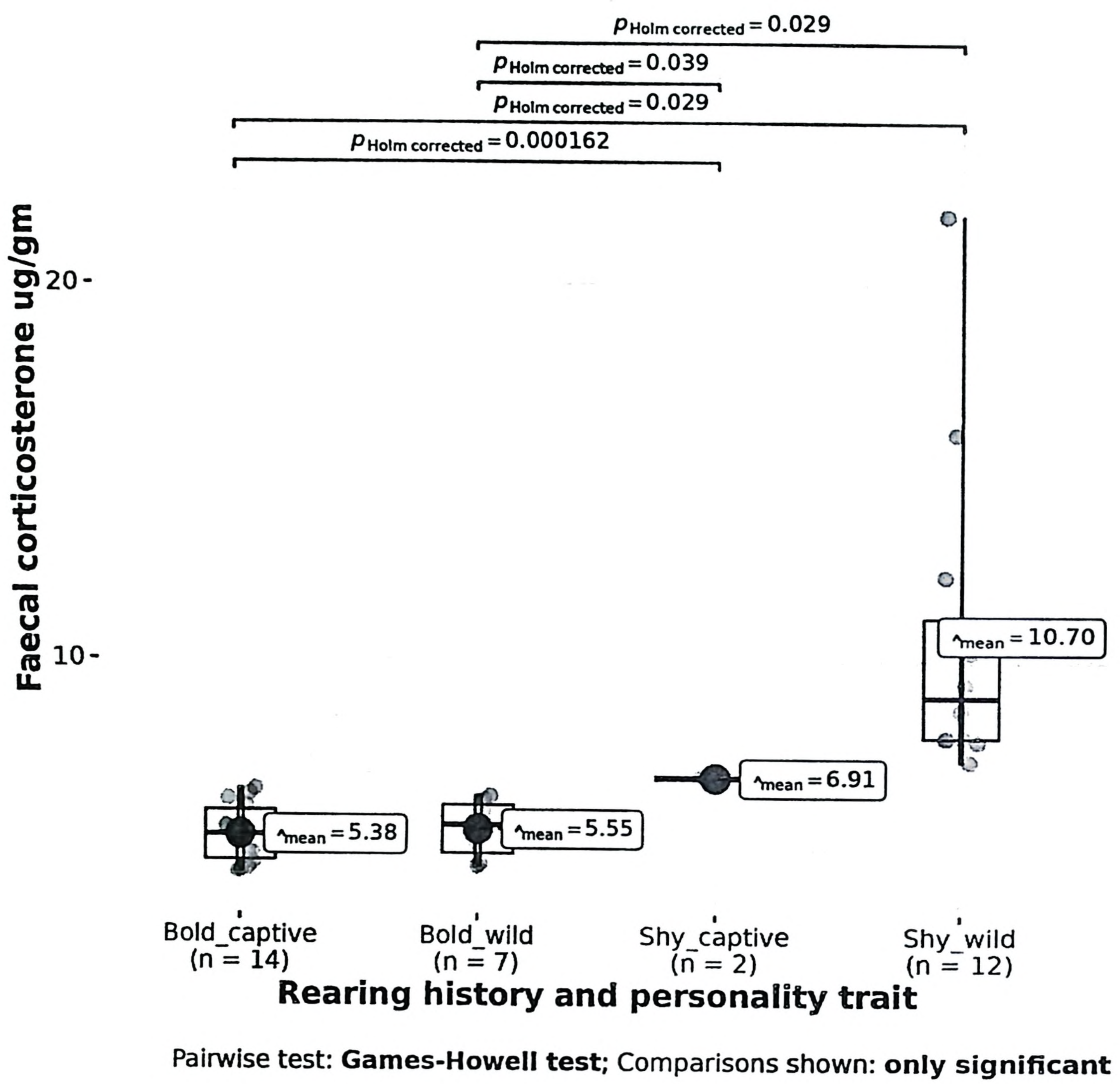

Figure 3.8: FGM levels across personality and rearing history categories

3.2b), suggesting that faecal extracts did not interfere with their metabolite measurement precisions. The intra-assay coefficient of variation (CV) was 9.8%, whereas the inter-assay CV was 8.99% (Table 3.4). Wild-rescued animals (M = 8.8 μg/gm, SD = 4.23, N = 19) showed a significantly higher amount of FGM as compared to the captive-raised animals (M = 5.57 μg/gm, SD = 4.23, N = 16) (F_{welch} (1, 19.88) = 10.51, p = 0, cohen's *d*= 2.0181243).

Bold lions (M = 5.44 μg/gm, SD = 0.76, N = 21) showed significantly lower levels of faecal corticosterone metabolites as compared to the shy lions (M = 10.16 μg/gm, SD = 4.17, N = 14) (F_{welch}(1, 13.58) = 17.58, p = 0.00, cohen's *d*= 3.01).

Table 3.12: Effect of personality and rearing history on faecal corticosterone metabolites (FGM)

	n	Mean	Std.Dev	Median
Bold_captive	14	5.38	0.78	5.38
Bold_wild	7	5.55	0.77	5.65
Shy_captive	2	6.90	0.06	6.90
Shy_wild	12	10.70	4.28	9.05

There was no difference between FGM levels across sex and social grouping (Table 3.7). When I combined personality traits and rearing history and compared the FGM levels across these groups. There was a significant difference across groups with different personality and rearing history groups for FGM levels ($F_{welch}(3, 15.39) = 24.73$, $p = 0$). Bold and captive ($M = 5.38$ μg/gm, $SD = 0.78$, $N = 14$) as well as bold and wild-rescued ($M = 5.55$ μg/gm, $SD = 0.77$, $N = 7$) lions had lower FGM levels as compared to the shy and wild-rescued animals ($M = 10.7$ μg/gm, $SD = 4.28$, $N = 12$) ($z = -4.7$, $p < 0.5$). Bold and wild rescued lions had significantly lower level of FGM as compared to the Shy and wild rescued animals ($z = -3.5$, $p < 0.5$, cohen's $d = 1.72$) The two shy captive-raised lions had lower FGM levels as compared to shy wild-rescued lions (Table 3.11).

I found no differences in FGM levels between male ($M = 6.34$ μg/gm , $SD = 1.99$, $N = 14$) and female ($M = 7.98$ μg/gm, $SD = 4.19$, $N = 21$) subjects as well as between group-housed ($M = 6.95$ μg/gm, $SD = 4.01$, $N = 18$) and pair-housed subjects ($M = 7.73$ μg/gm, $SD = 3.03$, $N = 17$).

3.4.3 Inter-relationship between welfare indices

Latency was positively correlated with enclosure usage bias(SPI) ($r = 0.59$, $N = 35$, $p<0.01$) , proportion of ARBs ($r = 0.57$, $N = 35$, $p<0.01$) and FGM levels ($r = 0.9$, $p <0.01$) (Table 3.13, Figure 3.9). Latency was negatively correlated to behaviour diversity ($r = -0.59$, $N = 35$, $p<0.01$). ARB was positively correlated with latency to novel objects, and enclosure usage ($r = 0.54$, $p = 0.01$) but was strongly negatively correlated with behaviour diversity ($r = -0.88$, $p = 0.01$). Behaviour diversity was negatively correlated with latency to novel objects, ARBs, enclosure usage ($r = -0.61$, $p = 0.01$) and FGM levels ($r = -0.6$, $N = 35$, $p<0.01$) (Table 3.13). Enclosure usage

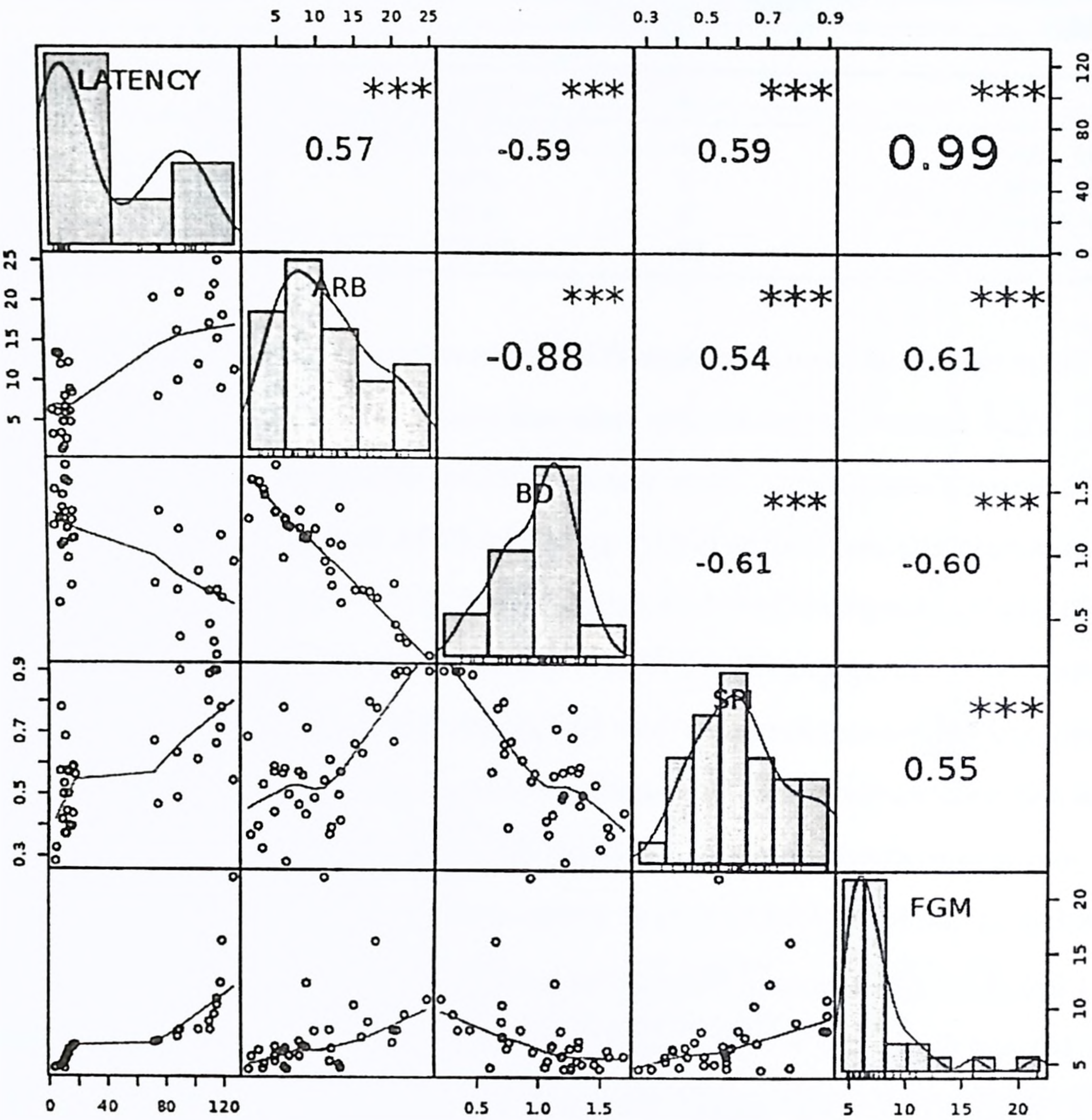

Figure 3.9: Correlation matrix(Spearman's rank) of different welfare indices, Latency, ARB = Aberrant repetitive behaviours, BD = Behaviour diversity, SPI = Enclosure usage and FGM = Faecal corticosterone metabolites
* significance at alpha 0.95, *** significance at alpha 0.99

bias was positively correlated with latency values, and proportion of ARBs ($r = 0.66$, $p<0.01$), but was negatively correlated with behaviour diversity ($r = -0.71$, $p<0.01$).

FGM levels showed a strong positive correlation with latency values of subjects ($r = 0.99$, $p = 0.01$) FGM levels had a positive correlation with enclosure usage values and ARB levels. As expected, FGM levels were negatively correlated with behaviour diversity and positively correlated with ARB. Therefore, it is evident that an increase in behaviour diversity is associated with a lowering in ARB, an increase in enclosure zone

Table 3.13: correlation matrix of welfare indices (Spearman's rank correlation)

	LATENCY	ARB	BD	SPI	FGM
LATENCY	1.00	0.57	-0.59	0.59	0.99
ARB	0.57	1.00	-0.88	0.54	0.61
BD	-0.59	-0.88	1.00	-0.61	-0.60
SPI	0.59	0.54	-0.61	1.00	0.55
FGM	0.99	0.61	-0.60	0.55	1.00

Table 3.14: Regression table for Behaviour diversity enclosure usage + ARB

Characteristic	**Beta**	**95% CI**	**p-value**
SPI	-0.45	-0.88, -0.02	0.039
ARB	-0.05	-0.06, -0.03	<0.001
FGM	0.00	-0.02, 0.01	0.8
R^2	0.851		
Adjusted R^2	0.836		
Sigma	0.154		
Statistic	58.9		
p-value	<0.001		
df	3		
Log-likelihood	17.8		
AIC	-25.7		
BIC	-17.9		
Deviance	0.740		
Residual df	31		
No. Obs.	35		

usage, and a lower FGM level. Interestingly, the FGM levels and latency measures were strongly positively correlated, which probably indicates a strong physiological relationship to the performance of lions in cognitive tasks.

Multiple regression analysis (Table 3.14) indicated that ARBs and space usage homogeneity explained 85% of the variance in the behaviour diversity ($R^2 = 0.85$, $F(2,32) = 90.92$, $p<0.01$) (Table 3.14). The predicted regression equation is Behaviour diversity = 1.8 + (-0.046) x (ARB) + (-0.46) x (Enclosure usage). These results suggest that lions who display low levels of ARB and use enclosure space more homogeneously are likely to have higher behaviour diversity.

3.5 Discussion

Personality traits of wild-rescued and captive-raised Asiatic lions were the single most important variable that led to variations across welfare indices. Our sample size constitutes 10% of the global captive stock of Asiatic lions, making these results relevant for the global conservation initiatives for this species. Several studies have asserted the importance of multiple indices for welfare assessment at zoos and conservation breeding programmes viz., behaviour diversity (Clark et al., 2012; Powell, 1995), enclosure usage (Kistler et al., 2010; Ross et al., 2016) and stereotypy in captive animals (Clegg, 2018; Dawkins, 2004; Kroshko et al., 2016). However, most *ex-situ* institutions continue to use uni-dimensional measures to assess welfare and seldom address individuality. I addressed this issue by showcasing the importance of an individual-focused multi-dimensional approach to welfare assessments. Overall, Asiatic lions with different personality traits (bold and shy) and rearing-history (captive-raised and wild-rescued) differed significantly on measures of welfare, which supports earlier studies linking animal welfare with individuality (Boissy et al., 2014; Carere et al., 2011; Gartner et al., 2016; Powell et al., 2011). I did not observe any sex-specific variations in behavioural and physiological welfare measures of the subjects, confirming our first hypothesis. Contrary to our second hypothesis, wild-rescued lions showed low behaviour diversity, high enclosure-use bias, increased stereotypy, higher latency to novel objects and elevated FGM levels as compared to captive-raised subjects. Our results challenge existing research findings that report wild-rescued animals to be less likely to develop stereotypies than captive-raised individuals, while not accounting for animal personality (Cooper et al., 1996; Schoenecker et al., 2001). Such a pattern in our study may be driven by a higher proportion of shy individuals (N = 12) in the wild-rescued category compared to the captive-raised subjects (N = 2). Nevertheless, these results clearly show that wild-rescued lions may not necessarily be in a better state of welfare by default when compared to captive-raised individuals under similar housing conditions. Further empirical studies with equal sampling across the bold and shy continuum between captive-raised and wild-rescued individuals are required to confirm these patterns. Our results supported the third hypothesis that lions with bold personalities are more resilient to functionally barren housing conditions than shy subjects, which supports earlier studies (Cole et al., 2014; Japyassú et al., 2014). Moreover, present welfare assessment protocols often do not consider individual requirements as modifiers for species-specific husbandry practices. The association of animal personality with welfare outcomes (Coelho et al., 2012; Izzo et al., 2011; Razal et al., 2016; Young et al., 2003) and its implications for post-release survival (Bremner-Harrison et al., 2004; Meehan et al., 2007) are well documented. This study aligns with the conservation goals for Asiatic lions by addressing individuality in welfare assessment (Coelho et al., 2012; Izzo et al., 2011; Rabin, 2003; Razal et al., 2016). Group-housed and pair-housed subjects were similar across all behavioural welfare indices, supporting our fourth hypothesis. Although the enclosures were aesthetically pleasing, appropriate in terms of size, naturalistic vegetation and social grouping of animals; the abject lack of multi-sensory stimulation in terms of enrichment and novel experiences rendered them functionally barren to the subjects (Reading et al., 2013). In this study, I measured the evenness of enclosure use, which represents the functional space of the enclosure rather than available space. I found that small variations in enclosure sizes do not associate with significant shifts in behavioural welfare of Asiatic lions, which is in line with previous studies that place more importance on enclosure design (Tan et al., 2013), complexity and species-appropriateness than enclosure area (Herrelko et al., 2012; Rose et al., 2013; Webb et al., 2019).

Bivariate correlations and regression model presented in this study underline strong inter-linkages between behaviour diversity, enclosure usage and ARBs (Kroshko et al., 2016; Melotti et al., 2011; Rabin, 2003; Rose et al., 2013). Interestingly we found that FGM levels of lions were strongly correlated with their latency measures. Our findings are in line with existing studies that show fear responses in chronically stressed animals are likely to lead to elevated plasma cortisol levels. Further investigations are required to understand the mechanisms that lead to this result. Our results provided evidence that behaviour diversity can be explained by the level of ARB and space usage patterns, which is in line with findings from existing studies (Clark et al., 2012; Watters et al., 2009; Wemelsfelder et al., 2000). From our results, it can be surmised that subjects that are under less stress (low ARB) are likely to show homogenous enclosure usage and a diverse behaviour repertoire and vice versa. When we categorized Asiatic lions based on their personality and rearing history, we found that there were only two subjects that were captive-shy. Most of the captive-raised subjects were categorized as bold (N = 14). We were surprised to see that the ratio of bold to shy animals was more balanced in wild animals (Bold = 12, Shy = 7). When we compared the welfare indices across these four groups, we found that the most significant differences lay between captive-bold and wild-shy Asiatic lions across all welfare indices across welfare indices. However, we also found some within-group differences within the wild-rescued animals across ARB and FGM levels. Therefore although there was a significant difference between wild-rescued and captive-raised animals across all the welfare indices, we found that bold-wild-rescued lions fared better on most welfare parameters as compared to shy-wild rescued lions. Interestingly, there was no difference between the shy-wild and the bold-wild animals across measures of behaviour diversity and enclosure usage. Therefore the limitations of captivity affected all the wild lions equally in terms of the opportunity to express species-typical behaviours and space usage patterns. But some lions (bold-wild-rescued) were able to cope with the stressor of the captive environment better than the shy-wild-rescued animals. Zoo managers must pay close attention to the development of high enclosure-zone biases (Ross et al., 2016) conjugated with low behaviour diversity (Clark et al., 2012; Rose et al., 2013) as that may develop into severe levels of ARBs (Konjević et al., 2015). Overall, these findings indicate that behavioural and physiological welfare measures (enclosure usage, behaviour diversity, ARB and FGM levels) have strong interlinkages and vary across inter-individual differences (viz., personality, rearing-history, sex, and social grouping). Zoo managers must take a proactive approach to improve the welfare status of captive Asiatic lions (Mendl et al. (2009)). Since enrichment interventions are effective in bringing complexity to sterile enclosures (Reading et al., 2013; Swaisgood et al., 2005), tailored-enrichment interventions must be integrated with husbandry practices for Asiatic lions (Cannon et al., 2016; Powell, 1995). Studies also show that positive keeper-animal relationships can improve welfare (Whitham et al., 2013). Finally, regular behaviour monitoring of captive wild animals should be incorporated into the husbandry practices to improve welfare and prevent the development of stereotypy (Watters et al., 2009).

4

Effects of enrichment interventions on Asiatic lion welfare

4.1 Introduction

The welfare status of Asiatic lions at the Indian conservation breeding programme has not received adequate research focus (Goswami et al., 2020; Pastorino et al., 2017). This chapter explores the effects of enrichment integrated with daily husbandry practices on the welfare of Asiatic lions. Enclosure enrichment has been considered an effective tool to improve welfare in captive animals. Despite the prevalence of incorporating enrichment with daily husbandry practices all over the world, Indian zoos have been slow to adopt such welfare-oriented measures into the daily husbandry practices. Studies conducted at the beginning of the twenty-first century by several zoo biologists (Mallapur & Chellam, 2002; Mallapur et al., 2007) showcased the importance of enclosure design and enrichment in improving animal welfare. Yet more than a decade later, the welfare status of animals housed across most Indian zoos remain vastly unchanged. Conservation breeding programmes for endangered species should be designed to promote species-typical behaviours and cognitive plasticity for better post-release fitness (Rabin, 2003). The deleterious impacts of sterile captive environments manifest with loss of species-typical behaviours and increase in psychosomatic disorders in animals (Broom, 2011; Dawkins, 2004; Fraser, 1999; Rabin, 2003). Therefore, welfare-based management practices are vital for any successful conservation breeding programme (Swaisgood, 2010). The benefits of an individual-centric welfare evaluation (Joslin, 1984) complemented with targeted enrichment interventions has been successfully demonstrated for several captive animals viz., ursids (Carlstead et al., 1991; McGowan et al., 2010), felids (Powell, 1995; Suárez et al., 2017), canids (Cloutier et al., 2014; Leonard, 2008), equids (Bulens et al., 2013), small mammals (Clark et al., 2012; Vargas et al., 1999), reptiles (DeGregorio et al., 2017), and amphibians (Michaels et al., 2014). Apart from improving welfare, enrichment interventions have been shown to play an important positive role in increasing post-release fitness in several species (Brown et al., 2003; Rabin, 2003; Reading et al., 2013). An earlier study on the welfare status of captive Asiatic lions reported that individual variations (personality and rearing history) are associated with differential welfare outcomes in lions housed under similar captive environments (Goswami et al., 2020), necessitating individually tailored husbandry regimen for the animals. This study focuses on identifying the areas for improvement of the current husbandry and management practices for Asiatic lions. Feeding, sensory and manipulable enrichments have been shown to improve the welfare of captive felids (Powell, 1995; Van Metter et al., 2008). I tested the efficacy of a combined enrichment intervention on the welfare of captive Asiatic lions at Sakkarbaug Zoological Garden (SZG). I measured several behavioural (species-typical behaviour diversity, enclosure usage and aberrant repetitive behaviours) and physiological (faecal corticosterone metabolites) welfare indices as a response to enrichment interventions. This is the first controlled trial study to incorporate both behavioural and physiological tools to measure the welfare status change in response to enrichment interventions of Asiatic lions housed at a conservation breeding centre. I hope that our findings assist in the improvement of incumbent husbandry and management practices for the species.

4.2 Methods

4.2.1 Study area

I conducted this study at the Asiatic lion conservation breeding centre at SZG. The conservation breeding programme of Asiatic lions was initiated in 1958 with nine founders and has since proliferated to house more than 47 individuals housed at SZG with several breeding pairs in other participating zoos across the world. Since SZG houses both captive-raised and wild-rescued individuals, it holds intrinsic value for the conservation of Asiatic lions. During the study period, the off-display conservation breeding facility housed 47 individuals in 21 large naturalistic enclosures. A schematic map of the facility is provided in Figure 3.1.

4.2.2 Subjects and housing

The Asiatic lion conservation breeding centre at SZG has 21 enclosures spread across two blocks (block-A= 15, Block-B = 6). Enclosures at block-A and Block-B are located 500 meters apart and provide identical housing and husbandry conditions to the subjects. All enclosures provided adequate space (above 400 m^2/animal in general with some having 1500 m^2/animal) for the animals. The naturalistic enclosures provided subjects with sufficient natural vegetation cover, simulating the habitat of Asiatic lions while protecting subjects from visitor disturbance. Enclosures were well covered with trees and shrubs, which provided subjects with a good combination of shade and sun throughout the day. Fresh drinking water was made available inside retiring cells adjoining the paddock area. Subjects were allowed free access to the paddock area and retiring cubicles throughout the day and night. However, these enclosures offered little in terms of novelty and cognitive enrichment. Our earlier study established low enclosure space utilization, low behaviour diversity, and a high incidence of aberrant repetitive behaviours prevalent in Asiatic lions housed at SZG (Goswami et al., 2020). We selected 15 (block-A = 12, block-B = 3) enclosures housing 35 Asiatic lions housed in pair (1:1 and 0:2) (N = 9) or heterosexual (1:2) (N =6) configurations (Table 3.1). Some of the subjects were wild-rescued (N = 19, Male = 11, Female = 8) , while the rest were born in captivity (N = 16, Male = 3, Female = 13). Subjects were primarily adult animals (N = 31) with a few sub-adults (N = 4) and were randomly assigned to test and control groups to ensure uniformity of treatment. The test group consisted 19 subjects (Male = 7, Female = 12) the control group consisted 16 subjects (Male = 7, Female = 9) , respectively. All subjects were housed in the same enclosure with the same enclosure mates for at least a year and were accustomed to identical enclosure and management practices.

A group of 10 animal keepers and contractual workers carried out all husbandry work for the lions. Keepers typically reported for duty at 0700 hours and cleaned all leftover food from retiring cells during 0730-1100 hours. The leftover food items were weighed to measure the food consumption by each individual. The keepers would provide the daily ration of buffalo meat to the subjects between 1700-1800 hours at the feeding cubicle, where each lion was fed separately. After confirming that each lion had consumed their ration, keepers opened the gate to the paddock area from the retiring cubicle. After the lions had been fed, the gate to the enclosure was kept open giving the animals a choice to stay inside or move out to the paddock area. Keepers were also tasked with behavioural monitoring of the subjects and recorded the commencement and cessation of mating events between subjects. The zoo managers and the animal keepers met regularly to ascertain the welfare need of the captive animals based on subjective evaluations. The approach to welfare assessment was preventative and based solely on incidental keeper observations hence difficult to quantify and address. We expected to observe the effect of novel enrichment devices on the behavioural and physiological welfare indices of these captive animals.

4.2.3 Study design

I hypothesized that enrichment interventions would lead to significant improvement in behavioural (Goswami et al., 2020) and physiological welfare measures for all test subjects in contrast to the controls. I divided the subjects into control (N = 16) and treatment (N = 19) groups by a blind draw of enclosures for enrichment interventions. In the last chapter, we found that sex, social vs paired grouping did not have any bearing on the welfare indices (Goswami et al., 2020).

However we did find that personality and rearing history could impact welfare indicators, which indicates the need for a controlled trial design for the present study. We divided the subjects into control (N = 16, seven enclosures) and treatment (N = 19, seven enclosures) groups by a blind draw of enclosures for enrichment interventions.

I conducted the study in two phases: baseline and post-enrichment. During the baseline period, I measured behaviour for 14 days/subject and collected two fresh faecal samples/subject/week. This was followed by a one-week exposure to the enrichment intervention to get test subjects accustomed to the new devices. During the testing period (14 days), we recorded the behaviour of test subjects as a response to the daily enrichment intervention. Apart from the enrichment interventions, housing and husbandry conditions remained unchanged for both groups throughout the course of the study.

4.2.4 Data collection

Behaviour observations

Two observers (Mr. Ashkar Bloch and I) collected all behaviour data, which necessitated accounting for inter-observer reliability. I created a detailed ethogram (Table ??) of the study subjects from one month of ad-*libitum* behaviour sampling and compared the behaviour data recorded from the same subject by both observers to ensure consistency (Goswami et al., 2020). I commenced data collection only after inter-observer reliability levels remained consistent across three consecutive sessions (Cronbach's alpha >0.9). I simultaneously video recorded all behaviour sessions to aid in data entry and reduce errors. I recorded behaviour during 0500-1100, 1200-1800, and 2200-0500 hours. I used instantaneous scan sampling (Altmann, 1974) at 1-minute intervals to record all behavioural states and all occurrences of behavioural events (Goswami et al., 2020). During every six-hour data collection period, I recorded four hours of behaviour data with 30 minutes rest for observers after every hour. Data for the observer-resting period were later tabulated from the video recordings. I collected 195 $\pm$ 12.3 hours of behaviour data per subject during the study period. I measured three behavioural welfare indices: species-typical behaviour diversity (Wemelsfelder et al., 2000), spread of participation index (Plowman, 2003) and aberrant repetitive behaviours or stereotypy (Mason et al., 2004) to measure the welfare of Asiatic lions (Goswami et al., 2020). I compared the behavioural welfare indices of all test and control subjects across the baseline and enrichment period. Baseline information for the diversity of behaviour repertoire in control and test subjects was collected using the Shannon-Weiner diversity index (SWI) (Goswami et al., 2020). I measured enclosure usage patterns of subjects based on the spread of participation index (SPI) (Plowman, 2003)to understand if the enclosures provided enough complexity to meet their welfare requirement (Plowman, 2003; Ross et al., 2016). I measured the proportion of aberrant repetitive behaviours (ARBs) or stereotypy as an indicator of poor welfare and compared their prevalence between control and test subjects across treatments.

Physiological measures

I measured faecal corticosterone metabolites of all subjects to contrast the physiological impacts of the enrichment interventions on control and test subjects. Faecal corticosterone is a reliable physiological indicator of stress and compromised welfare in captive felids (Ruskell et al., 2015; Schildkraut, 2016; Vaz et al., 2017; Young et al., 2004). I collected two fresh faecal samples/week from each subject (both control and test) during the entire study period (pre-enrichment and post-enrichment).

One day before the faecal sample collection, we cleaned all faecal materials from the enclosures. Before the day of the faecal sample, collection subjects were closely monitored and their defecating spot was marked. Samples that were close to one another were rejected to avoid the risk of cross-contamination. We rejected samples that were old and dry and selected only fresh faecal matter. Samples were collected using the dry sampling approach (Biswas et al., 2019) and stored in -20°C freezer onsite and later transported to the Wildlife Institute of India in dry ice. The downstream analysis to get the corticosterone metabolite concentrations μgm/gm was similar to the previous chapter.

4.2.5 Enrichment interventions

Enrichment interventions add cognitive complexity to enclosures and provide animals with the opportunity to express species-typical behaviours (Mellen et al., 1997; Skibiel et al., 2007). I used three types of enrichment devices: manipulable (Mellen et al., 1997; Powell, 1995), sensory (Skibiel et al., 2007), and feed (Powell, 1995; Skibiel et al., 2007).

Table 4.1: Description of enrichment devices

Sl. no	Enrichment device	Enrichment type	Description
1	Platform	Manipulable	A log platform made to enable subjects to access vantage points within the enclosure.
2	Sam ball	Manipulable	A hard-plastic ball with no holes or perforations.
3	Log branches	Manipulable	Tree logs sections with bark suspended from trees.

Table 4.1: Description of enrichment devices *(continued)*

Sl. no	Enrichment device	Enrichment type	Description
4	Lion bite ball	Manipulable	A hard-plastic ball with a rattle inside suspended by a bungee rope with canvas sheet that acts as a bite.
5	Buffalo shank	Feeding	Buffalo shank suspended from a zipline.
6	Chicken carcass	Feeding	Frozen Chicken carcass suspended from a tree by natural fibres.
7	Buffalo tail	Feeding	Skinned buffalo tail suspended from tree branches.
8	Buffalo blood frozen	Feeding	Frozen buffalo blood cubes, provided on a clean concrete platform.
9	Star anise oil mixed with garlic	Sensory	Sprayed on tree trunks or used to create scent trails to other enrichment devices.
10	Prey dung	Sensory	Sambar (Rusa unicolor) dung smeared on tree trunks or mixed with enclosure soil at certain zones.
11	Conspecific urine	Sensory	Earth drenched in the urine conspecifics from nearby enclosures smeared on tree trunks, or mixed with enclosure substrate at certain zones.

Manipulable enrichments included hanging lion-sam balls, burlap bags, wooden perches/ platforms, wooden planks etc., whereas the sensory enrichments included olfactory augmentations such as scent trails made from the blood of buffalo, urine of unknown conspecifics, and dung of other prey species (Sambar, *Rusa unicolour*) etc.

I also installed sensory enrichment (tactile) by wrapping rough coir rope on the bark of trees inside enclosures to promote auto-grooming and scent-marking behaviours in the subjects. Nutritional enrichment has been shown to garner the highest amount of attention in captive animals and can be useful for addressing neophobia to enrichment devices in shy individuals (Powell, 1995; Resende et al., 2009; Skibiel et al., 2007). I provided a range of nutritional enrichment devices, which included frozen dressed chicken, buffalo tails that were suspended by natural fibre ropes from trees at different parts of the enclosure. While manipulable and sensory enrichment devices were quasi-permanent and required little daily care, nutritional enrichment devices needed to be replenished every day. I installed and replenished enrichment devices between 0600-0700 hours at four enclosures every day. Keepers would arrive early and confine the

Figure 4.1: Enrichment interventions for Asiatic lions

subjects inside retiring cells with food rewards, while the enrichment interventions were placed at designated enclosures.

Table 4.2: Behaviours associated with enrichment devices

Sl. no	Behaviour	Description	Category
1	Aggressive Growl	Baring teeth with short vocalizations directed at conspecifics or humans, while interacting with enrichment.	Aggression
2	Charge	Run up to a conspecific at a rapid short charge as a result of being challenged while suing an enrichment device	Aggression
3	Strike	Hit conspecific with one paw, while defending an enrichment device.	Aggression
4	Bite	Bite conspecific, while asserting control over an enrichment device.	Aggression
5	Allogrooming	Lick conspecific, while interacting with enrichment device.	Social
6	Nuzzling	Rub noses with conspecifics with soft grunts, and inviting them to play with enrichment device.	Social
7	Roll	Rolling in the dirt with conspecific while interacting with a enrichment device.	Social
8	Rub	Rub body or neck against the enrichment device	Explore
9	Sniffing	Raise head and sniff near a sensory enrichment.	Explore

Table 4.2: Behaviours associated with enrichment devices *(continued)*

Sl. no	Behaviour	Description	Category
10	Flehmen	Bare teeth and sniff the air near an enrichment.	Explore
11	Scent mark	Spray urine or scratch the tree next to an enrichment device.	Explore
12	Digging	Dig near an enrichment device.	Explore
13	Scratching	Scratch the trees where enrichment devices are installed.	Explore
14	Chase	Chase away conspecifics trying to use or steal enrichment device successfully or unsuccessfully. Does not result in an overtly aggressive interaction.	Chase
15	Run	Run around the enclosure with the enrichment device in mouth. May or May not be chased by conspecific.	Play
16	Stalk	Stalk an enrichment device.	Play
17	Play	Kick around an enrichment device.	Play
18	Drag	Drag enrichment device along the enclosure	Play
19	Bite	Bite enrichment device.	Play
20	Eat	Eat a food based or sensory enrichment device.	Forage
21	Lick	Lick enrichment devices intently.	Forage
22	Neophobia	Approach enrichment device slowly and run away.	Fear
23	Fearful growl	Growling at enrichment device, ears back and cowering.	Fear
24	Circling	Warily circling around the enrichment device.	Fear

I furnished all enclosure zones with the same combination of manipulable, sensory and nutritional enrichment. To calculate enrichment device preferences, I collated all scans during which Asiatic lions interacted with the enrichment devices. I also recorded the type of behaviours that were performed by the subjects while interacting with the enrichment devices viz., exploration, social play, aggression, foraging and fear (Table 4.2). I counted the different types of behaviours performed per enrichment device by all subjects.

4.2.6 Data analysis

I analysed all data in R v4 (R Core Team, 2020)with R studio (Team, 2020). I used packages "tidyverse" (Wickham et al., 2019), "psych" (Revelle, 2019)and "dplyr" (Wickham et al., 2020),"ggpubr" (Kassambara, 2020), "sur" (Harel, 2020) and "ggplot2" (Wickham, 2016) to calculate statistical outputs and create graphical summaries of the findings. I conducted tests for normality and ensured that variances were similar between groups to select appropriate parametrical and non-parametrical statistical

Table 4.3: Enrichment device usage with behaviour performed (N = 109,238 scans)

Enrichment device	Feed (%)	Manipulable (%)	Sensory (%)
Aggression	2.94	11.51	0.00
Chase	11.54	1.89	0.00
Explore	3.05	5.95	11.77
Fear	0.00	1.96	0.00
Forage	11.48	0.00	0.00
Play	0.00	35.16	1.21
Social	1.16	0.35	0.00
Total	30.20	56.80	12.99

analysis. I used an unpaired t-test to compare the welfare indices between test and control groups during baseline and post-enrichment interventions. I used paired t-tests, to compare the differences in welfare indices within test and control groups and measured the amount of time spent by test subjects on different types of enrichment devices. Additionally, I calculated the effect size (Cohen, 1992) of the enrichment intervention for the test group, which is scale-independent and gives a measure of the magnitude of difference between baseline and post-treatment conditions.

4.3 Results

4.3.1 Enrichment preferences of study subjects

During the baseline period of 14 days, we collected 288,120 scans from all subjects (N = 35). During 14 days of enrichment intervention, we recorded 109,238 scans of test subjects (N = 19) using enrichment devices out of a total of 165,098 scans and 138,083 scans of control subjects (N = 16). In Figure 4.2, we show the results of the hourly percentages of enrichment use and enrichment-directed behaviours performed by test subjects.

The test subjects used manipulable enrichment devices for longer durations (56.8%) compared to all other types viz., sensory (12.99%) and feeding (30.20%) (Table 4.3). It was challenging to measure the usage levels of sensory enrichments accurately and hence these values may have been under-reported in our results. When subjects were released inside the enclosures, they approached the feed-based enrichment devices first

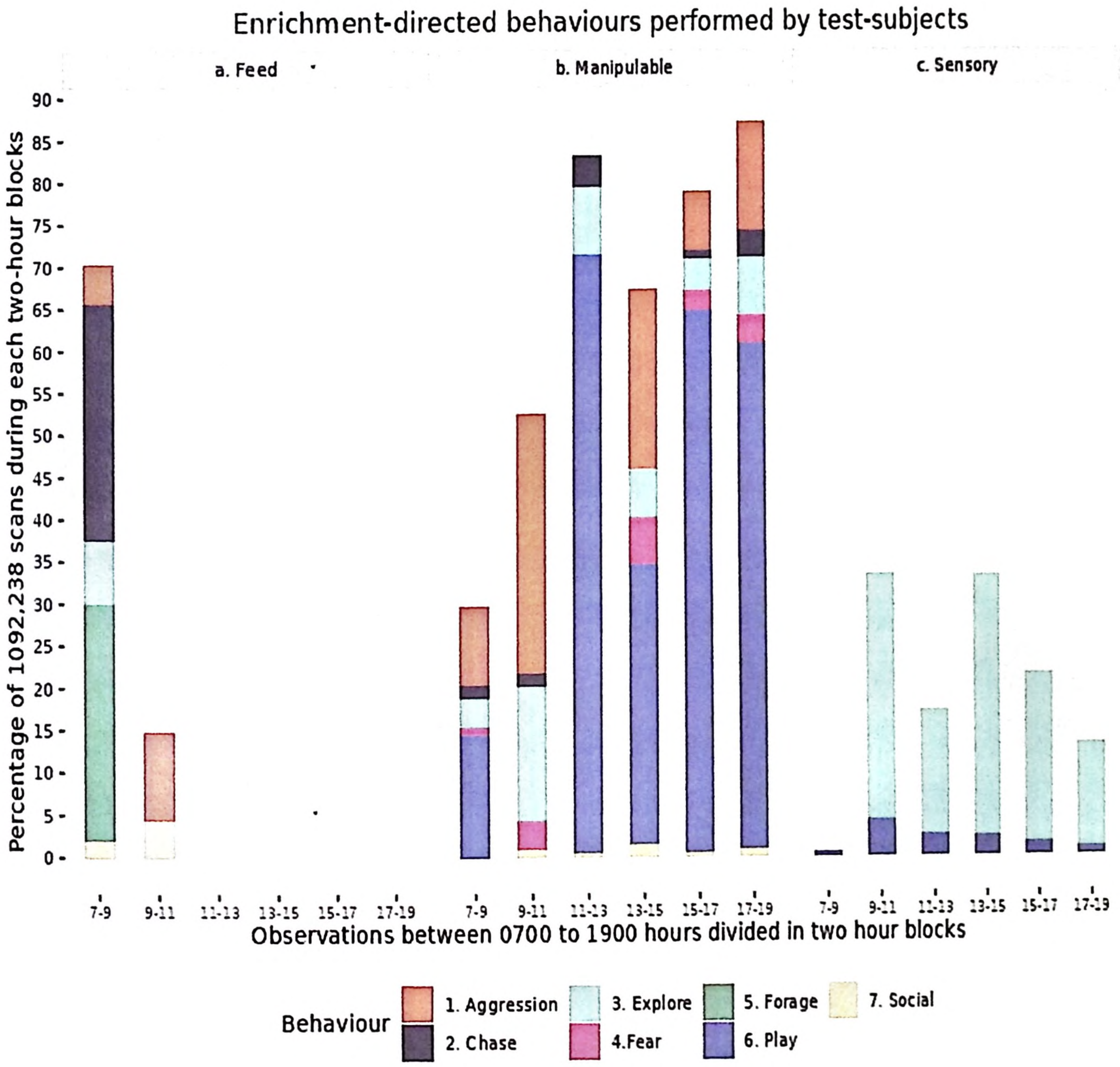

Figure 4.2: Temporal pattern of enrichment device use and enrichment-directed behaviours by Asiatic lions on a. Feeding, b. Manipulable, and c. Sensory enrichment devices. The Primary X-axis represent the hours of the observation period from 0700- 1900 hours divided into two-hour blocks. The primary Y-axis represents the percentage of scans(N = 109,238 scans) during each two-hour block spent performing a certain enrichment-directed behaviour. Enrichment-directed behaviours are represented as coloured stacked bars on the vertical axis (1:7), while enrichment devices associated with said behaviours are represented on separate panels.

Table 4.4: Hourly percentages of enrichment usage and enrichment-directed behaviours by test subjects during the interventions

Behaviours	0700-0900	0900-1100	1100-1300	1300-1500	1500-1700	1700-1900	Total
Enrichment usage during each two-hour block							
Feed	22.130	8.06	0.00	0.00	0.00	0.00	30.20
Manipulable	11.269	6.94	7.02	23.44	5.66	2.44	56.80
Sensory	1.900	2.00	3.18	0.99	3.81	1.09	12.99
Enrichment-directed behaviours performed during each two-hour block							
Aggression	6.780	1.91	1.97	0.95	1.91	1.00	14.57
Chase	7.690	4.31	0.92	0.00	0.15	0.30	13.40
Explore	6.350	2.80	3.71	2.39	4.11	1.40	20.78
Fear	0.000	0.49	0.16	0.48	0.16	0.65	1.96
Forage	7.660	3.83	0.00	0.00	0.00	0.00	11.48
Play	5.950	3.11	3.39	20.60	3.11	0.18	36.29
Social	0.860	0.53	0.08	0.00	0.00	0.03	1.52

followed by manipulable and sensory devices. This trend continued throughout the intervention period.

In terms of enrichment directed behaviours, we found that the peak of aggression (16.87%), chase (22.04%), exploratory (16.14%) and forage (20.04%) behaviours in test subjects occurred between 0700-1100 hours (Table 4.4). The next peak of enrichment-directed behaviours occurred between 1300-1700 hours constituting mostly of play (57.94%) and exploratory behaviours (25.25%) (Table 4.4). Early in the day (0700-0900 hours), when subjects were actively exploring around the enclosure for enrichment devices, aggressive dominant and displacement behaviours were common but subsided as the day progressed (Table 4.4 and Figure 4.2). We found that manipulable devices (11.51%) were more commonly associated with aggressive behaviours compared to food-based enrichment (2.94%) (Table 4.3). We also observed that positive social behaviours were more commonly associated with food-based enrichment devices (1.16%) rather than the manipulable devices (0.35%) (Table 4.3). Subjects usually monopolized the manipulable enrichment devices and showed high fidelity towards specific items. The feed-based enrichment devices were associated with the highest proportion of chase behaviour (11.54%), resulting in one animal monopolizing a device and chasing away others (Table 4.3). Some feed-based enrichment devices were consumed within minutes (e.g. dressed chicken) while others required more processing time (e.g. buffalo tail, frozen blood cubes). Once the feed enrichment devices were

Table 4.5: Summary statistics of welfare indices within control and test groups

Welfare index	Pre-enrichment	Post-enrichment	Statistic	Significance (p-value)	Effect size (Cohen's d)
Control Group (N =16)					
Behaviour diversity	0.9±0.31	0.77±0.35	1.11 (t30)	0.2900	0.39
Spread of participation index	0.82±0.09	0.8±0.13	0.51 (t30)	0.6100	0.17
Aberrant repetitive behaviours	12.81±6	13.31±6.07	0.23 (t30)	0.8100	0.08
Faecal corticosterone metabolites	7.1±4.08	6.96±3.93	0.09 (t30)	0.9200	0.03
Test Group (N = 19)					
Behaviour diversity	0.83±0.35	1.62±0.18	8.8 (t36)	0.0001	2.83
Spread of participation index	0.77±0.12	0.46±0.08	9.37 (t36)	0.0001	3.03
Aberrant repetitive behaviours	12.32±6.03	2.38±2.46	6.65 (t36)	0.0001	2.15
Faecal corticosterone metabolites	7.74±3.25	2.46±0.85	6.85 (t36)	0.0001	2.22

completely utilized, the lions concentrated on the manipulable and sensory enrichment and engaged in social and exploratory behaviours throughout the later parts of the day (Table 4.3, Figure 4.2). The manipulable enrichment devices were associated with the largest variety of species-typical behaviours, which included 35.16% play behaviour (Table 4.3) and were more commonly associated with aggressive interactions (11.51%) compared to all other enrichment types. Aggressive behaviours occurred when enclosure mates tried to steal or use a certain manipulable device from the lion that was playing with it. Since I installed more enrichment devices than subjects in every enclosure, displaced individuals had the opportunity to interact with other devices when displaced by conspecifics. Sensory enrichment devices contributed primarily to exploratory behaviours (11.7%) that led to increased enclosure usage (Table 4.3). However, it is important to point out that the true impacts of different types of enrichment devices cannot be assessed merely through measuring the amount of time the animal spent with it, but by comparing the welfare indices of test subjects with control and their baseline status. The results demonstrate that the presence of different types of enrichment devices was instrumental in mitigating the monopolization of resources by dominant individuals.

During pre-enrichment period, behaviour diversity levels of control subjects ($M = 0.9$, $SD = 0.3$) were similar to that of the test subjects ($M = 0.83$, $SD = 0.35$). After enrichment interventions, the behaviour diversity of control subjects remained unchanged ($M = 0.77$, $SD = 0.35$, $t(30) = 1.11$, $p = 0.29$) while that of the test subjects increased significantly ($M = 1.62$, $SD = 0.18$, $t(36) = -8.8$, $p < 0.001$, Cohen's $d = 2.83$) (Table 4.5 Figure 4.3a).

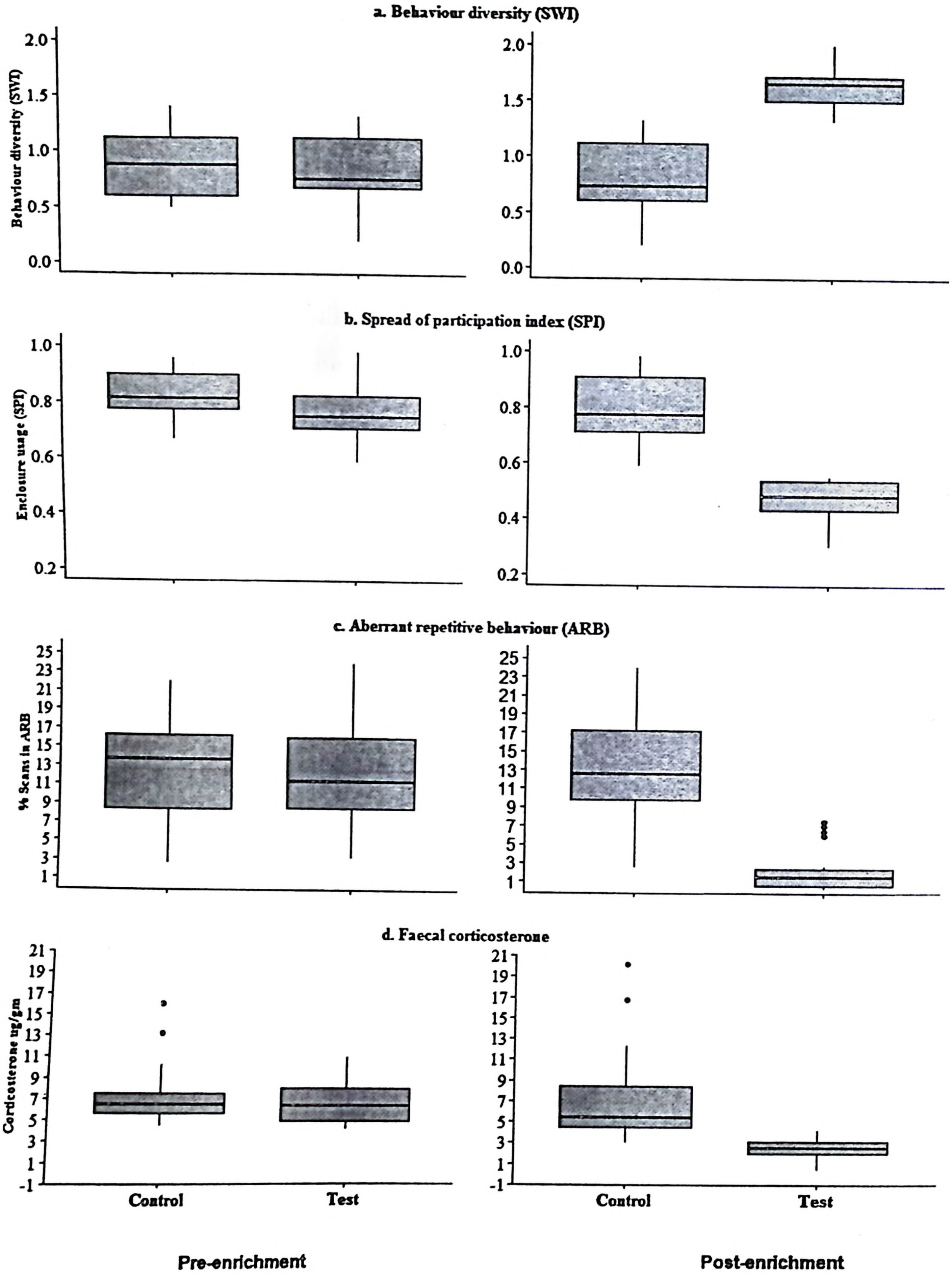

Figure 4.3: Effect of enrichment intervention on welfare indices]Effect of enrichment interventions on a. Behaviour diversity, b. Spread of participation index, c. Aberrant repetitive behaviours, d. Faecal corticosterone metabolites

During the baseline, I observed high SPI values in test (M = 0.77, SD = 0.12) and control groups (M = 0.82, SD = 0.09) indicating enclosure zone use bias. However, after enrichment intervention the SPI values decreased significantly indicating, enclosure usage homogeneity (M = 0.46, SD = 0.08) for test subjects (t(36) = 9.37, p <0.01, Cohen's d = 3.03) , while it remained unchanged for control subjects (M = 0.8, SD = 0.13, t(30) = 0.5, p = 0.61) (Table 4.5 and Figure 4.3b). During baseline, test (M = 12.32, SD = 6.03) , and control subjects (M = 12.8, SD = 6) showed similar levels of aberrant repetitive behaviours. After enrichment interventions, I observed a significant decrease in ARBs in test subjects (M = 2.38, SD = 2.46, t(36) = 6.65, p < 0.0001, Cohen's d= 2.15) while the levels remained unchanged for the control group (M = 13.31, SD = 6, t(30) = 0.23, p = 0.81) (Table 4.5 and Figure 4.3c).

4.3.2 Faecal corticosterone level

I compared the average faecal corticosterone levels of each subject across baseline and post-enrichment phases. Compared to baseline levels (M = 7.74 μg/gm, SD = 3.2) , the faecal corticosterone measures of test subjects decreased significantly (M = 2.46 μg/gm, SD= 0.85, t(36) = 5.33, p<0.0001, Cohen's d = 2.22) , while it remained unchanged from baseline (M = 7.1 μg/gm, SD = 4.08) for control subjects (M= 6.96 μg/gm, SD = 3.93, t(30) = 0.093, p = 0.92) (Table 4.5 , Figure 4.3d). These results confirm that the enrichment interventions led to significant improvement in behavioural and physiological welfare indices of test subjects as compared to the control group during the pre-enrichment period.

4.4 Discussion

Animal welfare encompasses but is not limited to the prevention of cruelty and amelioration of symptoms of stress, but also accords the opportunity to express species-typical behaviour patterns (Broom, 2011). The National Zoo Policy of India espouses animal welfare with captive animal management, yet welfare-centric management protocols remain divorced from husbandry practices across several zoos. Although few and far between, welfare research from Indian zoos report a prevalence of stereotypic behaviours (Goswami et al., 2020; Sanyal, 1892) and elevated faecal corticosterone levels (Vaz et al., 2017) across several captive felids, and prescribe species-appropriate enclosure design and enrichment interventions as remedial measures (Goswami et al., 2020; Mallapur et al., 2007; Vaz et al., 2017). Enrichment interventions have been shown to improve welfare conditions in several captive felids including African lions (Mellen et al., 1997; Powell, 1995; Skibiel et al., 2007), yet enrichment protocols are seldom incorporated in the daily management of captive Asiatic lions at Indian zoos. The Asiatic lion conservation breeding programme has successfully maintained a viable captive population insulated from stochastic ecological events. Most research pertaining to captive Asiatic lions investigate their genetic and demographic status (Bagatharia et al., 2013; Pastorino et al., 2017) (with only a handful mentioning their welfare) (Goswami et al., 2020; Pastorino et al., 2017; Vaz et al., 2017). This is the first study to assess the effects of enrichment protocols on the welfare of captive Asiatic lions. Welfare encompasses both internal and external conditions affecting an animal and hence must be measured using a multifarious approach. As highlighted by Miller et al. (2020a) the focus on negative welfare indicators has led to zoos adhering to minimum husbandry guidelines that attempt to suppress symptoms of poor welfare rather than trying to improve existing conditions. In this study, I measured two traditional negative welfare parameters like ARB (Mason et al., 2004) and faecal corticosterone levels (Schildkraut, 2016; Vaz et al., 2017) along with two positive welfare indicators (Miller et al., 2020a) viz., spread of participation index (SPI) (Cabana et al., 2018; Powell, 1995) and behaviour diversity (Pastorino et al., 2017).

Previous studies on captive African lions that establish the positive effects of enrichment interventions have primarily relied on behavioural welfare indices (Martínez-Macipe et al., 2015; Ncube et al., 2010; Powell, 1995; Regaiolli et al., 2019; Van Metter et al., 2008). Our study is the first to showcase both the behavioural and physiological impacts of enrichment interventions in captive Asiatic lions housed in a conservation breeding programme. The welfare evaluation framework used in this study can be applied universally (for other species) to measure the impacts of goal-oriented enrichment interventions. While previous studies have tested the welfare impacts of food (Powell, 1995; Van Metter et al., 2008) manipulable (Powell, 1995), sensory (Martínez-Macipe et al., 2015; Regaiolli et al., 2019) and social stimulation (Leonard, 2008; Ncube et al., 2010) individually. This is the first control trial study to test the efficacy of a combined enrichment strategy on the welfare of Asiatic lions. This has allowed us to understand the temporal usage pattern of enrichment devices in Asiatic lions, which can prove useful for tailoring enrichment strategies. For example, individual test subjects showed high fidelity towards manipulable enrichment devices, which were also associated with the highest amount of conspecific-directed aggression. Therefore, to reduce stress and aggressive behaviours I recommend providing more manipulable devices than the number of animals in an enclosure. Upon release, subjects rushed to the feeding enrichment devices, therefore to prevent aggression and injury, it is important to spread such devices far apart, which will reduce chances of monopolization and food-based aggression. While sensory enrichment devices are designed to encourage exploratory behaviours in lions, the feed-based enrichment devices create a positive association for these novel objects and reduce neophobia. Finally, I found that manipulable enrichment devices allow subjects to interact and exert control over the captive environment while engaging in species-typical behaviours. Our combined enrichment strategy brought novelty in a captive environment and presented animals with the choice to express species-typical behaviour patterns. All enrichment devices used in this study can be locally sourced or fabricated with minimal effort and require less than thirty minutes to install or replenish with a team of three people.

Post-enrichment intervention, I recorded a significant improvement in the welfare indices of test subjects compared to the control subjects. Subjects that would normally stay near the retiring cells and remain inactive all day started exploring and utilizing different parts of the enclosure and followed by a significant increase in behaviour diversity levels. Faecal corticosterone levels of test subjects decreased significantly from the baseline, while it remained unchanged for control subjects, showing the inter-linkages between behavioural and physiological indices of welfare. Our findings agree with existing research that associate enrichment interventions with increased behavioural diversity (Rabin, 2003; Wemelsfelder et al., 2000), lowered SPI (Nogueira et al., 2004; Rose et al., 2013; Traylor-Holzer et al., 1985), reduction of ARB (Clark et al., 2012; Mallapur et al., 2007; Vaz et al., 2017), and reduction of stress levels (Hutchinson et al., 2012; Marcon et al., 2018; Mitra et al., 2009; Nazar et al., 2011). However, it is necessary to point out that due to logistic constraints (sample size, time etc.) I did not address the variable responses of subjects with different personality types on enrichment interventions. Earlier work on captive Asiatic lions indicated that lions with different personality traits are likely to react differently to novel enrichment devices (Goswami et al., 2020). Future studies may explore this relationship and try answer how lions with different personality types react to different types of enrichment interventions.

5
Synthesis and recommendations

5.1 Introduction

In captivity, wild animals are likely to suffer from high allostatic loads and welfare is determined by how well they can cope with it (Hill et al., 2009). My thesis answered three questions about the external and internal factors affecting Asiatic lion welfare. I conducted three experiments to answer these questions. In this section, I discuss these findings that can be used to improve incumbent management practices at conservation breeding centres.

5.2 Key Findings

5.2.1 Role of enclosure complexity and visitor presence

Findings from the second chapter(first experiment) suggest that Asiatic lion welfare parameters and activity patterns are significantly affected by the lack of enclosure complexity.

The findings of this chapter demonstrate that, whereas visitor presence has been highlighted as a stressor by numerous writers, it has no substantial influence on Asiatic lion welfare.

Enclosure complexity is a constellation of several variables of enclosure design that come together to form the environment of the captive animals. The off-display and zoo-safari enclosures were more complex and provided more space to Asiatic lions than the on-display enclosures. However, the off-display and zoo safari enclosures evaluated by themselves scored far less than optimum on most parameters and averaged at 2.57 out of 5. The off-display enclosures were primarily aided by the large space and the natural features (Both factors on which they scored highly) that allowed Asiatic lions to cope better with the stressors of captivity. The on-display enclosures are more than 100 years old and do not meet modern minimum requirements for housing large felids as specified by the Central Zoo Authority (Gupta, 2008).

1. Enclosure complexity rating revealed that zoo-safari enclosures and off-display enclosures were more species-appropriate in comparison to the on-display enclosures of Asiatic lions.

2. All enclosures lacked an enrichment protocol linked with the husbandry regimen. Which should be addressed by the zoo management.

3. I found no difference in animal welfare between enclosures of the conservation breeding centre that had low visitor-presence as compared to those where visitors were not present. This means that the complexity of these off-display enclosures allows animals enough withdrawal distance to neutralize visitor effects.

4. Visitor disturbance and enclosure complexity were the key attributes separating the three enclosures. The on-display enclosures provided minimal withdrawal space and forced animals to stay close to visitors that could elevate stress levels. The zoo-safari enclosures, on the other hand, managed to offer enough withdrawal space for animals to demonstrate a behaviour repertoire and range of welfare indexes comparable to off-display enclosures, although not being completely isolated from visitor disturbance.

5. On the days in which visitors were absent, behaviour patterns of on-display and the zoo-safari lions did not shift significantly, which is perplexing. Therefore, we can surmise that visitor presence is just one of the stressors which are affecting the on-display animals, the bigger problems in this enclosure may be the abject lack of enrichment, withdrawal areas and most importantly the opportunity to express species-typical behaviours.

5.2.2 Role of personality and rearing history

In the second experiment, we looked at a number of internal characteristics that may account for the broad range of welfare results among Asiatic lions housed in comparable conditions.

We found that personality traits and rearing-history were the two most important factors determining the welfare requirement of Asiatic lions (Goswami et al., 2020). On the other hand, we also found that some other variables like sex and social grouping did not lead to differential welfare outcomes in the study subjects. Finally, I did an enrichment intervention at select enclosures (test-subjects) and found that lions exposed to a mix of different enrichment objects had better welfare outcomes than those who did not receive the intervention. I found that Asiatic lions housed at the same enclosure types had differential responses to similar husbandry practices (Goswami et al., 2020). This leads me to believe that animals housed under similar housing conditions are likely to react to these practices based on their traits. Such traits may include, factors like age, sex, personality and rearing history.

5.2.3 Effects of enrichment interventions on Asiatic lion welfare

Enrichment interventions are a well-established method of increasing welfare in captivity (Bulens et al., 2013). Apart from increasing novelty and a positive association with the paddock area, it also increases chances for animals to experience positive emotions (Hutchinson et al., 2012; Miller et al., 2020a). Ours is the first reported enrichment intervention from Indian conservation breeding programmes.

1. In this study we evaluated Asiatic lion welfare through latency to novel objects, aberrant repetitive behaviours, enclosure usage patterns, behaviour diversity and faecal corticosterone metabolite levels.

2. Enclosure enrichment treatment increased the quantity of species-typical behaviour variety in the test group considerably.

3. We designed the enrichment intervention to increase enclosure novelty while reducing chances of agonistic interactions

4. The rates of ARBs reduced significantly in test subjects as compared to the control. Lions that received the enrichment intervention, used the enclosure space more homogenously compared to control animals.

5. Moreover, the behavioural welfare indices were corroborated by the physiological measures of welfare. Test subjects showed a significant lowering in faecal corticosterone metabolites.

6. I found that a mixture of different enrichment devices is ideal for increasing engagement time and improving Asiatic lion welfare (Goswami et al., 2021).

5.3 Recommendations

Conservation breeding programmes for endangered species require a distinct set of husbandry methods than those used in on-display cages. The animals housed at these off-display facilities should be accorded all possible opportunities to express and maintain their repertoire of species-typical behaviour patterns. The first and foremost objective of any conservation breeding programme should be to conserve the species-typical genetic and behavioural attributes of the species. The points mentioned below shall assist zoo managers to achieve that objective.

5.3.1 Management strategies

1. Enclosures at the conservation breeding centre should be designed to ensure there is enough opportunity for the expression of species-typical behaviour repertoires.

2. Existing enclosures should be comprehensively evaluated for species-appropriateness. Followed by another round of evaluation based on individual welfare requirements (Goswami et al., 2020). While evaluating enclosures, the animal's response to the enclosure must be considered through the measurement of more than two welfare parameters, including measures of positive welfare (viz., Behaviour diversity). Based on the findings of the enclosure evaluation, enclosures and husbandry protocols must be redesigned to meet species-typical and individual-specific welfare requirements.

3. Conservation breeding programmes should maintain a quarterly schedule of welfare evaluation. This will ensure that a holistic picture of the existing welfare condition of the animals is obtained.

4. Existing enclosures should be periodically enriched with novel objects or stimuli to create opportunities for all animals to experience positive affective states.

5. Our results demonstrate that the Asiatic lions housed in high-complexity enclosures are less vulnerable to occasional visitor disturbances as compared to those housed in poorly-designed, on-display enclosures. Previous studies have emphasized the importance of species-appropriate features in encouraging the display of species-typical behaviours (Mallapur & Chellam, 2002). We can certainly state that high levels of visitor pressure without the ability to disengage is harmful to Asiatic lions' welfare. However, we can also say that animal welfare is unaffected when visitors are present in a sophisticated and suitably big enclosure.

6. Irrespective of the conservation status of housed animals, all poorly designed enclosures that do not provide enough space, illumination, ventilation or natural substrate should be decommissioned and the animals shifted to larger, species-appropriate enclosures. Such enclosure design and animal display practices should be abolished to pave way for more animal-centric enclosure design as seen at the zoo safari. Our results indicate that a complex and species-appropriate enclosure can offset the stress of visitor presence and allow the animals to express a species-typical behaviour repertoire leading to positive affective states or emotional well-being.

7. I found that life-history and personality traits were the dominant factors determining the welfare responses of animals to husbandry practices (Goswami et al., 2020). This finding has several implications for the welfare of captive animals across zoos and conservation breeding centres. As pointed out by Kaumanns et al. (2015), *ex-situ* conservation programmes for endangered species should

focus on the individual animal and look at a different set of welfare indices than just population numbers as a metric of success. Indian zoos and conservation breeding centres presently do not employ any individual-centric welfare measurement protocols. However, for the successful implementation of any *ex-situ* conservation breeding programme, it is crucial to take cognizance of these findings and inform future policies to ensure the welfare of captive endangered species. Frequent and holistic welfare evaluations are important to understand and mitigate the deleterious impacts of captivity on endangered species.

8. Our results also indicate that welfare indices are interrelated to one another. We found a strong inverse relationship between behaviour diversity and ARBs and reduced enclosure usage. This suggests that zoo managers should take cognizance of decreases in enclosure usage patterns in some individuals followed by lowering of behaviour diversity. This can help in preventing the development of stereotypic ARBs in captive animals. Regular behaviour monitoring is crucial to the successful implementation of any conservation breeding programme.

9. Documentation is a crucial aspect of zoo-biology that is often overlooked. A good *ex-situ* conservation breeding programme should have dedicated team members who will handle the collection, collation and interpretation of data specific to animal welfare. These team members would coordinate with zookeepers and biologists to find ways to improve the welfare status of captive animals under their care. Under the incumbent practices, every zoo in India has a single zoo-biologist, who has to design experiments, conduct them, collect the data, analyse it and implement the findings as well. I strongly recommend that large zoos should have at least two biologists per animal family and conservation breeding centres should have at least three biologists to oversee operations.

10. Studies show that positive keeper-animal relationships can improve welfare (Whitham et al., 2013). Finally, regular behaviour monitoring of captive wild animals

should be incorporated into the husbandry practices to improve welfare and prevent the development of stereotypy (Watters et al., 2009). So far, there have been no studies exploring the role of keeper-animal relationships in Indian zoos and their impacts on animal welfare. It will be interesting to understand the training gaps for zoo-keepers and fill them.

11. Keeper training and empowerment should be a top priority for all zoos and conservation breeding centres. Qualified, well-trained keepers can often understand the needs and want of animals as efficiently as any robust study design. As evidenced by our personality assessment system, keepers were able to predict the personality type of subjects with a significant level of certainty. Therefore it is imperative to treat keepers as important stakeholders for the positive welfare outcomes of captive animals.

12. Felids are among the most represented taxa across zoological institutions, which necessitates an effective and holistic welfare evaluation protocol (Szokalski et al., 2012). Our findings underline the importance of individual-tailored husbandry design (Boissy et al., 2014) to promote animal welfare at conservation breeding centers (Dawkins, 1990; Fraser et al., 1998; Fraser, 2009). Our results highlight that assessments of personality traits, enclosure usage patterns, behavioural diversity and stereotypy measurement as cost-effective and non-invasive tools that can reliably diagnose welfare needs in captive wild animals (Broom, 1996; Mason et al., 2007) and conduct post-occupancy evaluations of enclosures (Wilson et al., 2003). These assessments can also help in effective management of endangered species through personality-matched pairings for breeding success (Fox et al., 2014; Martin-Wintle et al., 2017), and profiling of individuals most suited for repatriation (Bremner-Harrison et al., 2004; Watters et al., 2009; Watters et al., 2012). More specifically for Asiatic lions, Indian and Southeast Asian zoos account for more than 60% of the global captive Asiatic lion population (Srivastav et al., 2018). Unlike many European or North American zoological institutions, most Indian zoos are state-funded and follow husbandry guidelines delineated

by governmental animal welfare agencies. Current governmental policies and guidelines for managing captive wild animals in Indian zoos do not explicitly consider inter-individual variations in animal welfare practices. Even existing studies on captive African (Powell et al., 2011; Powell et al., 2008; Powell, 1995) and Asiatic lions (Pastorino et al., 2016; Pastorino et al., 2017) have not translated into tangible shift in ongoing husbandry practises. We hope that our findings tip the balance in the favour of welfare-centric and individual-tailored husbandry and management regimens for captive animals at zoos and conservation breeding centres.

13. Daigle et al.,(2015) found reproductive success of captive African lions was determined by temperament, grouping patterns and positive human-animal interactions. Using our study in the backdrop of these findings can be an effective management tool that is worth exploring.

5.3.2 Enrichment strategies

1. At the beginning of the enrichment intervention, high-value food rewards should be provided along with manipulable and sensory enrichment devices. This reduces the chance of neophobia among shy animals. As the animals start to use the manipulable devices, the amount of food reward should be gradually reduced and sustained at a constant level. Food rewards should never be completely removed from the enrichment intervention unless there is a valid reason for the same.

2. Food rewards lead to infighting and agonistic interactions among zoo-housed group-living animals. Therefore, Food-rewards and enrichment devices should be provided in multiples and at different locations of the enclosure. This will prevent monopolization by dominant individuals.

3. If there is food aggression among conspecifics. the dominant individuals should be given high-value food items at certain locations of the enclosure that have a

long food-processing time. This will allow other animals to explore the enclosure unhindered.

4. We found a strong preference in subjects for food-based enrichment devices. However, the duration of usage was highest for manipulable enrichment. Since the subjects spent more time using manipulable devices, results showed manipulable enrichment devices as strongly associated with conspecific directed aggression. We recommend that a large number (at least two per animal) for each manipulable and feeding device be made and rotated across different areas of the enclosure to create more opportunities for play and socially affiliative behaviours.

5. The success of the conservation breeding program depends on repatriating endangered animals to reclaimed habitats, hence housing the animals in appropriate welfare conditions while persevering behaviour diversity is of utmost importance.

6. I studied the largest captive population of Asiatic lions, but the methodologies of this study can be scaled for smaller zoos housing fewer animals. Although I used at least three types of enrichment devices per intervention at multiple enclosure zones for experimental uniformity, at a smaller scale, zoo managers can try one enrichment device type per enclosure and rotate them with a new one every week.

7. Our results conclusively show that enrichment interventions can lead to better behavioural and physiological welfare for Asiatic lions. I have delineated a practical but holistic approach to enrichment interventions that can be implemented and tested with little effort and manpower. With this study, I hope to empower and enable zoo managers and regulatory agencies to mandate the incorporation of enrichment interventions into daily husbandry regimens and proactively improve the welfare status of the animals under their care.

8. With the incorporation of welfare-centric management practices, the Asiatic lions housed in the program can be ideal representatives of the species and great candidates for future reintroduction programmes. Until that day of repatriation, we should strive to create a cognitively enriching environment that preserves the species-specific traits of the Asiatic lions. Like the endangered Asiatic lion, the Indian ex-situ institutions run conservation breeding programmes for several endemic species.

9. So far, the welfare status of animals housed under conservation breeding programmes has received little research attention. I hope this study encourages zoo managers and regulators to incorporate enrichment interventions with animal management protocols.

10. I suggest that future studies should focus on addressing impacts of such individual variations on differential responses and enrichment preferences with a larger sample size.

5.3.3 Conservation breeding programmes at Indian zoos

Conservation breeding of endangered species has been one of the primary mandates of modern zoos that have managed to redeem them from a chequered past. Most conservation breeding programmes are borne out of the now commonplace narrative of the ark. Endangered animals threatened with habitat loss and other stochastic ecological or anthropological pressures can be brought into captivity and then bred to sustainable numbers to supplement the ailing natural fragmented populations. The growing number of conservation breeding programmes in the Indian zoological parks is intriguing. The surge in *ex-situ* CBP signals a growing concern for the future of the species resulting from a failure to conserve the species *in-situ.* It is also interesting to note the dearth of welfare research published from the findings of the aforementioned CBPs. This could indicate that the managers of these CBPs are already following best practices and have nothing new to contribute to science, or it could point towards a lack of scientific inquiry and data-driven decision-making at these CBPs. Presently Indian zoos manage the *ex-situ* conservation breeding programmes for over 29 species of endangered species. Since most zoos have to follow the policy framework laid down by the Central Zoo Authority (CZA). Policymakers must take cognizance of the wide research gaps in knowledge and expertise in husbandry and management practices at Indian zoos and CBPs. Our findings provide strong scientific evidence that the large-scale uptake of individual-focused welfare assessment practices at Indian zoos can lead to better welfare outcomes for all captive animals. Such policy-level changes to animal welfare guidelines will strengthen ex-situ conservation practices in this region. Future cross-institutional studies on how internal (physiology) or external factors (enrichment interventions) interact with personality traits to predict welfare outcomes can shed light on some of the trends highlighted in this study. We hope that this study encourages managers and biologists to revisit traditional husbandry protocols and change them to meet the cognitive needs of individual animals under their care.

5.3.4 Animal welfare as a measure of conservation success

The breeding programme for Asiatic lions at Sakkarbaug zoological garden was initiated in 1995 and continues to be the largest single population of captive Asiatic lions in the world. The zoo and the conservation programme is located within the geographic range of the species and has access to the wild population of founders, which is a luxury that few *ex-situ* conservation programmes can afford. Given these advantages, we must now summarise the efficacy of the Asiatic lion conservation breeding programme. I used the national studbook for Asiatic lions, maintained by Srivastav et al. (2018) to understand the demographic trends of the captive population in SZG. Since 1995, a total of 873 captive Asiatic lions have been housed at Indian zoos. Out of these individuals, 489 lions originated from SZG. Henceforth, I will restrict myself to these 489 individuals, since few other zoos have access to wild rescued Asiatic lions. I counted the number of new individuals brought into the records of SZG annually along with their points of origin (captive-born or rescued). I found that out of the 489 Asiatic lions entered into SZG records, 376 were born in captivity and 113 lions were wild-rescued. At first glance, this statistic might suggest that the Asiatic lion

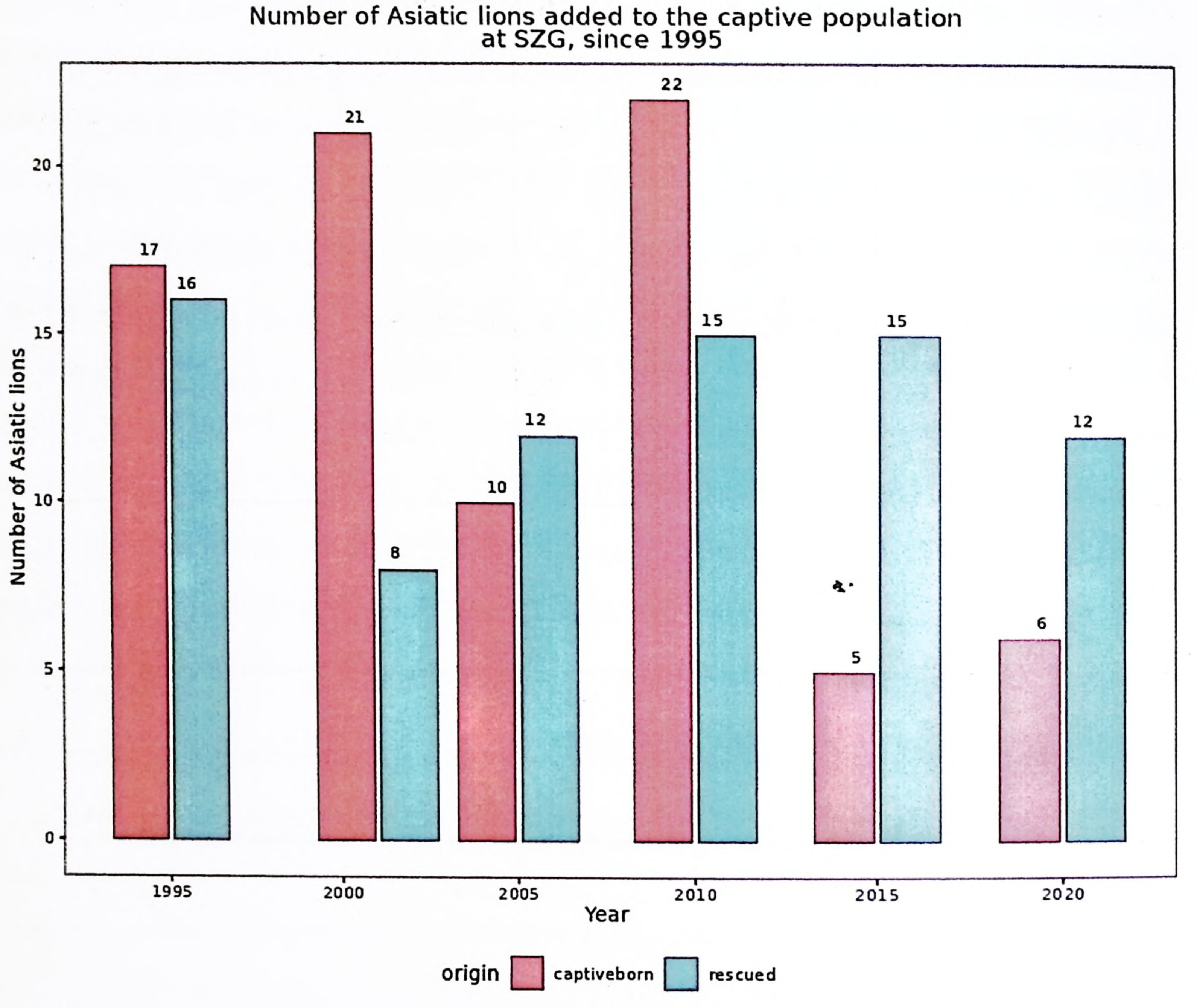

Figure 5.1: Population status of Asiatic lions at SZG

captive population is stable and capable of sustainable growth. I filtered the data to remove individuals born in captivity but died shortly (within one year) after birth (N = 251), which left us with only 125 individuals born in captivity. I plotted the five-yearly summaries of animal acquisition trends of SZG and made some observations (Figure 5.1). Since 1995, SZG has housed around 489 Asiatic lions and the ratio of captive-raised to wild-rescued animals is 1.10. Interestingly, my study subjects included 19 individuals that were wild-rescued and 16 animals that were born or raised in captivity. Every year, the conservation breeding programme has harvested roughly four individuals from the wild population (approximately 1% of the wild population) and has produced a roughly similar number of individuals in captivity that have grown beyond the age of one year. As evident from Figure 5.1, in the last ten years, the Asiatic lion conservation breeding programme has rescued more animals than were born in captivity. This probably indicates a rise in human-animal conflict driven by the healthy growth of the wild population, as indicated by Jhala et al. (2019). On the other hand, the birth rate and the demographic stability of the captive population shows a downward trend (Figure 5.1). Conservation breeding programmes are prohibitively expensive and take away resources that may be better spent on in-situ conservation. Therefore, conservation breeding programmes must be evaluated rigorously to assess their efficacy and viability in terms of the long-term conservation of the species. While evaluating conservation breeding programmes, the traditional outlook has been about measuring genetic diversity and ensuring a stable captive population. A similar metric has been applied to the Asiatic lion conservation breeding programme as well. However, my findings suggest that a conservation breeding programme should be made to adhere to strict short-term and long-term goals. In the case of Asiatic lions, the short-term goal can be ensuring optimum welfare for all individuals. The long-term goal should be to encourage the maintenance of species-typical behaviours so that these animals become ideal candidates for future reintroductions.

One of the primary goals of a CBP is to reintroduce or repatriate animals to restored habitats periodically and apart from one failed reintroduction attempt at Chandraprabha Sanctuary in 1957 (which also used wild-born individuals), the Asiatic lion CBP has conducted no reintroduction exercises. Additionally, the following metric should be used by policymakers to evaluate the long-term viability of ex-situ management programs (Fischer et al., 2000).

1. Appropriateness of approach.
2. Clear definition of success.
3. Constant monitoring of the reintroduced population or the measures of success.
4. Financial accountability.
5. Publication of results.

Based on our findings, animal welfare should be considered an indispensable metric of success or viability for any captive facility or breeding programme for wild endangered fauna. Housing the Asiatic lions at enriched enclosures can mitigate risks of chronic stress and provide enough opportunities to express species-typical behaviours. Good animal welfare will be necessary to ensure that once released, these lions will be able to survive in the wild. Therefore the management practices at the Asiatic lion CBP should be carefully re-evaluated to realign them with the conservation goals for the species. Unfortunately, barring a few, most conservation breeding programmes at Indian zoos do not have a welfare centric or individual-tailored management strategy. Long-term goals like repatriation success and demographic stability should always be considered but not at the expense of animal suffering.

The future survival and proliferation of Asiatic lions will depend on their (our) ability to expand their geographical range. A synergistic relationship between *ex-situ* and *in-situ* approaches is necessary to plan and support a successful long-term repatriation programme. Published research on Asiatic lions, seldom acknowledges or address the fate of the Asiatic lions in the conservation breeding programme. There is an urgent need to reframe the objective of the Asiatic lion conservation breeding programme to align it with the conservation goals for the species. This thesis will have served its purpose if the methodology discussed is used to improve the welfare of animals at other zoos and conservation breeding programmes.

References

Ahloy-Dallaire, J., Espinosa, J., & Mason, G. (2018). Play and optimal welfare: Does play indicate the presence of positive affective states? *Behavioural processes*, *156*, 3–15.

Ainsworth, M. S., & Bowlby, J. (1991). An ethological approach to personality development. *American psychologist*, *46*(4), 333.

Allport, F. H., & Allport, G. W. (1921). Personality Traits: Their Classification and Measurement. *The Journal of Abnormal Psychology and Social Psychology*, *16*(1), 6–40. https://doi.org/10.1037/h0069790

Alroy, J. (2015). Limits to captive breeding of mammals in zoos. *Conservation Biology*, *29*(3), 926–931. https://doi.org/10/f7dbv5

Altmann, J. (1974). Observational study of behavior: Sampling methods. *Behaviour*, *49*(3-4), 227–266.

Álvarez, M. P. (2009). The four causes of behavior: Aristotle and Skinner. *International Journal of Psychology and Psychological Therapy*, *9*(1), 45–57.

Amato, K. R., Belle, S. V., & Wilkinson, B. (2013). A Comparison of Scan and Focal Sampling for the Description of Wild Primate Activity, Diet and Intragroup Spatial Relationships. *Folia Primatologica*, *84*(2), 87–101. https://doi.org/10/gjqnjr

Ashraf, N., Chellam, R., Molur, S., Sharma, D., & Walker, S. (1995). *Asiatic lion, population and habitat viability assessement, (PHVA) and global animal survival plan workshop* (CBSG workshop). Baroda, India.

Atkinson, K. E., Kitchener, A. C., Tobe, S. S., & O'Donoghue, P. (2018). An assessment of the genetic diversity of the founders of the European captive population of Asian lion (Panthera leo leo), using microsatellite markers and studbook analysis. *Mammalian Biology*, *88*, 138–143. https://doi.org/10.1016/j.mambio.2017.10.001

Bagatharia, S. B., Joshi, M. N., Pandya, R. V., Pandit, A. S., Patel, R. P., Desai, S. M., Sharma, A., Panchal, O., Jasmani, F. P., & Saxena, A. K. (2013). Complete mitogenome of Asiatic lion resolves phylogenetic status within Panthera. *BMC Genomics*, *14*(1), 572. https://doi.org/10.1186/1471-2164-14-572

Ball, V. (1880). Observations on Lion-Breeding in the Gardens of the Royal Zoological Society of Ireland. *The Transactions of the Royal Irish Academy*, *28*, 723–758.

Banerjee, K., Jhala, Y. V., Chauhan, K. S., & Dave, C. V. (2013). Living with Lions: The Economics of Coexistence in the Gir Forests, India. *PLoS ONE*, *8*(1), 1–11. https://doi.org/10.1371/journal.pone.0049457

Baratay, E., & Hardouin-Fugier, E. (2004). *Zoo: A History of Zoological Gardens in the West*. Reaktion Books.

Bashaw, M. J., Kelling, A. S., Bloomsmith, M. A., & Maple, T. L. (2007). Environmental Effects on the Behavior of Zoo-housed Lions and Tigers, with a Case Study of the Effects of a Visual Barrier on Pacing. *Journal of Applied Animal Welfare Science*, *10*(2), 95–109. https://doi.org/10/cxk77g

Ben-Shachar, M. S., Lüdecke, D., & Makowski, D. (2020). Effectsize: Estimation of effect size indices and standardized parameters. *Journal of Open Source Software*, *5*(56), 2815. https://doi.org/10/ghq8sk

Birke, L. (2002). Effects of browse, human visitors and noise on the behaviour of captive orang utans. *Animal Welfare*, *11*(2), 189–202.

Biswas, S., Bhatt, S., Paul, S., Modi, S., Ghosh, T., Habib, B., Nigam, P., Talukdar, G., Pandav, B., & Mondol, S. (2019). A practive faeces collection protocol for multidisciplinary research in wildlife science. *Current Science*, *116*(11), 1878–1885. https://doi.org/10.18520/cs/v116/i11/1878-1885

Boissy, A., & Erhard, H. W. (2014). How Studying Interactions Between Animal Emotions, Cognition, and Personality Can Contribute to Improve Farm Animal Welfare. *Genetics and the Behavior of Domestic Animals* (pp. 81–113). Elsevier. https://doi.org/10.1016/B978-0-12-394586-0.00003-2

Boissy, A., Manteuffel, G., Jensen, M. B., Moe, R. O., Spruijt, B., Keeling, L. J., Winckler, C., Forkman, B., Dimitrov, I., & Langbein, J. (2007). Assessment of positive emotions in animals to improve their welfare. *Physiology & behavior*, *92*(3), 375–397.

Brambell, F. W. R. (1965). *Report of the technical committee to enquire into the welfare of animals kept under intensive livestock husbandry systems*.

Braverman, I. (2014). Conservation without nature: The trouble with in situ versus ex situ conservation. *Geoforum*, *Complete*(51), 47–57. https://doi.org/10/f5stdh

Breithoff, E., & Harrison, R. (2020). From ark to bank: Extinction, proxies and biocapitals in ex-situ biodiversity conservation practices. *International Journal of Heritage Studies*, *26*(1), 37–55. https://doi.org/10/gjf9hz

Bremner-Harrison, S., Prodohl, P. A., & Elwood, R. W. (2004). Behavioural trait assessment as a release criterion: Boldness predicts early death in a reintroduction programme of captive-bred swift fox (Vulpes velox). *Animal Conservation*, *7*(3), 313–320. https://doi.org/10.1017/S1367943004001490

Broom, D. (1986). Indicators of Poor Welfare. *British veterinary Journal*, *142*, 524–526. http://endcap.eu/wp-content/uploads/2015/06/Broom-1986-Indicators-of-poor-animal-welfare.pdf

Broom, D. (1996). Animal Welfare Defined in Terms of Attempts to Cope with the Environment. *Acta Agriculturae Scandinavica, Section A - Animal Science*, *27*, 22–28.

Broom, D. (2014). *Sentience and Animal Welfare*. CABI.

Broom, D., & Fraser, A. (2008, December). *Domestic Animal Behaviour and Welfare* . (Vol. 83). https://doi.org/10.1086/596242

Broom, D. M. (2007). Cognitive ability and sentience: Which aquatic animals should be protected? *Diseases of aquatic organisms*, *75*(2), 99–108. https://doi.org/10/b5fdcz

Broom, D. M. (2011). A history of animal welfare science. *Acta Biotheoretica*, *59*(2), 121–37. https://doi.org/10.1007/s10441-011-9123-3

Brown, C., Davidson, T., & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery□reared Atlantic salmon. *Journal of Fish Biology*, *63*, 187–196.

Bulens, A., Van Beirendonck, S., Van Thielen, J., & Driessen, B. (2013). The enriching effect of non-commercial items in stabled horses. *Applied Animal Behaviour Science*, *143*(1), 46–51.

Cabana, F., Maguire, R., Hsu, C.-D., & Plowman, A. (2018). Identification of possible nutritional and stress risk factors in the development of marmoset wasting syndrome. *Zoo Biology*, *37*(2), 98–106. https://doi.org/10.1002/zoo.21398

Canino, W., & Powell, D. (2010). Formal behavioral evaluation of enrichment programs on a zookeeper's schedule: A case study with a polar bear (Ursus Maritimus) at the Bronx Zoo. *Zoo Biology*, *29*(4), 503–508.

Cannon, T. H., Heistermann, M., Hankison, S. J., Hockings, K. J., & McLennan, M. R. (2016). Tailored Enrichment Strategies and Stereotypic Behavior in Captive Individually Housed Macaques (Macaca spp.) *Journal of Applied Animal Welfare Science, 19*(2), 171–182. https://doi.org/10.1080/10888705.2015.1126786

Carere, C., & Locurto, C. (2011). Interaction between animal personality and animal cognition. *Current Zoology, 57*(4), 491–498.

Carlstead, K., Seidensticker, J., Baldwin, R., & Park, N. Z. (1991). Environmental Enrichment for Zoo Bears. *Zoo Biology, 16*, 3–16.

Carlstead, K., Shepherdson, D. J., Sliepherdson, D., Park, N. Z., Dc, K. C., & Shepherdson, D. J. (1994). Effects of environmental enrichment on reproduction. *Zoo Biology, 13*(5), 447–458. https://doi.org/10.1002/zoo.1430130507

Caro, T. M., Roper, R., Young, M., & Dank, G. R. (1979). Inter-observer reliability. *Behaviour*, 303–315. https://doi.org/10.1163/156853979X00520

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: What are behavioural ecologists measuring? *Biological reviews of the Cambridge Philosophical Society, 88*(2), 465–75. https://doi.org/10.1111/brv.12007

Casas, P. (2019). funModeling: Exploratory data analysis and data preparation tool-box. *R package version, 1*(3).

Chakrabarti, S., Bump, J. K., Jhala, Y. V., & Packer, C. (2021). Contrasting levels of social distancing between the sexes in lions. *Iscience, 24*(5), 102406. https://doi.org/10/gk7sxz

Chakrabarti, S., & Jhala, Y. V. (2017). Selfish partners: Resource partitioning in male coalitions of Asiatic lions. *Behavioral Ecology, 28*(6), 1532–1539.

Chamove, A. S., Hosey, G. R., & Schaetzel, P. (1988). Visitors excite primates in zoos. *Zoo biology, 7*(4), 359–369. https://doi.org/10/c97s7t

Chauvet, J.-M., Brunel Deschamps, E., & Hillaire, C. (1996). *Dawn of art: The Chauvet Cave: The oldest known paintings in the world.*

Chellam, R., & Johnsingh, A. J. T. (1993). Management of Asiatic lions in the Gir forest, India. *Symposia of the Zoological Society of London, 65*, 409–424.

Chellam, R., Joshua, J., Williams, C. A., & Johnsingh, A. J. T. (1995). Survey of potential sites for reintroduction of Asiatic Lions. *Unpublished Report, Wildlife Institute of India, Dehra Dun, India.*

Chrulew, M. (2018). My place, my duty: Zoo biology as field philosophy in the work of Heini Hediger. *Parallax, 24*(4), 480–500.

Clark, F. E., & Melfi, V. A. (2012). Environmental enrichment for a mixed-species nocturnal mammal exhibit. *Zoo Biology, 31*(4), 397–413. https://doi.org/10.1002/zoo.20380

Clegg, I. L. (2018). Cognitive bias in zoo animals: An optimistic outlook for welfare assessment. *Animals, 8*(7), 1–25. https://doi.org/10.3390/ani8070104

Cloutier, T. L., & Packard, J. M. (2014). Enrichment options for African painted dogs (*Lycaon pictus*). *Zoo biology, 33*(5), 475–480.

Coelho, C. M., de Azevedo, C. S., & Young, R. J. (2012). Behavioral responses of maned wolves (Chrysocyon brachyurus, Canidae) to different categories of environmental enrichment stimuli and their implications for successful reintroduction. *Zoo Biology, 31*(4), 453–469. https://doi.org/10/cm67jx

Cohen, J. (1992). A power primer. *Psychological bulletin, 112*(1), 155.

Cole, E. F., & Quinn, J. L. (2014). Shy birds play it safe: Personality in captivity predicts risk responsiveness during reproduction in the wild. *Biology Letters, 10*(5), 20140178–20140178. https://doi.org/10.1098/rsbl.2014.0178

Cooper, J. J., & Nicol, C. J. (1996). Stereotypic behaviour in wild caught and laboratory bred bank voles (Clethrionymus glareolus). *Animal Welfare*, *5*(3), 245–257.
Corsetti, S., Borruso, S., Traglia, M. D., Lai, O., Alfieri, L., Villavecchia, A., Cariola, G., Spaziani, A., & Natoli, E. (2018). Bold personality makes domestic dogs entering a shelter less vulnerable to diseases. https://doi.org/10.1371/journal.pone.0193794
Crocq, M.-A., & Crocq, L. (2000). From shell shock and war neurosis to posttraumatic stress disorder: A history of psychotraumatology. *Dialogues in Clinical Neuroscience*, *2*(1), 47–55. Retrieved September 7, 2021, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3181586/
Crump, A., Arnott, G., & Bethell, E. (2018). Affect-Driven Attention Biases as Animal Welfare Indicators: Review and Methods. *Animals*, *8*(8), 136–136. https://doi.org/10.3390/ani8080136
Daigle, C. L., Brown, J. L., Carlstead, K., Pukazhenthi, B., Freeman, E. W., & Snider, R. J. (2015). Multi-institutional survey of social, management, husbandry and environmental factors for the SSP African lion Panthera leo population: Examining the effects of a breeding moratorium in relation to reproductive success. *International Zoo Yearbook*, *49*(1), n/a–n/a. https://doi.org/10.1111/izy.12073
Darwin, C. (1872). *The expression of the emotions in man and animals by Charles Darwin.* John Murray.
Darwin's, C. (1859). On the origin of species. *published on*, *24*.
Davey, G. (2007). Visitors' effects on the welfare of animals in the zoo: A review. *Journal of Applied Animal Welfare Science*, *10*(2), 169–183. https://doi.org/10/fb36m5
Dawkins, M. S. (2004). Using behaviour to assess animal welfare. *Animal welfare*, *13*(1), 3–7.
Dawkins, M. S. (2017). Animal welfare with and without consciousness. *Journal of Zoology*, *301*(1), 1–10. https://doi.org/10/f9nx8c
Dawkins, M. S. (1990). From an animal's point of view: Motivation, fitness, and animal welfare. *Behavioral and brain sciences*, *13*(1), 1–9. https://doi.org/10/dg2xx2
Dawkins, M. S. (2021). *The Science of Animal Welfare: Understanding What Animals Want.* Oxford University Press, USA.
DeGregorio, B. A., Sperry, J. H., Tuberville, T. D., & Weatherhead, P. J. (2017). Translocating ratsnakes: Does enrichment offset negative effects of time in captivity? *Wildlife Research*, *44*(5), 438–448.
Delacre, M., Leys, C., Mora, Y. L., & Lakens, D. (2019). Taking Parametric Assumptions Seriously: Arguments for the Use of Welch's *F*-test instead of the Classical *F*-test in One-Way ANOVA. *International Review of Social Psychology*, *32*(1), 13. https://doi.org/10/gf6ht8
de Oliveira, D., & Keeling, L. J. (2018). Routine activities and emotion in the life of dairy cows: Integrating body language into an affective state framework. *PloS one*, *13*(5), e0195674.
Duncan, I. J. (2006a). The changing concept of animal sentience. *Applied Animal Behaviour Science*, *100*(1-2), 11–19. https://doi.org/10.1016/j.applanim.2006.04.011
Duncan, I. J. (2006b). The changing concept of animal sentience. *Applied animal behaviour science*, *100*(1-2), 11–19. https://doi.org/10/cqphgn
Engel, G. L. (1980). The clinical application of the biopsychosocial model. *The American journal of psychiatry*, *137*(5), 535–544. https://doi.org/10.1176/ajp.137.5.535
Englefield, B., Blackman, S. A., Starling, M., & McGreevy, P. D. (2019). A Review of Australian Animal Welfare Legislation, Regulation, Codes of Practice, and Policy, and Their Influence on Stakeholders Caring for Wildlife and the Animals for Whom

They Care. *Animals*, *9*(6), 335. https://doi.org/10.3390/ani9060335
Fàbregas, M. C., Guillén-Salazar, F., & Garcés-Narro, C. (2012). Do naturalistic enclosures provide suitable environments for zoo animals? *Zoo Biology*, *31*(3), 362–373. https://doi.org/10/b4dz66
Farrand, A. (2007). The effect of zoo visitors on the behaviour and welfare of zoo mammals.
Farrand, A., Hosey, G., & Buchanan-Smith, H. M. (2014). The visitor effect in petting zoo-housed animals: Aversive or enriching? *Applied Animal Behaviour Science*, *151*, 117–127. https://doi.org/10/f5s9cz
Favre, D., & Tsang, V. (1993). The development of anti-cruelty laws during the 1800's. *Det. CL Rev.*, 1.
Fernandez, E. J., Upchurch, B., & Hawkes, N. C. (2021a). Public Feeding Interactions as Enrichment for Three Zoo-Housed Elephants. *Animals*, *11*(6), 1689. https://doi.org/10/gkfw82
Fernandez, E. J., Upchurch, B., & Hawkes, N. C. (2021b). Public Feeding Interactions as Enrichment for Three Zoo-Housed Elephants. *Animals*, *11*(6), 1689. https://doi.org/10/gkfw82
Fischer, J., & Lindenmayer, D. B. (2000). An assessment of the published results of animal relocations. *Biological conservation*, *96*(1), 1–11.
Flandreau, E. I., & Toth, M. (2017). Animal models of PTSD: A critical review. *Behavioral Neurobiology of PTSD*, 47–68. https://doi.org/10/gmqhj2
Fox, R. A., & Millam, J. R. (2014). Personality traits of pair members predict pair compatibility and reproductive success in a socially monogamous parrot breeding in captivity. *Zoo Biology*, *33*(3), 166–172. https://doi.org/10/f569t2
Francione, G. L. (2010). Animal welfare and the moral value of nonhuman animals. *Law, Culture and the Humanities*, *6*(1), 24–36. https://doi.org/10/cncgmc
Franks, B., Higgins, E. T., & Champagne, F. A. (2014). A Theoretically Based Model of Rat Personality with Implications for Welfare. *PLOS ONE*, *9*(4), e95135. https://doi.org/10/f588c9
Franks, N. R., Wilby, A., Silverman, B. W., & Tofts, C. (1992). Self-organizing nest construction in ants: Sophisticated building by blind bulldozing. *Animal behaviour*, *44*, 357–375.
Fraser, A. F., & Broom, D. M. (1997). *Farm animal behaviour and welfare.* CAB international.
Fraser, D. (1995). Science, Values and Animal Welfare: Exploring the 'Inextricable Connection'. *Animal Welfare*, *4*(2), 103–117. https://doi.org/10.1098/rsbl.2010.1057
Fraser, D., & Duncan, I. J. (1998). 'Pleasures','pains' and animal welfare: Toward a natural history of affect. *Animal welfare*, *7*(4), 383–396.
Fraser, D. (1999). Animal ethics and animal welfare science: Bridging the two cultures. *Applied Animal Behaviour Science*, *65*(3), 171–189. https://eds.a.ebscohost.com/eds/detail/detail?vid=4&sid=fe576c97-01ae-43cb-9354-2b8d3bb61ca2@sessionmgr4004&hid=4105&bdata=JnNpdGU9ZWRzLWxpdmU=#db=edswsc&AN=000084642600001
Fraser, D. (2009). Animal behaviour, animal welfare and the scientific study of affect. *Applied Animal Behaviour Science*, *118*(3-4), 108–117. https://doi.org/10.1016/j.applanim.2009.02.020
Frost, A. J., Winrow-giffen, A. A., Ashley, P. J., & Sneddon, L. U. (2007). Plasticity in animal personality traits: Does prior experience alter the degree of boldness? *Proceedings. Biological sciences / The Royal Society*, *274*(1608), 333–339.

https://doi.org/10.1098/rspb.2006.3751
Funder, D. C. (1995). On the accuracy of personality judgment: A realistic approach. *Psychological Review*, *102*(4), 652–670. https://doi.org/10.1037/0033-295X.102.4.652
Funder, D. C., & Colvin, C. R. (1988). Friends and strangers: Acquaintanceship, agreement, and the accuracy of personality judgment. *Journal of Personality and Social Psychology*, *55*(1), 149–158. https://doi.org/10.1037//0022-3514.55.1.149
Gartner, M. C., Powell, D. M., & Weiss, A. (2016). Comparison of subjective well-being and personality assessments in the clouded leopard (Neofelis nebulosa), snow leopard (Panthera uncia), and African lion (Panthera leo). *Journal of Applied Animal Welfare Science*, *19*(3), 294–302. https://doi.org/10/gk36g2
Gartner, M., & Powell, D. M. (2012). Personality assessment in snow leopards (Uncia uncia). *Zoo Biology*, *31*(2), 151–165. https://doi.org/10.1002/zoo.20385
Gartner, M., Powell, D., & Weiss, A. (2014). Personality structure in the domestic cat (Felis silvestris catus), Scottish wildcat (Felis silvestris grampia), clouded leopard (Neofelis nebulosa), snow leopard (. *Journal of Comparative Psychology*, *128*(4), 414–426. http://psycnet.apa.org/journals/com/128/4/414/
Gilby, I. C., Pokempner, A. A., & Wrangham, R. W. (2010). A Direct Comparison of Scan and Focal Sampling Methods for Measuring Wild Chimpanzee Feeding Behaviour. *Folia Primatologica*, *81*(5), 254–264. https://doi.org/10/chpp65
Glatston, A. R., Geilvoet-Soeteman, E., Hora-Pecek, E., & Van Hooff, J. (1984). The influence of the zoo environment on social behavior of groups of cotton-topped tamarins, Saguinus oedipus oedipus. *Zoo biology*, *3*(3), 241–253. https://doi.org/10/bbjkq9
Gliem, J. A., & Gliem, R. R. (2003). Calculating, interpreting, and reporting Cronbach's alpha reliability coefficient for Likert-type scales.
Gonyou, H. W. (1994). Why the study of animal behavior is associated with the animal welfare issue. *Journal of animal science*, *72*(8), 2171–2177. https://doi.org/10/gmq2m4
Goold, C., & Newberry, R. C. (2017). Modelling personality, plasticity and predictability in shelter dogs. *Royal Society Open Science*, *4*, 170618–170618. https://doi.org/10.1101/145367
Gosling, S. D., & John, O. P. (1999). Personality Dimensions in Nonhuman Animals: A Cross-Species Review. *Current Directions in Psychological Science*, *8*(3), 69–75. https://doi.org/10/bk3s2v
Gosling, S. D., & Vazire, S. (2002). Are we barking up the right tree? Evaluating a comparative approach to personality. *Journal of Research in Personality*, *36*(6), 607–614. https://doi.org/10/b74sk5
Goswami, S., Patel, S. K., Kadivar, R., Tyagi, P. C., Malik, P. K., & Mondol, S. (2021). Effects of a combined enrichment intervention on the behavioural and physiological welfare of captive Asiatic lions (Panthera leo persica). *Applied Animal Behaviour Science*, *236*, 105222. https://doi.org/10/gjq5dv
Goswami, S., Tyagi, P. C., Nigam, P., Srivastav, A., & Malemleima, N. (2016). Enrichment Manual for Selected Species in Indian Zoos Enrichment Manual for Selected Species in Indian Zoos. https://doi.org/10.13140/RG.2.2.33437.31200
Goswami, S., Tyagi, P. C., Malik, P. K., Pandit, S. J., Kadivar, R. F., Fitzpatrick, M., & Mondol, S. (2020). Effects of personality and rearing-history on the welfare of captive Asiatic lions (*Panthera leo persica*). *PeerJ*, *8*, e8425.

https://doi.org/10.7717/peerj.8425
Greenwald, K. R., & Dabek, L. (2003). Behavioral development of a polar bear cub (Ursus maritimus) in captivity. *Zoo Biology, 22*(5), 507–514. https://doi.org/10.1002/zoo.10095
Griffin, A. S., Guillette, L. M., & Healy, S. D. (2015). Cognition and personality: An analysis of an emerging field. *Trends in Ecology and Evolution, 30*(4), 207–214. https://doi.org/10.1016/j.tree.2015.01.012
Gupta, B. K. (2008). *Barrier designs for zoos.* Central Zoo Authority.
Hanson, E., Anne. (1996). *Nature civilized: A cultural history of American zoos, 1870-1940.* University of Pennsylvania. Retrieved July 14, 2021, from https://www.proquest.com/openview/0621f040d0dd911680aea181db892e0f/1?pq-origsite=gscholar&cbl=18750&diss=y
Harding, E. J., Paul, E. S., & Mendl, M. (2004). Cognitive bias and affective state. *Nature, 427*, 312–312. https://doi.org/10.1038/427312a
Harel, D. (2020). *Sur: Companion to "Statistics Using R: An Integrative Approach* (Version 1.0.3). https://CRAN.R-project.org/package=sur
Harrison, R. (1964). *Animal machines.* Cabi.
Haskell, M., Wemelsfelder, F., Mendl, M. T., Calvert, S., & Lawrence, A. B. (2018). The Effect of Substrate-Enriched and Substrate-Impoverished Housing Environments on the Diversity of Behaviour in Pigs Published by : Brill Stable URL : http://www.jstor.org/stable/4535392 REFERENCES Linked references are available on JSTOR for this artic. *Behaviour, 133*(9), 741–761.
Healy, E., & Marples, N. (2000). The effect of enclosure complexity on activity levels and stereotypic behaviour of three species of the genus Panthera at Dublin Zoo. *Proceedings of the 2nd Annual Symposium on Zoo Research, Paignton Zoo Environmental Park, Paignton, Devon, UK, 6-7th July 2000*, 35–41.
Hediger, H. (1955). Studies of the psychology and behavior of captive animals in zoos and circuses.
Hediger, H. (1964). Man as a social partner of animals and vice versa. *Animal Behaviour, 12*(2-3), 395. https://doi.org/10/c73gc4
Hediger, H. (1968). *The psychology and behaviour of animals in zoos and circuses.*
Herborn, K. A., Macleod, R., Miles, W. T., Schofield, A. N., Alexander, L., & Arnold, K. E. (2010). Personality in captivity reflects personality in the wild. *Animal Behaviour, 79*(4), 835–843. https://doi.org/10/ft65dt
Herrelko, E. S., Vick, S.-J., & Buchanan-Smith, H.-M. (2012). Cognitive Research in Zoo-Housed Chimpanzees: Influence of Personality and Impact on Welfare. *American Journal of Primatology, 74*(9), 828–840. https://doi.org/10.1002/ajp.22036
Highfill, L., Hanbury, D., Kristiansen, R., Kuczaj, S., & Watson, S. (2010). Rating vs. coding in animal personality research. *Zoo biology, 29*(4), 509–16. https://doi.org/10.1002/zoo.20279
Higley, J. D., Hasert, M. F., Suomi, S. J., & Linnoila, M. (1991). Nonhuman primate model of alcohol abuse: Effects of early experience, personality, and stress on alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America, 88*(16), 7261–5. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=52274&tool=pmcentrez&rendertype=abstract
Hill, S. P., & Broom, D. M. (2009). Measuring zoo animal welfare: Theory and practice. *Zoo Biology, 28*(6), 531–544. https://doi.org/10.1002/zoo.20276
Hinde, R. A. (1966). Animal behavior: A synthesis of ethology and comparative psychology.

Hodos, W., & Campbell, C. B. G. (1969). Scala naturae: Why there is no theory in comparative psychology. *Psychological Review*, *76*(4), 337. https://doi.org/10/c8fdnm

Hosey, G., Melfi, V., Formella, I., Ward, S. J., Tokarski, M., Brunger, D., Brice, S., & Hill, S. P. (2016). Is wounding aggression in zoo-housed chimpanzees and ring-tailed lemurs related to zoo visitor numbers? *Zoo Biology*, *35*(3), 205–209. https://doi.org/10/f87ttn

Hosey, G. (2000). Zoo Animals and Thier Human Audiences : What is the Visitors Effect? *Animal Welfare*, *9*, 343–357.

Hunter, L. T., White, P., Henschel, P., Frank, L., Burton, C., Loveridge, A., Balme, G., Breitenmoser, C., & Breitenmoser, U. (2013a). Walking with lions: Why there is no role for captive-origin lions Panthera leo in species restoration. *Oryx*, *47*(1), 19–24. https://doi.org/10/gk8f3h

Hunter, L. T., White, P., Henschel, P., Frank, L., Burton, C., Loveridge, A., Balme, G., Breitenmoser, C., & Breitenmoser, U. (2013b). Walking with lions: Why there is no role for captive-origin lions Panthera leo in species restoration. *Oryx*, *47*(1), 19–24. https://doi.org/10/gk8f3h

Hutchins, M., Willis, K., & Wiese, R. J. (1995). Strategic collection planning: Theory and practice. *Zoo Biology*, *14*(1), 5–25. https://doi.org/10/bt22th

Hutchinson, K. M., McLaughlin, K. J., Wright, R. L., Ortiz, J. B., Anouti, D. P., Mika, A., Diamond, D. M., & Conrad, C. D. (2012). Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. *Neurobiology of learning and memory*, *97*(2), 250–260.

Izzo, G. N., Bashaw, M. J., & Campbell, J. B. (2011). Enrichment and individual differences affect welfare indicators in squirrel monkeys (Saimiri sciureus). *Journal of comparative psychology*, *125*(3), 347–352. https://doi.org/10.1037/a0024294

Jamieson, P. (1990). Duty and the beast: The movement in reform of animal welfare law. *U. Queensland LJ*, *16*, 238.

Japyassú, H. F., & Malange, J. (2014). Plasticity, stereotypy, intra-individual variability and personality: Handle with care. *Behavioural processes*, *109*, 40–47.

Jensvold, M. L. A., Sanz, C. M., Fouts, R. S., & Fouts, D. H. (2001). Effect of enclosure size and complexity on the behaviors of captive chimpanzees (Pan troglodytes). *Journal of Applied Animal Welfare Science*, *4*(1), 53–69. https://doi.org/10/bdtcmt

Jha, A. K. (2011). Release and Reintroduction of Captive-bred Red Pandas into Singalila National Park, Darjeeling, India. *Red panda* (pp. 435–446). Elsevier.

Jhala, Y. V., Banerjee, K., Chakrabarti, S., Basu, P., Singh, K., Dave, C., & Gogoi, K. (2019). Asiatic Lion: Ecology, Economics, and Politics of Conservation. Front. Ecol. Evol. 7: 312. doi: 10.3389/fevo. 2019.00312 Asiatic Lion: Ecology. *Economics, and Politics of Conservation Yadvendradev V. Jhala, 1.*

Jhala, Y. V., Singh, A. P., Gogoi, K., Chakrabarti, S., Singh, P., & Nala, R. R. (2018). *Spatial Analysis of Livestock Predation by Lions in the Greater Gir Landscape*. Technical report of Gujarat Forest Department & Wildlife Institute of India ....

Jhala, Y. V., Mukherjee, S., Shah, N., Chauhan, K. S., Dave, C. V., Meena, V., & Banerjee, K. (2009). Home range and habitat preference of female lions (Panthera leo persica) in Gir forests, India. *Biodiversity and Conservation*, *18*(13), 3383–3394. https://doi.org/10.1007/s10531-009-9648-9

Johnsingh, A. J. T., Goyal, S. P., & Qureshi, Q. (2007). Short Communication Preparations for the reintroduction of Asiatic lion Panthera leo persica into Kuno Wildlife Sanctuary , Madhya Pradesh , India. *Oryx*, *41*(1), 93–96.

https://doi.org/10.1017/S0030605307001512
Joslin, P. (1984). The environmental limitations and future of the Asiatic lion. *Journal of the Bombay Natural History Society. Bombay*, *81*(3), 648–664.
Joslin, P. (1973). The Asiatic Lion: A Study of Ecology and Behaviour, 498–498.
Jule, K. R., Leaver, L. A., & Lea, S. E. (2008). The effects of captive experience on reintroduction survival in carnivores: A review and analysis. *Biological conservation*, *141*(2), 355–363. https://doi.org/10/d4s7cb
Kassambara, A. (2020). *Ggpubr: 'ggplot2' Based Publication Ready Plots* (Version 0.4.0). https://CRAN.R-project.org/package=ggpubr
Kaumanns, W., & Singh, M. (2015). Towards more individual-based and fitness-oriented captive mammal population management. *Current Science*, 1121–1129.
Kessel, A. L., & Brent, L. (1996). Space utilization by captive-born baboons (Papio sp.) before and after provision of structural enrichment. *Animal Welfare*, *5*(1), 37–44.
Keulartz, J. (1993). An Ethics of Captivity for the Anthropocene Zoos at a Crossroads, 1–11.
Kind, C.-J., Ebinger-Rist, N., Wolf, S., Beutelspacher, T., & Wehrberger, K. (2014). The smile of the Lion Man. Recent excavations in Stadel Cave (Baden-Württemberg, southwestern Germany) and the restoration of the famous Upper Palaeolithic figurine. *Quartär*, *61*, 129–145.
Kistler, C., Hegglin, D., Würbel, H., & König, B. (2010). Structural enrichment and enclosure use in an opportunistic carnivore: The red fox (Vulpes vulpes). *Animal Welfare*, *19*(4), 391.
Konjević, D., Bata, I., Sindičić, M., & Njemirovskij, V. (2015). Zoochosis as a cause of excessive dental wear in captive Siberian tiger - a case report. *Veterinarska Stanica*, *46*(1), 59–63. Retrieved August 23, 2021, from https://www.cabdirect.org/cabdirect/abstract/20153088717
Koolhaas, J., Korte, S., De Boer, S., Van Der Vegt, B., Van Reenen, C., Hopster, H., De Jong, I., Ruis, M., & Blokhuis, H. (1999). Coping styles in animals: Current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews*, *23*(7), 925–935.
Kroshko, J., Clubb, R., Harper, L., Mellor, E., Moehrenschlager, A., & Mason, G. (2016). Stereotypic route tracing in captive Carnivora is predicted by species-typical home range sizes and hunting styles. *Animal Behaviour*, *117*, 197–209. https://doi.org/10.1016/j.anbehav.2016.05.010
Kry, K., & Casey, R. (2007). The effect of hiding enrichment on stress levels and behaviour of domestic cats (Felis sylvestris catus) in a shelter setting and the implications for adoption potential. *Animal Welfare*, *16*(3), 375–383.
Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*, 1–12. https://doi.org/10.3389/fpsyg.2013.00863
Leonard, D. A. (2008). *An evaluation of environmental enrichment for two highly social and endangered canid species, the African wild dog (*Lycaon pictus*) and Mexican wolf (*Canis lupus baileyi*)*. Saint Louis University.
Life, W., Government, C., Rules, Z., Gazette, O., & Life, W. (1992). RECOGNITION OF ZOO RULES 1992. *1972*(1).
Locurto, C. (2006). Individual Differences and Animal Personality. *Comparative Cognition & Behavior Reviews*, *2*, 67–78. https://doi.org/10.3819/ccbr.2008.20004
Loehlin, J. C. (1992). *Genes and environment in personality development*. Sage Publications, Inc.

Loehlin, J. C., McCrae, R. R., Costa Jr, P. T., & John, O. P. (1998). Heritabilities of common and measure-specific components of the Big Five personality factors. *Journal of research in personality*, *32*(4), 431–453. https://doi.org/10/fmm44w

Lucht, M. (2012). The Irony of Animal Cruelty Legislation. *Society & Animals*, *20*(4), 411–412. https://doi.org/10/gmbgwj

Lukas, K. E., Hoff, M. P., & Maple, T. L. (2003). Gorilla behavior in response to systematic alternation between zoo enclosures. *Applied Animal Behaviour Science*, *81*(4), 367–386. https://doi.org/10.1016/S0168-1591(02)00237-X

Lyons, J., Young, R. J., & Deag, J. M. (1997). The effects of physical characteristics of the environment and feeding regime on the behavior of captive felids. *Zoo Biology*, *16*(1), 71–83. https://doi.org/10/crxzzb

MacIntosh, A. J. J., Alados, C. L., & Huffman, M. A. (2011). Fractal analysis of behaviour in a wild primate: Behavioural complexity in health and disease. *Journal of the Royal Society Interface*, *8*(63), 1497–1509. https://doi.org/10.1098/rsif.2011.0049

MacKenzie, J. M. (2017). The empire of nature: Hunting, conservation and British imperialism.

Mallapur, A., & Chellam, R. (2002). Environmental influences on stereotypy and the activity budget of Indian leopards (Panthera pardus) in four zoos in Southern India. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *21*(6), 585–595.

Mallapur, A., Qureshi, Q., & Chellam, R. (2002). Enclosure Design and Space Utilization by Indian Leopards (*Panthera pardus*) in Four Zoos in Southern India. *Animal Welfare*, *5*(2), 111–124.

Mallapur, A., Sinha, A., & Waran, N. (2005). Influence of visitor presence on the behaviour of captive lion-tailed macaques (Macaca silenus) housed in Indian zoos. *Applied Animal Behaviour Science*, *94*(3-4), 341–352. https://doi.org/10/dg295b

Mallapur, A., Waran, N., & Sinha, A. (2005). Use of enclosure space by captive lion-tailed macaques (Macaca silenus) housed in Indian zoos. *Journal of Applied Animal Welfare Science*, *8*(3), 175–186. https://doi.org/10/ccxxgc

Mallapur, A., Waran, N., & Sinha, A. (2007). A note on enrichment for captive lion-tailed macaques (*Macaca silenus*). *Applied Animal Behaviour Science*, *108*(1-2), 191–195. https://doi.org/10.1016/j.applanim.2006.11.020

Marcet-Rius, M., Fàbrega, E., Cozzi, A., Bienboire-Frosini, C., Descout, E., Velarde, A., & Pageat, P. (2019). Are Tail and Ear Movements Indicators of Emotions in Tail-Docked Pigs in Response to Environmental Enrichment? *Animals*, *9*(7), 449. https://doi.org/10.3390/ani9070449

Marcon, M., Mocelin, R., Benvenutti, R., Costa, T., Herrmann, A. P., de Oliveira, D. L., Koakoski, G., Barcellos, L. J., & Piato, A. (2018). Environmental enrichment modulates the response to chronic stress in zebrafish. *Journal of Experimental Biology*, *221*(4).

Marinath, L., Vaz, J., Kumar, D., Thiyagesan, K., & Baskaran, N. (2019). Drivers of stereotypic behaviour and physiological stress among captive jungle cat (Felis chaus Schreber, 1777) in India. *Physiology & behavior*, *210*, 112651.

Marshall, A. R., Deere, N. J., Little, H. A., Snipp, R., Goulder, J., & Mayer-Clarke, S. (2016). Husbandry and enclosure influences on penguin behavior and conservation breeding. *Zoo biology*, *35*(5), 385–397. https://doi.org/10/gmwrnk

Martin, P., & Bateson, P. (1993). Measuring behaviour. https://doi.org/10.1017/CBO9781139168342

Martínez-Macipe, M., Lafont-Lecuelle, C., Manteca, X., Pageat, P., & Cozzi, A. (2015). Evaluation of an innovative approach for sensory enrichment in zoos: Semiochemical stimulation for captive lions (*Panthera leo*). *Animal Welfare, 24*(4), 455–461.

Martin-Wintle, M. S., Shepherdson, D., Zhang, G., Huang, Y., Luo, B., & Swaisgood, R. R. (2017). Do opposites attract? Effects of personality matching in breeding pairs of captive giant pandas on reproductive success. *Biological Conservation, 207*, 27–37. https://doi.org/10/f9zssb

Mason, G., & Mendl, M. (2007). Why is there no simple way of measuring animal welfare? *Animal Welfare, 2*, 301–319. papers://031fc22f-ec67-46dc-8154-9b1109ad3ac4/Paper/p94

Mason, G. J. (2006). *Stereotypic Animal Behaviour: Fundamentals and applications to welfare and beyond.* https://doi.org/10.1079/9780851990040.0325

Mason, G. J., & Latham, N. R. (2004). Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? *Animal Welfare, 13*, 57–69. https://doi.org/10.2307/4493573

McCall, D. F. (1973). The prevalence of lions: Kings, deities and feline symbolism in Africa and Elsewhere. *Paideuma*, 130–145.

McFarland, S. E., & Hediger, R. (2009). *Animals and agency: An interdisciplinary exploration* (Vol. 8). Brill.

McGowan, R. T. S., Robbins, C. T., Alldredge, J. R., & Newberry, R. C. (2010). Contrafreeloading in grizzly bears: Implications for captive foraging enrichment. *Zoo Biology, 29*(4), 484–502.

Meehan, C. L., & Mench, J. A. (2007). The challenge of challenge: Can problem solving opportunities enhance animal welfare? *Applied Animal Behaviour Science, 102*(3-4), 246–261. https://doi.org/10.1016/j.applanim.2006.05.031

Meehan, C., & Mench, J. (2002). Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. *Applied Animal Behaviour Science, 79*(1), 75–88.

Meena, V. (2009). Variation in social organisation of lions with particular reference to the Asiatic Lions Panthera leo persica (Carnivora: Felidae) of the Gir forest, India. *Journal of Threatened Taxa, 1*(3), 158–165. https://doi.org/10.11609/JoTT.o2095.158-65

Meena, V., Macdonald, D. W., & Montgomery, R. A. (2014). Managing success: Asiatic lion conservation, interface problems and peoples' perceptions in the Gir Protected Area. *Biological Conservation, 174*, 120–126. https://doi.org/10/f57fpv

Mehta, P. H., & Gosling, S. D. (2008). Bridging human and animal research: A comparative approach to studies of personality and health. *Brain, Behavior, and Immunity, 22*(5), 651–661. https://doi.org/10.1016/j.bbi.2008.01.008

Melfi, V. A. (2009). There are big gaps in our knowledge, and thus approach, to zoo animal welfare: A case for evidence-based zoo animal management. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association, 28*(6), 574–588.

Mellen, J. D., & Shepherdson, D. J. (1997). Environmental enrichment for felids: An integrated approach. *International Zoo Yearbook, 35*(1), 191–197. https://doi.org/10/d7kfsz

Mellor, D. J., & Reid, C. S. W. (1994). Concepts of animal well-being and predicting the impact of procedures on experimental animals.

Mellor, D. J. (2016). Moving beyond the "five freedoms" by updating the "five provisions" and introducing aligned "animal welfare aims". *Animals, 6*(10), 59. https://doi.org/10/gg6p9v

Mellor, D. J. (2017). Operational details of the five domains model and its key applications to the assessment and management of animal welfare. *Animals*, *7*(8), 60.
Mellor, D. J. (2019). Welfare-aligned Sentience: Enhanced Capacities to Experience, Interact, Anticipate, Choose and Survive. *Animals*, *9*(7), 440. https://doi.org/10.3390/ani9070440
Mellor, D. J., & Beausoleil, N. J. (2015). Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. *Anim. Welf*, *24*(3), 241. https://doi.org/10/f7p26r
Mellor, D. J., Beausoleil, N. J., Littlewood, K. E., McLean, A. N., McGreevy, P. D., Jones, B., & Wilkins, C. (2020). The 2020 five domains model: Including human–animal interactions in assessments of animal welfare. *Animals*, *10*(10), 1870. https://doi.org/10/gh55dt
Melotti, L., Oostindjer, M., Bolhuis, J. E., Held, S., & Mendl, M. (2011). Coping personality type and environmental enrichment affect aggression at weaning in pigs. *Applied Animal Behaviour Science*, *133*(3-4), 144–153.
Mendl, M., Burman, O. H., Parker, R. M., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science*, *118*(3-4), 161–181. https://doi.org/10.1016/j.applanim.2009.02.023
Merkies, K., Ready, C., Farkas, L., & Hodder, A. (2019). Eye Blink Rates and Eyelid Twitches as a Non-Invasive Measure of Stress in the Domestic Horse. *Animals*, *9*(8), 562. https://doi.org/10.3390/ani9080562
Michaels, C. J., Downie, J. R., & Campbell-Palmer, R. (2014). The importance of enrichment for advancing amphibian welfare and conservation goals. *Amphibian Reptile Conservation*, *8*, 7–23.
Miller, L. J., Vicino, G. A., Sheftel, J., & Lauderdale, L. K. (2020a). Behavioral Diversity as a Potential Indicator of Positive Animal Welfare. *Animals*, *10*(7), 1211. https://doi.org/10.3390/ani10071211
Miller, L. J., Vicino, G. A., Sheftel, J., & Lauderdale, L. K. (2020b). Behavioral diversity as a potential indicator of positive animal welfare. *Animals*, *10*(7), 1211. https://doi.org/10/gg7sxd
Mills, D. (1998). Personality and individual differences in the horse, their significance, use and measurement. *Equine Veterinary Journal*, *30*(S27), 10–13.
Mitra, R., & Sapolsky, R. M. (2009). Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. *Stress*, *12*(4), 305–312.
Moe, R. O., Bakken, M., Kittilsen, S., Kingsley-Smith, H., & Spruijt, B. M. (2006). A note on reward-related behaviour and emotional expressions in farmed silver foxes (Vulpes vulpes)-Basis for a novel tool to study animal welfare. *Applied Animal Behaviour Science*, *101*(3-4), 362–368. https://doi.org/10.1016/j.applanim.2006.02.004
Mondol, S., Booth, R. K., & Wasser, S. K. (2020). Fecal stress, nutrition and reproductive hormones for monitoring environmental impacts on tigers (*Panthera tigris*). *Conservation physiology*, *8*(1), coz091.
Moneta, G. B., & Spada, M. M. (2009). Coping as a mediator of the relationships between trait intrinsic and extrinsic motivation and approaches to studying during academic exam preparation. *Personality and Individual Differences*, *46*(5), 664–669. https://doi.org/10/bq4x3n
Moorhouse, T. P., Dahlsjö, C. A. L., Baker, S. E., & Cruze, N. C. D. (2015). The Customer Isn ' t Always Right — Conservation and Animal Welfare Implications of the Increasing

Demand for Wildlife Tourism, 1–15. https://doi.org/10.1371/journal.pone.0138939
Morris, E. K., Higgins, S. T., Bickel, W. K., & Braukmann, C. J. (1987). An Introduction to Contemporary Behaviorism. *Behavioral Approaches to Crime and Delinquency* (pp. 61–105). Springer.
Morton, F. B., Lee, P. C., & Buchanan-Smith, H. M. (2013). Taking personality selection bias seriously in animal cognition research: A case study in capuchin monkeys (Sapajusapella). *Animal Cognition, 16*(4), 677–684. https://doi.org/10/f48sw2
Muehlmann, A. M., & Lewis, M. H. (2012). Abnormal repetitive behaviours: Shared phenomenology and pathophysiology. *Journal of Intellectual Disability Research, 56*(5), 427–440. https://doi.org/10.1111/j.1365-2788.2011.01519.x
Murphy, L. B. (1977). Responses of domestic fowl to novel food and objects. *Applied Animal Ethology, 3*(4), 335–349.
Narayan, E., Baskaran, N., & Vaz, J. (2017). Conservation Physiology of Tigers in Zoos: Integrating Stress Physiology and Behaviour to Monitor Their Health and Welfare. *Big Cats*, 35–44.
Nazar, F. N., & Marin, R. H. (2011). Chronic stress and environmental enrichment as opposite factors affecting the immune response in Japanese quail (*Coturnix coturnix japonica*). *Stress, 14*(2), 166–173.
Ncube, S., & Ndagurwa, H. G. T. (2010). Influence of social upbringing on the activity pattern of captive lion (*Panthera leo*)cubs: Benefits of behavior enrichment. *Current Zoology, 56*(4), 389–394.
Nogueira, S. S., Bernardi, L. G., & Nogueira-Filho, S. L. (2004). A note on comparative enclosure facility usage by wild and captive-born capybaras (*Hydrochoerus hydrochaeris*). *Applied Animal Behaviour Science, 89*(1-2), 139–143.
O'Donovan, D., Hindle, J. E., McKeown, S., & O'Donovan, S. (1993). Effect of visitors on the behaviour of female cheetahs Acinonyx jubutus and cubs. *International Zoo Yearbook, 32*(1), 238–244.
Owen, M. A., Swaisgood, R. R., & Blumstein, D. T. (2017). Contextual influences on animal decision-making: Significance for behavior-based wildlife conservation and management. *Integrative Zoology, 12*(1), 32–48. https://doi.org/10.1111/1749-4877.12235
Pastorino, G. Q., Soares, N. R. P., Mazzola, S., & Preziosi, R. (2016). Personality assessment and feline – keepers relationship in lions (Panthera leo). *International Journal of Health Aniimal Science & Food Safety, 3*(1), 1–2. file:///C:/Users/siten/Downloads/Documents/7102-21761-1-PB.pdf
Pastorino, G. Q., Viau, A., Curone, G., Pearce-Kelly, P., Faustini, M., Vigo, D., Mazzola, S. M., & Preziosi, R. (2017). Role of personality in behavioral responses to new environments in captive Asiatic lions (*Panthera leo persica*). *Veterinary medicine international, 2017.*
Patel, F., Wemelsfelder, F., & Ward, S. J. (2019). Using Qualitative Behaviour Assessment to Investigate Human-Animal Relationships in Zoo-Housed Giraffes (Giraffa camelopardalis). *Animals, 9*(6), 381. https://doi.org/10.3390/ani9060381
Patil, I., & Powell, C. (2018). Ggstatsplot:"ggplot2" based plots with statistical details. *CRAN.*
Paulson, M. (1999). Pride in place: Reintroduction of Asiatic lions in Gir Forest, India.
Plowman, A. (2003). A note on a modification of the spread of participation index allowing for unequal zones. https://doi.org/10.1016/S0168-1591(03)00142-4
Portions, R., Wildlife, N., Plan, A., The, P. B., Wildlife, N., Plan, A., Board, I., National, T., Policy, F., Wildlife, N., & Plan, A. (2016). National Wildlife Action Plan (2002-2016

).
Powell, D. M., & Gartner, M. C. (2011). Applications of personality to the management and conservation of nonhuman animals. *From genes to animal behavior* (pp. 185–199). Springer.
Powell, D. M., & Svoke, J. T. (2008). Novel Environmental Enrichment May Provide a Tool for Rapid Assessment of Animal Personality: A Case Study With Giant Pandas (Ailuropoda melanoleuca). *Journal of Applied Animal Welfare Science, 11*(4), 301–318. https://doi.org/10/fkkzv4
Powell, D. (1995). Preliminary evaluation of environmental enrichment techniques for African lions (*Panthera Leo*). *Animal Welfare, 4*(4), 361–370. http://apps.webofknowledge.com/full_record.do?product=UA&search_mode=Refine&qid=3&SID=P26jSUXD7jxRZsxWBnJ&page=1&doc=9&cacheurlFromRightClick=no
Preece, R. (2006). *Awe for the tiger, love for the lamb: A chronicle of sensibility to animals.* Routledge.
Proctor, H. S., Carder, G., & Cornish, A. R. (2013). Searching for animal sentience: A systematic review of the scientific literature. *Animals, 3*(3), 882–906. https://doi.org/10/gcbxxj
Quirke, T., & O'Riordan, R. M. (2011). The effect of different types of enrichment on the behaviour of cheetahs (Acinonyx jubatus) in captivity. *Applied animal behaviour science, 133*(1-2), 87–94.
R Core Team. (2020). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria* (Version 4). https://www.r-project.org/
Rabin, L. A. (2003). Maintaining behavioural diversity in captivity for conservation: Natural behaviour management. *Animal Welfare, 12*(1), 85–94.
Rachlin, H. (1999). Teleological behaviorism. *Handbook of behaviorism* (pp. 195–215). Elsevier.
Ramanathan, A., Malik, P. K., & Prasad, G. (2007). Seroepizootiological survey for selected viral infections in captive Asiatic lions (Panthera leo persica) from western India. *Journal of Zoo and Wildlife Medicine, 38*(3), 400–409.
Rangarajan, M. (2013). Animals with rich histories: The case of the lions of Gir Forest, Gujarat, India. *History and Theory, 52*(4), 109–127.
Rashkow, E. (2015). Resistance to Hunting in Pre-independence India: Religious environmentalism, ecological nationalism or cultural conservation? *Modern Asian Studies, 49*(2), 270–301.
Razal, C. B., Pisacane, C. B., & Miller, L. J. (2016). Multifaceted approach to personality assessment in cheetahs (Acinonyx jubatus). *Animal behaviour, 3*(1), 22–31. https://doi.org/10.12966/abc.03.01.2016
Reading, R. P., Miller, B., & Shepherdson, D. (2013). The value of enrichment to reintroduction success. *Zoo Biology, 32*(3), 332–341. https://doi.org/10.1002/zoo.21054
Regaiolli, B., Rizzo, A., Ottolini, G., Miletto Petrazzini, M. E., Spiezio, C., & Agrillo, C. (2019). Motion illusions as environmental enrichment for zoo animals: A preliminary investigation on lions (Panthera leo). *Frontiers in Psychology, 10*, 2220.
Resende, L. S., Remy, G. L., Ramos Jr, V. d. A., & Andriolo, A. (2009). The influence of feeding enrichment on the behavior of small felids (Carnivora: Felidae) in captivity. *Zoologia (Curitiba), 26*(4), 601–605.
Revelle, W. (2019). *Psych: Procedures for Psychological, Psychometric, and Personality*

Research (Version 1.9.12). Evanston, Illinois. https://CRAN.R-project.org/package=psych
Rollin, B. E. (1990). Animal welfare, animal rights and agriculture. *Journal of animal science*, *68*(10), 3456–3461. https://doi.org/10/gmq2mm
Rose, P., & Robert, R. (2013). Evaluating the activity patterns and enclosure usage of a little-studied zoo species, the sitatunga (*Tragelaphus spekii*). *Journal of Zoo and Aquarium Research*, *1*(1), 14–19. http://www.jzar.org/jzar/article/view/12/4
Ross, S. R. (2006). Issues of choice and control in the behaviour of a pair of captive polar bears (Ursus maritimus). *Behavioural processes*, *73*(1), 117–120.
Ross, S. R., Schapiro, S. J., Hau, J., & Lukas, K. E. (2009). Space use as an indicator of enclosure appropriateness: A novel measure of captive animal welfare. *Applied Animal Behaviour Science*, *121*(1), 42–50. https://doi.org/10.1016/j.applanim.2009.08.007
Ross, S. R., & Shender, M. A. (2016). Daily travel distances of zoo-housed chimpanzees and gorillas: Implications for welfare assessments and space requirements. *Primates*, *57*(3), 395–401. https://doi.org/10.1007/s10329-016-0530-6
Rothfels, N. (2002). *Savages and beasts: The birth of the modern zoo*. JHU Press.
Rouff, J. H., Sussman, R. W., & Strube, M. J. (2005). Personality traits in captive lion-tailed macaques (Macaca silenus). *American journal of primatology*, *67*(2), 177–198. https://doi.org/10.1002/ajp.20176
Ruskell, A. D., Meiers, S. T., Jenkins, S. E., & Santymire, R. M. (2015). Effect of bungee-carcass enrichment on behavior and fecal glucocorticoid metabolites in two species of zoo-housed felids. *Zoo Biology*, *34*(2), 170–177. https://doi.org/10.1002/zoo.21192
Saberwal, V. K., Gibbs, J. P., Chellam, R., & Johnsingh, A. J. T. (1994). Lion-human conflict in the Gir Forest, India. *Conservation Biology*, *8*(2), 501–507.
Sanyal, R. B. (1892). *A Hand-book of the Management of Animals in Captivity in Lower Bengal*. Printed at the Bengal Secretariat Press.
Schildkraut, R. (2016). *Characteristation of positive welfare indices in captive african lions (*Panthera leo*)* (Master's Thesis). University of Sydney.
Schoenecker, B., & Heller, K. E. (2001). The involvement of dopamine (DA) and serotonin (5-HT) in stress-induced stereotypies in bank voles (Clethrionomys glareolus). *Applied Animal Behaviour Science*, *73*(4), 311–319. https://doi.org/10/cj8gnw
Sekar, M., Rajagopal, T., & Archunan, G. (2008). Influence of zoo visitor presence on the behavior of captive Indian gaur (Bos gaurus gaurus) in a zoological park. *Journal of Applied Animal Welfare Science*, *11*(4), 352–357. https://doi.org/10/b5shwt
Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in ecology & evolution*, *19*(7), 372–8. https://doi.org/10.1016/j.tree.2004.04.009
Singer, P. (1979). Killing humans and killing animals. *Inquiry*, *22*(1-4), 145–156. https://doi.org/10/btjzgz
Sinha, S. P. (1987). *Ecology of wildlife with special reference to the lion (Panthera leo persica) in Gir wildlife sanctuary, Saurashtra, Gujarat* (PhD Thesis). Ph. D. Partial fulfillment of the requirements for the degree of Doctor of ….
Skibiel, A. L., Trevino, H. S., & Naugher, K. (2007). Comparison of several types of enrichment for captive felids. *Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association*, *26*(5), 371–381.
Skinner, B. F. (1963). Behaviorism at fifty. *Science*, *140*(3570), 951–958.

https://doi.org/10/ctjk3n
Skrzypczak, N. (2016). *Personality traits in the Atlantic spotted dolphin (Stenella frontalis): Syndromes and predictors of neophilia.* http://proxy.bc.edu/login?url=http://search.proquest.com/docview/1847953019?accountid=9673%0Ahttp://bc-primo.hosted.exlibrisgroup.com/openurl/BCL/services_page??url_ver=Z39.88-2004&rft_val_fmt=info:ofi/fmt:kev:mtx:dissertation&genre=dissertations+%26+thes
Smith, G. L. (1984). *International Studbook: Indian Lion,* Panthera leo persica *(Meyer, 1826).* Knoxville Zoological Park.
Sneddon, L. U., Braithwaite, V. A., & Gentle, M. J. (2003). Animal Studies Repository Novel Object Test: Examining Nociception and Fear in the Rainbow Trout Novel Object Test: Examining Nociception and Fear in the Rainbow Trout. *The Journal of Pain, 4*(8), 431–440. http://animalstudiesrepository.org/acwp_vsm
Snyder, N. F., Derrickson, S. R., Beissinger, S. R., Wiley, J. W., Smith, T. B., Toone, W. D., & Miller, B. (1996). Limitations of Captive Breeding in Endangered Species Recovery. *Conservation Biology, 10*(2), 338–348. https://doi.org/10/chx6v3
Spiezio, C., Valsecchi, V., Sandri, C., & Regaiolli, B. (2018). Investigating individual and social behaviour of the Northern bald ibis (Geronticus eremita): Behavioural variety and welfare. *PeerJ, 6,* e5436–e5436. https://doi.org/10.7717/peerj.5436
Squibb, K., Griffin, K., Favier, R., & Ijichi, C. (2018). Poker Face: Discrepancies in behaviour and affective states in horses during stressful handling procedures. *Applied Animal Behaviour Science, 202,* 34–38.
Srivastav, A. (2014). *International Studbook of Asiatic lion (*Panthera leo persica *).* Srivastav_2014_International_Studbook_of_Asiatic_lion.pdf
Srivastav, A., Nigam, P., & Begum, N. (2018). Indian National Studbook of Asiatic Lion (*Panthera leo persica*).
Stamps, J., & Groothuis, T. G. G. (2010). The development of animal personality The development of animal personality : Relevance , concepts and perspectives. https://doi.org/10.1111/j.1469-185X.2009.00103.x
Stanton, M. A., Heintz, M. R., Lonsdorf, E. V., Santymire, R. M., Lipende, I., & Murray, C. M. (2015). Maternal Behavior and Physiological Stress Levels in Wild Chimpanzees (Pan troglodytes schweinfurthii). *International Journal of Primatology, 36*(3), 473–488. https://doi.org/10.1007/s10764-015-9836-2
Storey, W. K. (1991). Big cats and imperialism: Lion and tiger hunting in Kenya and Northern India, 1898-1930. *Journal of World History, 2*(2), 135–173.
Suárez, P., Recuerda, P., & Arias-de-Reyna, L. (2017). Behaviour and welfare: The visitor effect in captive felids. *Animal Welfare, 26*(1), 25–34.
Swaisgood, R. R. (2010). The conservation-welfare nexus in reintroduction programmes: A role for sensory ecology. *Animal Welfare, 19*(2), 125–137.
Swaisgood, R. R., & Shepherdson, D. J. (2005). Scientific Approaches to Enrichment and Stereotypies in Zoo Animals : What ' s Been Done and Where Should We Go Next ? *Zoo Biology, 518,* 499–518. https://doi.org/10.1002/zoo
Sweeney, N. (2017). *A Practical Approach to Animal Welfare Law.* 5m Books Ltd.
Szokalski, M. S., Litchfield, C. A., & Foster, W. K. (2012). Enrichment for captive tigers (Panthera tigris): Current knowledge and future directions. *Applied Animal Behaviour Science, 139*(1-2), 1–9.
Tan, H. M., Ong, S. M., Langat, G., Bahaman, A. R., Sharma, R. S. K., & Sumita, S. (2013). The influence of enclosure design on diurnal activity and stereotypic behaviour in captive Malayan Sun bears (Helarctos malayanus). *Research in veterinary science,*

94(2), 228–239.

Team, R. (2020). *RStudio: Integrated development for R (Version 1.3.776)* (Version 1.3.776).

Tilbrook, A. J., & Ralph, C. R. (2018). Hormones , stress and the welfare of animals, 408–415.

Tinbergen, N. (1963). *On aims and methods of Ethology.*

Traylor-Holzer, K., & Fritz, P. (1985). Utilization of space by adult and juvenile groups of captive chimpanzees (*Pan troglodytes*). *Zoo Biology*, *4*(2), 115–127. https://doi.org/10.1002/zoo.1430040205

Valle, E., Raspa, F., Giribaldi, M., Barbero, R., Bergagna, S., Antoniazzi, S., Mc Lean, A. K., Minero, M., & Cavallarin, L. (2017). A functional approach to the body condition assessment of lactating donkeys as a tool for welfare evaluation. *PeerJ*, *5*, e3001–e3001. https://doi.org/10.7717/peerj.3001

Van der Harst, J. E., & Spruijt, B. M. (2007). Tools to measure and improve animal welfare: Reward-related behaviour. *Animal Welfare*, *16*, 67–73.

Van Metter, J. E., Harriger, M. D., & Bolen, R. H. (2008). Environmental enrichment utilizing stimulus objects for African lions (*Panthera leo leo*) and Sumatran tigers (*Panthera tigris sumatrae*). *Beta Beta Beta Biological Society*, *79*(1), 7–16.

Vargas, A., & Anderson, S. H. (1999). Effects of experience and cage enrichment on predatory skills of black-footed ferrets (*Mustela nigripes*). *Journal of Mammalogy*, *80*(1), 263–269.

Vaz, J., Narayan, E. J., Dileep Kumar, R., Thenmozhi, K., Thiyagesan, K., & Baskaran, N. (2017). Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. *PloS one*, *12*(4), e0174711.

Veasey, J. S. (2020). Can Zoos Ever Be Big Enough for Large Wild Animals? A Review Using an Expert Panel Assessment of the Psychological Priorities of the Amur Tiger (Panthera tigris altaica) as a Model Species. *Animals : an open access journal from MDPI.* https://doi.org/10/gh55dr

Veissier, I., & Forkman, B. (2008). *The nature of animal welfare science* (Vol. 10). https://doi.org/10.5016/1806-8774.2008.v10pT15

Vigne, J.-D. (2011). The origins of animal domestication and husbandry: A major change in the history of humanity and the biosphere. *Comptes Rendus Biologies*, *334*(3), 171–181. https://doi.org/10.1016/j.crvi.2010.12.009

Visser, E., Van Reenen, C., Van Der Werf, J., Schilder, M., Knaap, J., Barneveld, A., & Blokhuis, H. (2002). Heart rate and heart rate variability during a novel object testand a handling test in young horses. *Physiology and Behavior*, *76*, 289–296.

Walker, S. (2007). Population and Habitat Viability Assessment PHVA for Asiatic Lion (Panthera leo persica), Executive Summary and Summary Recommendations. *ZOO'S PRINT*, *22*(7), 9–12.

Wasser, S. K., Azkarate, J. C., Booth, R. K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., & Rodríguez-Luna, E. (2010). Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. *General and comparative endocrinology*, *168*(1), 1–7.

Watanabe, S. (2007). How animal psychology contributes to animal welfare. *Applied Animal Behaviour Science*, *106*(4), 193–202. https://doi.org/10.1016/j.applanim.2007.01.003

Watson, J. B. (1913). Psychology as the behaviorist views it. *Psychological review*, *20*(2), 158.

Watters, J. V., Margulis, S. W., & Atsalis, S. (2009). Behavioral monitoring in zoos and aquariums: A tool for guiding husbandry and directing research. *Zoo Biology*, *28*(1), 35–48. https://doi.org/10.1002/zoo.20207

Watters, J. V., & Powell, D. M. (2012). Measuring animal personality for use in population management in zoos: Suggested methods and rationale. *Zoo biology*, *31*(1), 1–12. https://doi.org/10.1002/zoo.20379

Webb, S. J. N., Hau, J., & Schapiro, S. J. (2019). Does group size matter? Captive chimpanzee (Pan troglodytes) behavior as a function of group size and composition. *American Journal of Primatology*, *81*(1), e22947. https://doi.org/10/gmjstj

Welberg, L., Thrivikraman, K. V., & Plotsky, P. M. (2006). Combined pre-and postnatal environmental enrichment programs the HPA axis differentially in male and female rats. *Psychoneuroendocrinology*, *31*(5), 553–564.

Wemelsfelder, F. (1997). The scientific validity of subjective concepts in models of animal welfare. *Applied Animal Behaviour Science*, *53*(1-2), 75–88. https://doi.org/10/csbjqn

Wemelsfelder, F., Haskell, M., Mendl, M. T., Calvert, S., & Lawrence, A. B. (2000). Diversity of behaviour during novel object tests is reduced in pigs housed in substrate-impoverished conditions.

Whitham, J., & Wielebnowski, N. (2013). New directions for zoo animal welfare science. *Applied Animal Behaviour Science*, *147*(3), 247–260. https://doi.org/10/f496tt

Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis*. https://ggplot2.tidyverse.org

Wickham, H., & Averick. (2017). Welcome to the tidyverse. *R package version*, *1*(1).

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., & Hester, J. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686.

Wickham, H., Henry, L., & Miller, K. (2020). *Dplyr: A Grammar of Data Manipulation* (Version 1.0.0). https://CRAN.R-project.org/package=dplyr

Williams, L. J., Finch, K., Agnew, R., & Holmes, L. (2021). Effects of Nearby Construction Work on the Behavior of Asiatic Lions (Panthera leo persica). *Journal of Zoological and Botanical Gardens*, *2*(1), 66–74. https://doi.org/10/gjnh8x

Wilson, D. S., Coleman, K., Clark, A. B., Biederman, L., Wilson, D. S., Coleman, K., & Clark, A. B. (1993). Shy-Bold Continuum in Pumpkinseed Sunfish (Lepomis gibbosus): An Ecological Study of a Psychological Trait. *107*(3), 250–260.

Wolfensohn, S., Shotton, J., Bowley, H., Davies, S., Thompson, S., & Justice, W. S. (2018). Assessment of welfare in zoo animals: Towards optimum quality of life. *Animals*, *8*(7), 1–16. https://doi.org/10.3390/ani8070110

Young, K. M., Walker, S. L., Lanthier, C., Waddell, W. T., Monfort, S. L., & Brown, J. L. (2004). Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. *General and comparative endocrinology*, *137*(2), 148–165.

Young, R., de Azevedo, C. S., & Cipreste, C. F. (2003). Environmental enrichment. *Zoo Animal Learning and Training*, 101.

A
Appendix

A.1 Permits and ethical clearance

- Research permit for this study was granted by the Gujarat Forest Department, India (Permit no: WLP/28/A/1316-21/2015-16). This study complies with the regulations of zoo animal welfare standards set by the Central Zoo Authority, Government of India.
- This study was conducted in accordance with CZA norms on the welfare of animals housed in conservation breeding centres. I did not conduct any invasive sampling procedures on the subjects for experimental setup and data collection. All necessary precautions were taken to ensure that enrichment interventions were integrated with the daily husbandry regimens of the test subjects.

A.2 Conference certificates

A.2.1 International conferences-1

International Conference on
Recent Trends in Zoology, Biodiversity, Genetics & Environmental Sciences 2018

CHOOSE TO BE HUGE

Govt of India Approved Conference MHA Vide F.No 42180123/CC-603 ; MEA Vide F.No No. AA/162/01/2018-1583

Certificate of Participation & Presentation

This is to certify that

MR. SITENDU GOSWAMI

has virtually / participated and presented in the International Conference on Recent Trends in Zoology, Biodiversity, Genetics & Environmental Sciences 2018 with a paper titled

EFFECTS OF COGNITIVE ENRICHMENT ON WELFARE MEASURES OF ASIATIC LIONS AT SAKKARBAUG ZOO

at Dept of Zoology, JB Campus, Bangalore University, Karnataka, Dec 04-05, 2018.

Dr. Bela Zutshi
Organizing Secretary,
Chairperson, Dept of Zoology
JB Campus, Bangalore University

Dr. Ratnakar D Bala
Conference Chairman

A.2.2 International conferences-2

VIRTUAL MEETING

Certificate of Attendance

Sitendu Goswami

has attended the

ABS VIRTUAL MEETING

August 1 - 7, 2021

Animal Behavior Society

August 15, 2021

DATE

Timothy Wright, PhD

CONFERENCE CHAIR

A.2.3 National conference-2

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

Certificate of Participation

This is to certify that

Sitendu Goswami

presented a paper during the 12th Internal Annual Research Seminar held on 26th September 2016 at the Wildlife Institute of India.

In recognition thereof, this **Certificate of Participation** is hereby awarded on this 30th day of September, 2016 at Dehradun.

Director

Dean

Research Coordinator

PeerJ

Effects of personality and rearing-history on the welfare of captive Asiatic lions (*Panthera leo persica*)

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Background. The long-term success of ex-situ conservation programmes depends on species-appropriate husbandry and enrichment practices complemented by an accurate welfare assessment protocol. Zoos and conservation breeding programmes should employ a bottom-up approach to account for intraspecific variations in measures of animal welfare. We studied 35 (14:21) captive Asiatic lions in Sakkarbaug Zoological Garden, Junagadh, India to understand the implications of individual variations on welfare measures. We categorized the subjects based on personality traits (bold or shy), rearing history (wild-rescued or captive-raised), sex, and social-grouping. We explored the association of these categorical variables on welfare indices such as behavioural diversity, latency to approach novel objects, enclosure usage and aberrant repetitive behaviours. Further, we assessed the inter-relationships between different behavioural measures of welfare.

Results. Our results show that intraspecific variations based on rearing-history and personality traits are significantly associated with the welfare states of captive Asiatic lions. Asiatic lions with bold personality traits ($M = 0.50, SD = 0.12, N = 21$) and those raised in captivity ($M = 0.47, SD = 0.12, N = 16$) used enclosure space more homogenously compared to shy ($M = 0.71, SD = 0.15, N = 14$) and wild-rescued ($M = 0.67, SD = 0.15, N = 19$) animals. Behaviour diversity was significantly higher in captive-raised ($M = 1.26, SD = 0.3, N = 16$) and bold ($M = 1.23, SD = 0.26, N = 21$) subjects compared to wild-rescued ($M = 0.83, SD = 0.35, N = 19$) and shy ($M = 0.73, SD = 0.34, N = 14$) individuals. Aberrant repetitive behaviours (stereotypy) were significantly lower in bold ($M = 7.01, SD = 4, N = 21$) and captive-raised ($M = 7.74, SD = 5.3$) individuals compared to wild-rescued ($M = 13.12, SD = 6.25, N = 19$) and shy ($M = 16.13, SD = 5.4, N = 16$) lions. Sex and social-grouping of subjects did not show significant associations with behavioural welfare indices. Interestingly, behaviour diversity was reliably predicted by the enclosure usage patterns and aberrant repetitive behaviours displayed by subjects.

Discussion. Our findings underline the importance of individual-centric, behaviour-based, and multi-dimensional welfare assessment approaches in ex-situ conservation programmes. The results suggest that behavioural welfare indices complemented with individual variations can explain inter-individual differences in behavioural welfare measure outcomes of Asiatic lions. These findings also provide zoo managers with a non-invasive tool to reliably assess and improve husbandry practices for Asiatic lions.

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Additional Information and Declarations can be found on page 18

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Understanding the unique welfare requirement of individuals in captivity will be crucial for the survival of the species.

Subjects Animal Behavior, Conservation Biology
Keywords Animal personality, Cognition, Ex-situ conservation, Behaviour diversity, Latency, Stereotypy, Captive animal welfare

INTRODUCTION

Welfare defines a fine balance between pathophysiology and affective states, or the state of the animal as it copes with its environment (*Broom, 1991*; *Spruijt, Bos & Pijlman, 2001*; *Meehan & Mench, 2007*; *Boissy et al., 2007*; *Butterworth, Mench & Wielebnowski, 2011*; *Panksepp, 2011*). Modern welfare science advocates the creation of opportunities for animals to experience positive emotions (*Fraser & Duncan, 1998*; *Fraser, 1999*; *Fraser, 2009*; *Dawkins, 2004*; *Whitham & Miller, 2016*). Pro-welfare husbandry practices are vital for the biopsychosocial health of captive animals and long-term success of conservation breeding programmes (*Hediger, 1968*; *Rabin, 2003*; *Broom, 2011*). Studies show that animals housed under poor welfare conditions experience allostatic overload and chronic stress, that manifest as loss of behaviour diversity and cognitive abilities (*Sheperdson, Carlstead & Wielebnowski, 2004*; *Mendl et al., 2009*; *Kroshko et al., 2016*; *Razal, Pisacane & Miller, 2016*), ultimately reducing their survival and reproductive potential (*Broom, 1991*; *Schreck, 2010*). Ideally conservation breeding programs should conduct periodic welfare evaluations for the improvement of incumbent housing and husbandry practices, and realign with conservation goals (*Engel, 1980*; *Korte, Olivier & Koolhaas, 2007*; *Broom, 2011*; *Moorhouse et al., 2015*). In practice, ex-situ institutions continue to rely on unidimensional measures such as keeper ratings, physiological, and behavioural measures without accounting for individuality (*Mason & Mendl, 2007*; *Boissy & Erhard, 2014*; *Chadwick, 2014*). Intraspecific variations originating from personality (*Locurto, 2006*) and early-life experiences (*Watters & Powell, 2012*; *Gartner, Powell & Weiss, 2016*) determine the umwelt of individuals (*Loehlin, 1992*; *Stamps & Groothuis, 2010*), affective states (*Harding, Paul & Mendl, 2004*; *Boissy & Erhard, 2014*), and ultimately welfare (*Carere & Locurto, 2011*; *Izzo, Bashaw & Campbell, 2011*). Inter-individual differences in bold/shy personality traits (*Mills, 1998*; *Gosling & John, 1999*; *Gartner, Powell & Weiss, 2016*; *Gartner & Powell, 2011*; *Gartner, Powell & Weiss, 2014*) are associated with differential decision-making abilities (*Carter et al., 2013*), cognition (*Morton, Lee & Buchanan-Smith, 2013*; *Griffin, Guillette & Healy, 2015*) and coping responses (*Wilson et al., 1993*) to welfare deprivation (*Koolhaas et al., 1999*; *Moneta & Spada, 2009*; *Goold & Newberry, 2017*; *Franks, Higgins & Champagne, 2014*), and ultimately have a bearing on post-release fitness (*Bremner-Harrison, Prodohl & Elwood, 2004*). Early-life experiences can also have a bearing on the personality development of animals (*Ainsworth & Bowlby, 1991*; *Higley et al., 1991*; *Loehlin, 1992*; *Frost et al., 2007*; *Stamps & Groothuis, 2010*; *Watters & Powell, 2012*). Therefore, addressing early-life experiences and personality traits in welfare evaluation protocols can be vital to the success of ex-situ conservation breeding programmes (*Wemelsfelder, 1997*; *Rabin,* *2003*; *Reading, Miller & Shepherdson, 2013*). Unfortunately, parameters of individuality are seldom addressed while designing housing and husbandry protocols for wild animals at conservation breeding programmes. Focused multi-species studies are required to understand how personality and early life-experiences (rearing-history) may be associated with behavioural welfare measures. Using captive Asiatic lions as a study system, we tried to understand the relationship between individual variations (viz., bold-shy traits, rearing history, sex, social grouping) with behavioural welfare measures.

The endangered Asiatic lion (*Panthera leo persica*) is now relegated to a fraction of its historic range, across scattered patches of the Greater Gir landscape of Gujarat, India (*Banerjee et al., 2013*). With a global wild and captive population of about 523 and 359 individuals (*Srivastav, 2014*; *Pant, 2015*), the future survival of Asiatic lions can be secured through a successful conservation-breeding program complemented by repatriation across historic ranges (*Jhala et al., 2006*; *Meena, 2009*). While extensive research on population ecology (*Joslin, 1973*; *Jhala et al., 2009*), behaviour (*Meena, 2008*), social dynamics (*Meena, 2009*; *Chakrabarti & Jhala, 2017*), and human-animal interaction (*Joslin, 1973*; *Banerjee, Jhala & Pathak, 2010*; *Banerjee et al., 2013*) of wild Asiatic lions has been conducted, the captive populations and their welfare needs have received relatively less attention. *Pastorino et al. (2016)* and *Pastorino et al. (2017)* studied feline-keeper interactions, personality variations, and behavioural aspects of welfare in captive Asiatic lions at London Zoo, but were limited by a small sample size ($N = 4$) and a short study period. There is a paucity of information on detailed welfare status of captive Asiatic lions despite a large ex-situ population spread among global zoological institutions. It is vital to standardize the welfare evaluation practices for this species to meet its long-term conservation goals. Since Indian ex-situ facilities account for more than 60 percent of the global captive Asiatic lion population (*Srivastav, 2014*), holistic welfare assessments at these sites can have a tangible impact on the conservation goals for the species.

We studied 35 Asiatic lions housed in the ex-situ conservation breeding centre of Sakkarbaug Zoological Garden (SZG), Gujarat, India to understand if rearing-history and personality (*Allport & Allport, 1921*) are important factors associated with intraspecific variations in behavioural welfare indices (*Broom, 1986*; *Fraser, 2009*). We categorized these subjects based on their rearing histories (wild-rescued and captive-raised), sex, social grouping (pair-housed and group-housed) and personality traits (bold and shy) (*Wilson et al., 1994*). We measured species-typical behaviour diversity (*Powell, 1995*; *Wemelsfelder et al., 2000*; *Rabin, 2003*; *Clark & Melfi, 2012*; *Miller, Pisacane & Vicino, 2016*; *Watanabe, 2007*; *Skrzypczak, 2016*; *Owen, Swaisgood & Blumstein, 2017*), space usage patterns (*Kessel & Brent, 1996*; *Mallapur, Qureshi & Chellam, 2002*; *Ross & Shender, 2016*), latency to novel objects (*Murphy, 1977*; *Meehan & Mench, 2002*; *Sneddon, Braithwaite & Gentle, 2003*), and proportion of aberrant behaviours (*Mason & Rushen, 2006*; *Tan et al., 2013*; *Japyassú & Malange, 2014*; *Kroshko et al., 2016*) understand the relationship between individual variations and welfare measures. We believe that this study will address knowledge gaps in animal welfare evaluation procedures, leading to the adoption of individual-focused husbandry and management practices at ex-situ endangered species conservation programmes.

MATERIALS & METHODS

Research permit and ethical considerations

Research permit for this study was granted by the Gujarat Forest Department, India (Permit no: WLP/28/A/1316-21/2015-16). This study complies with the regulations of zoo animal welfare standards set by the Central Zoo Authority, Government of India.

Study area

We conducted the study at Sakkarbaug Zoological Garden (SZG), which is situated within the natural range of Asiatic lions (*Panthera leo persica*). SZG is the coordinating zoo for the Asiatic lion conservation-breeding programme in India and hosts the largest captive population ($N = 60$) with the highest reported number of wild founders (*Srivastav, 2014*) The conservation breeding programme aims to stock a healthy population of captive Asiatic lions for possible repatriation to lost range habitats. The zoo has a separate off-display conservation breeding facility, which houses 47 Asiatic lions. A map (unscaled) of the off-display conservation breeding enclosures of SZG is provided in Fig. S1.

Subjects and housing

We collected data from 38 (Male = 15, Female = 23) healthy Asiatic lions housed in the conservation breeding facility of SZG. During the study we removed three individuals (Male = 1, Female = 2) due to ongoing veterinary treatments, reducing our sample size to 35 individuals (Male = 14, Female = 21) (Table 1). The study subjects were either born in captivity ($N = 16$; Male = 3, Female = 13) or rescued from wild ($N = 19$; Male = 11, Female = 8). Individuals born in the zoo ($N = 14$) and rescued as cubs ($N = 2$) were categorized as captive-raised since they have similar life experiences. Lions that were rescued at a young age and spent most of their lives in captivity cannot have similar life-experiences as adult wild-rescued lions and hence were grouped in the captive-raised category. Most wild-rescued lions were rehabilitated as adults for treatment of injuries incurred due to infighting, and after making full recovery were assimilated in the conservation breeding programme. Some wild-rescued animals ($N = 3$) were rescued to ameliorate conflict caused by livestock depredation. All subjects were either housed in pairs ($N = 17$) or housed in a sex ratio of 1:2 ($N = 18$). All subjects (including the wild-rescued lions) were in socially cohesive groups and were housed in the same enclosure (with the same enclosure mates) for at least a year prior to the commencement of the study. This facility provided us with a unique opportunity to study the behaviour of wild-rescued and captive-raised lions under similar housing conditions.

Subjects were housed in 15 naturalistic enclosures spread across 8-ha area resembling the habitat of Asiatic lions. All enclosures were similar in design, devoid of enrichment devices, evenly populated with leafy trees (for shade and cover) and provided similar enclosure space per animal (400 m^2), ensuring uniformity of housing conditions for all subjects. Due to the absence of complexity and an active enrichment intervention programme, all enclosures were deemed functionally barren to the subjects. The enclosure sizes ranged from 1,100–6,542 m^2, with an average size of (M = 1,970 m^2, SD = 1,685.24m^2). Only one enclosure was 6,542 m^2 in size, and most other enclosures were similar in sizes (M thickets. Subjects were confined to feeding cubicles only during feeding time and had free access to all enclosure areas (including feeding cubicles) for the rest of the day. Subjects were fed separately at the indoor cubicles between 1,700–1,900 h six days a week, with a fast on Sundays. Subjects were fed in-house slaughtered and quality-inspected buffalo meat. The average meat consumption was 3.5 kg (SD = 0.5 kg) for females ($N = 21$) and 4.9 kg (SD = 1.4 kg) for males ($N = 14$). Most subjects were group-housed (1:2) ($N = 18$) or pair-housed (1:1) ($N = 20$). Four subjects were iso-sexually paired which included two male lions (2:0) and a mother-daughter dyad (0:2).

A group of animal keepers carried out all husbandry work for the subjects on a rotational basis, which meant that all subjects were accustomed to the same group of keepers. Since the conservation breeding area is off-display and restricts access to unauthorized personnel, subjects' interactions with humans were limited to keeper interactions. The animal-keepers had trained the subjects to respond to their house names and vocal instructions for moving in and out of the feeding cubicles.

Study design

We aimed to answer two broad research questions in this study; (a) how differences in bold/shy personality traits, rearing history, sex, and social grouping are associated with variations in behavioural welfare outcomes in a group of captive Asiatic lions? (b) How are the behavioural welfare indices (viz., enclosure usage, behaviour diversity, and aberrant repetitive behaviours) interlinked?

To answer the above questions, we categorized subjects and recorded outcomes of welfare measures. The detailed design for the study is given below.

Personality assessment

We adopted a combination of keeper-rating and behaviour-coding techniques to reliably assess personality traits (*Funder & Colvin, 1988; Funder, 1995; Gosling & Vazire, 2002; Highfill et al., 2010; Gartner & Powell, 2011*). We separately interviewed three animal keepers with at least ten years of work experience to rate 38 subjects (15:23) on a scale of 1–9 (1-very low and 9- very high) for pre-selected bold ($N = 10$) and shy traits ($N = 10$) (Table S1). We found that all keepers agreed on their ratings for subjects (high inter-rater reliability, Cronbach's alpha > 0.8). First, we averaged all keeper ratings ($N = 3$) subject-wise for all personality traits. Next, we calculated the average rating on bold ($N = 10$) and shy ($N = 10$) traits for each subject. Subjects that received an average score above seven on bold traits were categorized as bold, whereas an average rating above seven on shy traits were categorized as shy.

To validate keeper ratings for personality traits of subjects (bold/shy), we conducted novel-object tests in day kraals for ten minutes using video recorders in the absence of keepers and observers (*Highfill et al., 2010*). Naive observers ($N = 3$) with no prior exposure to study subjects, recorded the latency of subjects to interact with novel objects (*Sih, Bell & Johnson, 2004; Frost et al., 2007*) and calculated the percentage of bold vs shy behaviours (*Powell & Svoke, 2008; Gartner & Powell, 2011; Corsetti et al., 2018*) performed by the subjects during these tests (Tables S2 & S3). During these tests, the subjects were exposed to (a) unknown conspecifics, (b) unknown person and (c) non-food novel objects (lion-sam ball and bungee cord). All novel-object tests were conducted in an open-air day kraal adjacent to the paddock area of the enclosures. For the first test, we simultaneously released two subjects (same sex but unknown to one another) at adjacent day kraals and recorded their reactions to encountering a same-sex unknown conspecific. The latency counter was started as soon as both lions were released inside their respective day kraals. For the second test, we released the subject inside the day kraal and had a volunteer (unknown to the lion and not wearing a keeper's uniform) approach the kraal, and stop at the median section facing the day kraal for ten minutes. The volunteer did not make any eye contact or vocal communication with the animal. The latency counter was started as soon as the volunteer reached the day kraal. For the final test, we placed a novel object (lion-sam ball or bungee cord) at the centre of the enclosure, and then released the subject inside the day kraal. The latency counter was started when the subject was released inside the day kraal. Observers used focal animal sampling (*Altmann, 1974*; *Martin & Bateson, 1993*) to calculate duration of all behavioural states and events performed by subjects during each of these tests. These focal observations were used to calculate the percentage of bold and shy behaviour performed by subjects (Table S3). We tested each subject separately to avoid confounding personality with dominance. We conducted the latency tests simultaneously for 12 individuals daily between 0900-1100 h. Since we did not want to overwhelm the animals with multiple novel stimuli on a single day, three sessions of novel-object tests were conducted for each subject on consecutive days. The novel object tests were conducted for ten minutes, and if subjects failed to approach the novel object after five minutes, they were categorized as shy. We repeated the novel object tests with unknown human and novel object after a month to check for trait consistency and calculated the average latency values for each subject. In the first session, lion-sam ball was used as the novel object which was replaced in the second session with a hanging bungee cord. The order of the latency tests was kept the same for all subjects. Three Asiatic lions undergoing veterinary treatments for physical injuries (Male = 1, Female = 2) showed inconsistencies in trait measures across different sessions. We excluded these animals from the study, thus reducing the number of subjects to 35 individuals (Male = 14, Female = 21). We found that keepers ($N = 3$) and observers ($N = 3$) reliably agreed on the personality type (Cronbach's alpha > 0.9) of these 35 subjects. These subjects were further categorized based on rearing history (wild-caught = 19, captive-raised = 16), sex (Male = 14, Female = 21), and social grouping (pair-housed = 17, group-housed = 18).

Behaviour data collection

For behaviour data collection, we used pre-existing ethograms for felids (*Powell, 1995*; *Stanton, Sullivan & Fazio, 2015*) and modified them to include unique behaviours displayed by subjects (Table 2). Two independent observers collected all behaviour data. To minimize inter-observer bias, behaviour recording was commenced after inter-observer reliability reached satisfactory levels from the same group of animals (Cronbach's $\alpha > 0.9$) (*Caro et al., 1979*; *Gliem & Gliem, 2003*). We recorded ten hour-long behaviour observation sessions (for four subjects) and found one-minute instantaneous scans (*Altmann, 1974*; *Martin*

& Bateson, 1993; Amato, Van Belle & Wilkinson, 2013) were comparable to focal animal behaviour observation data (*Altmann, 1974; Gilby, Pokempner & Wrangham, 2010; Amato, Van Belle & Wilkinson, 2013*) in recording behavioural states and events for multiple subjects. We chose instantaneous scans as it provided a good balance between data-accuracy and observer fatigue. We recorded behaviour at three different time periods: 0500-1100 h, 1300–1800 h and 2200-0500 h in six-hour blocks. During each six-hour block, we conducted four one-hour sessions of instantaneous scans at one-minute intervals for one-hour duration followed by a 15-minute rest. During each scan, we recorded the behavioural state of the subject and its location in the enclosure. All occurrences of behaviour events were recorded separately. We used the frequencies of behavioural states and events to measure behaviour diversity of each subject during an observation session (one hour). We measured the directionality of all social interactions between subjects to gain a better understanding of the social cohesiveness of each enclosure group. We also video recorded behaviour observation sessions, which were used to fill any potential gaps in observer recording of instantaneous scans. We gathered a total of 2,009 h of behaviour observation data (average data of 57 h/subject) across 486 observation days. We collected information on the following behavioural welfare indices:

Enclosure usage

Enclosure use is a critical behavioural parameter that is influenced by the biological relevance of different zones of the captive environment (*Traylor-Holzer & Fritz, 1985; Plowman, 2003; Rose & Robert, 2013; Ross & Shender, 2016*). Homogenous usage is indicative of a complex and novel enclosure design (*Ross & Shender, 2016; Mallapur, Qureshi & Chellam, 2002; Rose & Robert, 2013*) which are considered more important drivers of welfare than enclosure area (*Traylor-Holzer & Fritz, 1985*). We divided each enclosure into ten equal zones, which included three broad zones viz. (a) proximal, (b) medial, and (c) distal zones. Each of these broad zones was further subdivided into three smaller zones as (i) left, (ii) middle, and (iii) right. The tenth zone was the paddock area next to the retiring cell (Fig. 1). We recorded the enclosure zone location of subjects during each scan. We calculated the spread of participation index (SPI) (*Plowman, 2003*) of enclosure usage for all 35 subjects across 486 observation days using instantaneous scan data. We calculated the SPI values using the following formula:

$$SPI = \frac{\sum |fo - fe|}{2(N - femin)}$$

where f_o stands for the observed frequency of usage of enclosure zones, f_e stands for the expected frequency of enclosure usage. N stands for gross observations in all zones of the enclosure and f_{emin} stands for the expected frequency of observation for the smallest zone (*Plowman, 2003*). SPI measures indicate the homogeneity of space usage. A high SPI value (close to 1) indicates that subjects are biased towards certain areas of the enclosure, while a lower SPI value (close to 0.5 or lower) indicates that lions use most areas of the enclosure equitably.

It is noteworthy to point out that social animals like lions are likely to have hierarchies and dominant animals are likely to monopolize preferred areas, but an ideal enclosure should provide equal opportunities for exploration and free movement to all individuals.

Table 2 Ethogram showing Asiatic lion behaviour (states and events) used in this study.

Behaviour class	Behavior	Description
Behavioural states	Locomotion	Walking or moving inside the enclosure.
	Rest	In a reclined position, head up or down.
	Sit	Haunches on the ground.
	Sleep	Reclined position, eyes closed.
	Climb	Climbing trees.
Discrete Behaviours	Defecate	Passing urine or fecal matter.
	Drink	Drinking water with distinct lapping sound.
	Groom	Lick or bite or scratch with paw self or conspecific.
	Lick	Running tongue over lips and nose multiple times in quick succession.
	Mark	Spraying object via perianal secretions or rubbing paws on the ground.
	Grab	Cautiously reaching or touching an object or conspecific with the forepaw in jabbing fashion.
	Roll	Body in the prostate position and rolling from side to side usually with the belly up.
	Rub	Pushing head or body against an object with head or part of the body.
	Scratch	Rubbing claws on an object (e.g. tree).
	Sniff	Inhaling scent from the air or an object.
	Vocalize	growling, roaring, grunting, humming, chuffing.
	Yawn	Opening mouth wide while showing canines and inhaling deeply.
	Stalk	Silently shadowing an object, conspecific, birds or keepers.
	Other	Any other behaviour observed.
Stereotypy	Pacing	Walking up and down on a fixed path occasionally raising its head to look up.
	Swaying	Subject moves head and body from side to side while standing next to a wall and shifting bodyweight from left to right foot.
	Head bobbing	Nodding head up and down while the animal is stationery.
	Nose rubbing	Subject rubs nose on enclosure wall or enclosure barrier continuously without any aim or purpose.

Notes.
*Ethogram showing Asiatic lion behaviour (states and events) used in this study.

In this study, we aimed to measure the enclosure zone usage pattern of each subject in a social configuration to ascertain how it met individual welfare requirements and related to other welfare indices.

Species-typical behaviour diversity

Behaviour diversity is indicative of the scope of novelty, and complexity afforded to animals in captivity (*Wemelsfelder et al., 2000; Haskell et al., 2018*). Maintaining behaviour diversity of captive animals housed at breeding programmes is essential for the preservation of essential learned behaviours required for post-release survival (*Rabin, 2003*). Complex and cognitively enriching enclosures have been shown to stimulate captive animals to display

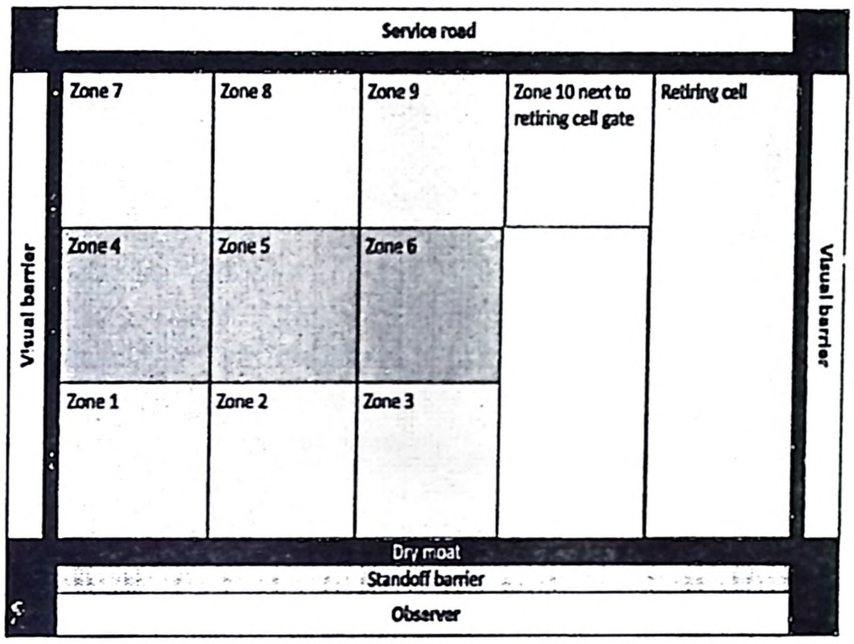

Figure 1 Schematic representation of an enclosure in Sakkarbaug zoological garden with the layout of zones for behavioural observations of enclosure use by study subjects.
Full-size DOI: 10.7717/peerj.8425/fig-1

a diverse behaviour repertoire (*Spiezio et al., 2018*). We used Shannon-Weiner diversity index (SWI) to measure species-typical behaviour diversity as this approach considers both richness and evenness of species-typical behaviours in the data set (*Clark & Melfi, 2012; Miller, Pisacane & Vicino, 2016; Spiezio et al., 2018*). We compiled an ethogram of all behaviour states and events observed from all subjects during the study period (Table 2). We pooled all behaviour observations of each subject to calculate behaviour diversity. We excluded aberrant repetitive behaviours from the calculations since they did not qualify as species-typical behaviours.

Aberrant repetitive behaviours (ARB)

Aberrant repetitive behaviours (ARB) are reliable measures of poor welfare conditions (*Dawkins, 2004; Watters, 2009; Kroshko et al., 2016*) as they are precursors of cognitive dysfunction and neurophysiological changes (*Muehlmann & Lewis, 2012*). For this study, we measured the proportion of scans spent by each subject performing ARBs as an indicator of poor welfare (*Mason & Latham, 2004*). ARBs mostly included, stereotypic swaying, pacing, and nose rubbing behaviours (Table 2). We did not record anticipatory displacement behaviours, for example, pacing before feeding (Watters, 2014) or during interaction with conspecifics as ARB. We considered behaviours persisting over five consecutive scans and without an observer-discernible cause as ARB. Therefore, displacement behaviours performed before feeding time, or in response to keeper activities were not considered as aberrant repetitive behaviours.

Data analysis

We tested the following hypotheses in this study:

1. There would be no variations in behaviour indices between male and female subjects and the measures would perform uniformly for both sexes.
2. The wild-rescued lions would display higher behaviour diversity than captive-raised animals.
3. Bold individuals would differ in behavioural welfare indices compared to shy individuals.
4. There would be no difference in behavioural welfare parameters between pair-housed and group-housed subjects.
5. There will be no significant prediction of behavioural diversity by the proportion of aberrant repetitive behaviour and the enclosure usage patterns of subjects.

We used R statistical software version 3.4 and 3.5.2 through RStudio (*RStudio, 2015*) using packages, dplyr (*Wickham, 2016*), ggplot2 (*Wickham, 2016*), lubridate (*Spinu, Grolemund & Wickham, 2018*), tidyverse (*Wickham, 2017*), and funModeling (*Casas, 2019*). For exploratory data analyses, we used Shapiro–Wilk test and Levene's test to ascertain the normal distribution and homogeneity of variance in SPI, SWI, latency and ARB values, respectively. We conducted bivariate Pearson's correlation to ascertain the strength of association between four above-mentioned welfare indices. We also checked for correlation between enclosure area and zone usage bias.

For statistical analysis, we had four categorical predictor variables (personality trait, rearing history, sex, and social grouping), each with two levels (viz. bold & shy, wild-rescued & captive-raised, male & female and pair-housed & group-housed). We compared welfare indices across groups (categorical predictor variables) using independent samples *t*-tests for normally distributed dependent variables (enclosure usage, behaviour diversity, and ARBs). We used non-parametric Kolmogorov–Smirnov test for latency measures and the proportion of bold and shy behaviours performed by subjects during the novel object test. When comparing between means of two groups with different sample sizes, it is important to report effect sizes in addition to p-values to indicate the scale-independent degree of difference. We calculated effect sizes to quantify differences in welfare measures between groups (*Cohen, 1992*; *Lakens, 2013*).

Finally, we conducted multiple regression analysis to understand how the behaviour diversity of captive animals were predicted by their enclosure usage patterns and ARB levels. Before conducting a regression analysis, we checked for multicollinearity between independent variables using measures of VIF (variance inflation factor). Since enclosure usage and ARBs were not highly correlated, we used them as predictors for behaviour diversity in regression analysis.

RESULTS

Validation of keeper ratings

The mean latency times for all subjects after averaging both trials was 47.76 s (SD = 46.85). Subjects categorized as bold by keepers ($M = 11.13$, SD = 3.65, $N = 21$) showed significantly lower latency values ($z = 2.89$, $p < 0.01$, Cohen's $d = 7.28$) compared to subjects categorized as shy ($M = 102.71$, SD = 17.4, $N = 14$)(Table 3, Fig. 2). Bold subjects

Table 3 Comparison of welfare indices (viz., enclosure usage, behaviour diversity, aberrant behaviours, and latency to novel objects) between Asiatic lions of different categories (captive-raised vs wild-rescued, bold vs shy, male vs female, and pair-housed vs group-housed).

Life-histories	Captive-raised	Wild-rescued	t(33)	*p*-value	Effect size (Cohen's d)
Enclosure usage	0.47 ± 0.12	0.67 ± 0.15	4.28	<0.000	1.47
Behaviour diversity	1.26 ± 0.3	0.83 ± 0.35	−3.94	<0.00	1.35
Aberrant behaviours	7.74 ± 5.3	13.12 ± 6.25	2.71	0.10	0.92
Latency to novel object	18.61 ± 21.55	72.30 ± 48.7	2.89*	0.000	1.42
Personality	***Bold***	***Shy***	**t(33)**	***p*-value**	***Effect size (Cohen's d)***
Enclosure usage	0.5 ± 0.12	0.71 ± 0.15	−4.572	<0.000	1.54
Behaviour diversity	1.23 ± 0.26	0.73 ± 0.34	4.897	<0.000	1.64
Aberrant behaviours	7.01 ± 3.9	16.13 ± 5.4	−5.825	<0.000	1.94
Latency to novel object	11.13 ± 3.65	102.71 ± 17.4	−4.95*	<0.000	7.28
Sex	***Male***	***Female***	**t(33)**	***p*-value**	***Effect size (Cohen's d)***
Enclosure usage	0.61 ± 0.2	0.57 ± 0.15	5.28	0.60	0.17
Behaviour diversity	0.96 ± 0.43	1.1 ± 3.5	−0.85	0.4	0.28
Aberrant behaviours	11.04 ± 7.05	10.41 ± 6.02	0.282	0.78	0.09
Latency to novel object	37.02 ± 45	54.91 ± 47.8	−1.11*	0.27	0.38
Social grouping	**Pair housed (*n* = *17*)**	**Group housed (*n* = *18*)**	**t(33)**	***p*-value**	**Effect size (Cohen's d)**
Enclosure usage	0.6 ± 0.13	0.56 ± 0.19	−0.69	0.49	0.22
Behaviour diversity	0.99 ± 0.33	1.06 ± 0.42	0.46	0.64	0.18
Aberrant behaviours	11.33 ± 6.12	10.02 ± 6.69	−0.6	0.55	0.2
Latency to novel object	55.14 ± 48.2	40.78 ± 45.81	0.95*	0.31	0.3

Notes.
*Z values from Kolmogorov Smirnov test for independent samples.

also showed significantly higher percentage of bold behaviours ($M = 87.24$, $SD = 8.74$) than shy individuals ($M = 15.86$, $SD = 9.5$) ($t(33) = -10.57$, $p < 0.01$) (Table S3). These results validate the keeper rating of subjects on the bold-shy scale.

Comparison of welfare measures across categorical independent variables

Latency to novel objects

Captive-raised individuals ($M = 18.61$, $SD = 21.55$, $N = 16$) displayed significantly ($z = 1.86$, $p = 0.02$, Cohen's $d = 1.42$) lower latency compared to wild-rescued individuals ($M = 72.30$, $SD = 48.7$, $N = 19$) (Table 3, Fig. 2). We found no difference in latency scores between male ($M = 37.02$, $SD = 45$, $N = 14$) and female ($M = 54.9$, $SD = 47.8$, $N = 21$) subjects ($z = 0.89$, $p = 0.39$, Cohen's $d = 0.38$). Latency values did not vary significantly between pair-housed ($M = 55.14$, $SD = 48.2$, $N = 17$) and group-housed ($M = 40.78$, $SD = 45.81$, $N = 18$) lions ($z = 0.9$, $p = 0.31$, Cohen's $d = 0.3$).

Enclosure usage

Enclosure space usage patterns varied significantly between subjects with different personality types and rearing histories. Wild-rescued individuals used enclosure space less homogeneously ($M = 0.67$, $SD = 0.15$, $N = 19$) than captive-raised individuals ($M = 0.47$, $SD = 0.12$, $N = 16$) (t (33) = 4.28, $p < 0.01$, Cohen's $d = 1.47$) (Table 3, Fig. 2). Subjects with bold personality traits showed significantly less enclosure-zone bias ($M = 0.5$, SD

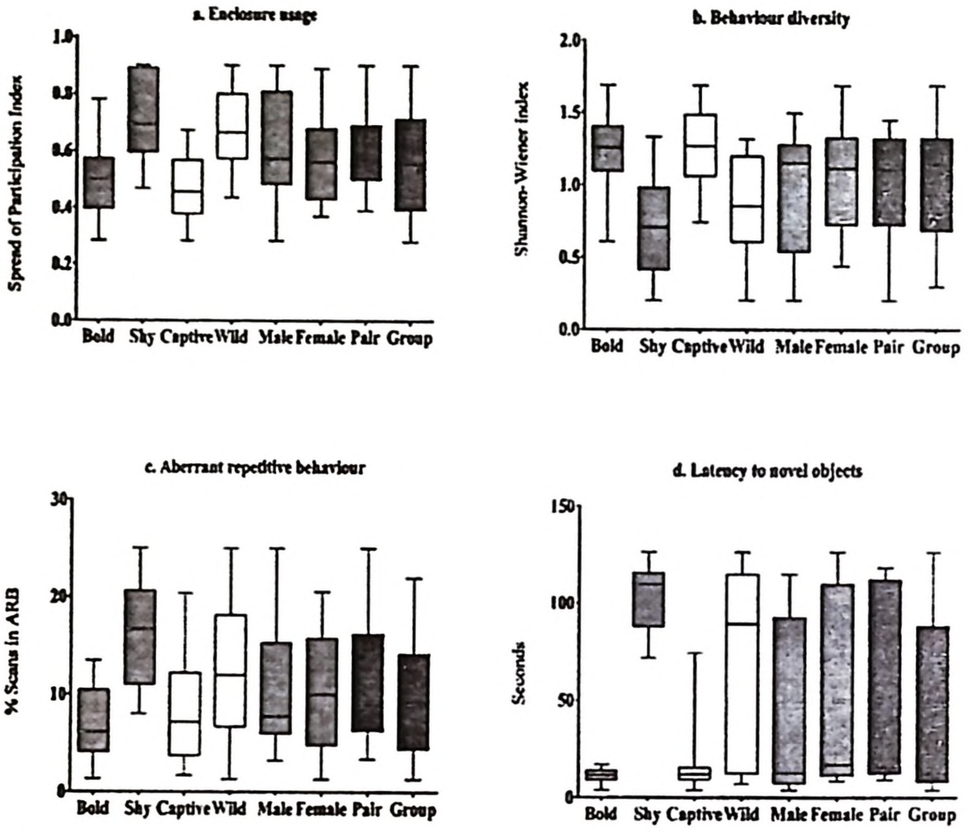

Figure 2 Comparison of behavioural welfare indices of Asiatic lions across personality (bold and shy), life-history (wild and captive), sex (male and female), and social grouping (pair-housed vs group-housed) categories. The behavioural welfare indices used here are (A) Enclosure usage; (B) Behaviour diversity; (C) Aberrant repetitive behaviour; and (D) Latency to novel objects.

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= 0.12, $N = 21$) compared to individuals with shy traits ($M = 0.71$, SD = 0.15, $N = 14$) ($t(33) = -4.572$, $p < 0.01$, Cohen's $d = 1.54$). Overall, the SPI value of males ($M = 0.61$, SD = 0.20, $N = 14$) was not significantly different from female ($M = 0.57$, SD = 0.15, $N = 21$) lions (t (33) = 5.28, $p = 0.6$, Cohen's $d = 0.17$). The enclosure usage patterns of group-housed ($M = 0.56$, SD = 0.19, $N = 18$) and pair-housed subjects ($M = 0.60$, SD = 0.13, $N = 17$) ($t(33) = -0.69$, $p = 0.49$, Cohen's $d = 0.22$) were similar.

Species-typical behaviour diversity

We found that species-typical behaviour diversity of captive-raised animals ($M = 1.26$, SD = 0.3, $N = 16$) was significantly higher than wild-rescued animals ($M = 0.83$, SD = 0.35, $N = 19$) ($t(33) = -3.94$, $p < 0.01$, Cohen's $d = 1.35$) (Table 3, Fig. 2). Further, bold subjects displayed higher behaviour diversity ($M = 1.23$, SD = 0.26, $N = 21$) than shy individuals ($M = 0.73$, SD = 0.34, $N = 14$) ($t(33) = 4.89$, $p < 0.01$, Cohen's $d = 1.64$) (Table 3, Fig. 2). Behaviour diversity levels were similar between male ($M = 0.96$, SD = 0.43, $N = 14$) and female ($M = 1.1$, SD = 0.35, $N = 21$) lions ($t(33) = -0.85$, $p = 0.4$). Group-housed ($M = 1.06$, SD = 0.42, $N = 18$) and pair housed subjects ($M = 0.99$, SD = 0.33, $N = 17$) showed similar levels of behaviour diversity ($t(33) = 0.64$, $p = 0.64$, Cohen's $d = 0.18$).

Table 4 Pearsons bivariate correlations between behavioural welfare indices, age of subjects and enclosure size.

	Enclosure usage	Behaviour diversity	Aberrant behaviours	Latency to novel object	Age	Enclosure size
Enclosure usage						
Behaviour diversity	−0.71***					
Aberrant repetitive behaviours	0.66***	−0.91***				
Latency to novel object	0.66***	−0.67***	0.70***			
Age	0.26	0.04	−0.19	−0.09		
Enclosure size	0.36	−0.3	0.26	0.16	0.14	

Notes.
***$p < 0.001$.
**$p < 0.01$.
*$p < 0.05$.

Aberrant repetitive behaviours (ARB)

Wild-rescued individuals ($M = 13.12$, SD = 6.25, $N = 19$) expressed higher proportions of ARBs than captive-raised individuals ($M = 7.74$, SD = 5.3, $N = 16$) ($t(33) = 2.71$, $p = 0.01$, Cohen's $d = 0.92$) (Table 3, Fig. 2). Bold individuals ($N = 21$) showed significantly lower levels of stereotypic behaviour such as pacing and swaying ($M = 7.01$, SD = 4, $N = 21$) compared to shy individuals ($M = 16.13$, SD = 5.4, $N = 14$) ($t(33) = -5.82$, $p < 0.01$, Cohen's $d = 1.94$) (Table 3, Fig. 2). We found no difference in the expression of ARBs between male ($M = 11.04$, SD = 7.05, $N = 14$) and female ($M = 10.41$, SD = 6.02, $N = 21$) subjects ($t(33) = 0.282$, $p = 0.78$, Cohen's $d = 0.09$), as well as between group-housed ($M = 10.02$, SD = 6.69, $N = 17$) and pair-housed subjects ($M = 11.33$, SD = 6.12, $N = 17$) (t(33)=-0.6, $p = 0.55$, Cohen's $d = 0.2$).

Inter-relationship between welfare indices

Latency was positively correlated (Fig. S2, Table 4) to enclosure zone bias ($r = 0.67$, $N = 35$, $p < 0.01$), and proportion of ARBs ($r = 0.70$, $N = 35$, $p < 0.01$). Latency was negatively correlated to behaviour diversity (r=−0.67, $N = 35$, $p < 0.01$). Enclosure zone bias was positively correlated with latency values ($r = 0.66$, $p < 0.01$), and proportion of ARBs ($r = 0.66$, $p < 0.01$) (Fig. S2, Table 4), but was negatively correlated with behaviour diversity ($r = -0.71$, $p < 0.01$). We found that enclosure zone bias was weakly positively correlated to enclosure size ($r = 0.36$, $p = 0.05$). Behaviour diversity was negatively correlated with latency to novel objects, ($r = -0.67$, $p = 0.01$), ARBs ($r = 0.91$, $p = 0.01$), and enclosure usage ($r = -0.71$, $p = 0.01$) (Supplementary Fig. 2, Table 4). ARB was positively correlated with latency to novel objects ($r = 0.70$, $p = 0.01$), and enclosure usage ($r = 0.66$, $p = 0.01$) but was negatively correlated with behaviour diversity ($r = -0.91$, $p = 0.01$) (Supplementary Fig. 2, Table 4). Multiple regression analysis (Table 5) indicated that ARBs and space usage homogeneity explained 85% of the variance in the behaviour diversity ($R^2 = 0.85$, $F(2,32) = 90.92$, $p < 0.01$) (Table 5). The predicted regression equation is Behaviour diversity $= 1.8 + (-0.046)x(ARB) + (-0.46)x$ (Enclosure usage). The results from the regression indicate that subjects that show less ARB and use enclosure space more homogenously are likely to have higher behaviour diversity.

Table 5 Multiple regression results for inter-relationships between behavioural welfare measures.

Independent variables	Estimates	Std error	*t*	*p* values	*R*-squared	F	Durbin-Watson
		Behaviour diversity					
(Intercept)	1.793	0.09	18.870	2e−			
Enclosure space usage	−0.459	0.20	−2.258	0.030	0.85	90.92	1.78
Aberrant repetitive behaviour	−0.046	0.005	−8.49	1.04e−09			

DISCUSSION

To the best of our knowledge, this is the first empirical study to showcase the association of personality traits in wild-rescued and captive-raised Asiatic lions across multiple behavioural welfare measures. Our sample size constitutes 10% of the global captive stock of Asiatic lions, making the results relevant for the global conservation initiatives for this species. Several studies have asserted the importance of multiple indices for welfare assessment at zoos and conservation breeding programmes viz., behaviour diversity (*Powell, 1995; Clark & Melfi, 2012*), enclosure usage (*Ross & Shender, 2016; Kistler et al., 2010*) and stereotypy in captive animals (*Dawkins, 2004; Kroshko et al., 2016; Clegg, 2018*). However, most ex-situ institutions continue to use uni-dimensional measures to assess welfare and seldom address individuality (*Van der Harst & Spruijt, 2007; Volpato et al., 2009; Hill & Broom, 2009; McMahon et al., 2013*). We addressed this issue by showcasing the importance of an individual-focused multi-dimensional approach to welfare assessments. Overall, Asiatic lions with different personality traits (bold and shy) and rearing-history (captive-raised and wild-rescued) differed significantly on measures of welfare, which supports earlier studies linking animal welfare with individuality (*Carere & Locurto, 2011; Gartner & Powell, 2011; Boissy & Erhard, 2014; Gartner, Powell & Weiss, 2016*). We did not observe any sex-specific variations in behavioural welfare measures of the subjects, confirming our first hypothesis. Contrary to our second hypothesis, wild-rescued lions showed low behaviour diversity, high enclosure-use bias, increased stereotypy and higher latency to novel objects compared to captive-raised subjects. Our results challenge existing research findings that report wild-rescued animals to be less likely to develop stereotypies than captive-raised individuals, while not accounting for animal personality (*Cooper & Nicol, 1996; Schoenecker, Heller & Freimanis, 2000*). It is possible that such pattern in our study is driven by higher proportion of shy individuals ($N = 12$) in the wild-rescued category compared to the captive-raised subjects ($N = 2$). Nevertheless, these results clearly show that wild-rescued lions may not necessarily be at a better state of welfare by default when compared to captive-raised individuals under similar housing conditions. Further empirical studies with equal sampling across bold and shy continuum between captive-raised and wild-rescued individuals are required to confirm these patterns. Our results supported the third hypothesis that lions with bold personalities are more resilient to functionally barren housing conditions than shy subjects, which supports earlier studies (*Cole & Quinn, 2014; Japyassú & Malange, 2014*). Moreover, present welfare assessment protocols often do not consider individual requirements as modifiers for species-specific husbandry practices. The effects of animal personality on welfare (*Izzo, Bashaw & Campbell, 2011; Coelho, De Azevedo & Young, 2012; Razal, Pisacane & Miller, 2016*) and its implications for post-release survival (*Bremner-Harrison, Prodohl & Elwood, 2004; Watters, 2009*) are well documented. This study aligns with the conservation goals for Asiatic lions by addressing individuality in welfare assessment (*Rabin, 2003; Izzo, Bashaw & Campbell, 2011; Coelho, De Azevedo & Young, 2012; Razal, Pisacane & Miller, 2016*). Group-housed and pair-housed subjects were similar across all behavioural welfare indices, supporting our fourth hypothesis. Although the enclosures were aesthetically pleasing, appropriate in terms of size, naturalistic vegetation and social grouping of animals; the abject lack of multisensory stimulation in terms of enrichment and novel experiences rendered them functionally barren to the subjects (*Reading, Miller & Shepherdson, 2013*). In this study we measured the evenness of enclosure use, which represents the functional space of the enclosure rather than available space. We found that small variations in enclosure sizes do not associate with significant shifts in behavioural welfare of Asiatic lions, which is in line with previous studies that place more importance on enclosure design (*Tan et al., 2013*), complexity and species-appropriateness than enclosure area (*Rose & Robert, 2013; Herrelko, Buchanan-Smith & Vick, 2015; Neal Webb, Hau & Schapiro, 2018*). The correlation between enclosure size and space usage bias was weak but positive, which means that increasing enclosure sizes were associated with higher zone-usage bias. This underlines the urgent need to provide complex captive environments that promote homogenous space usage and stimulate expression of species-typical behaviours.

Bivariate correlations and regression model presented in this study underline strong inter-linkages between behaviour diversity, enclosure usage and ARBs (*Rabin, 2003; Melotti et al., 2011; Rose & Robert, 2013; Kroshko et al., 2016*). Our results provided evidence that behaviour diversity can be explained by level of ARB and space usage patterns, which is in line with findings from existing studies (*Wemelsfelder et al., 2000; Watters, Margulis & Atsalis, 2009; Clark & Melfi, 2012*). From our results it can be surmised that subjects that are under less stress (low ARB) are likely to show homogenous enclosure space usage and a diverse behaviour repertoire and vice versa. Zoo managers must pay close attention to the development of high enclosure-zone biases (*Ross & Shender, 2016*) conjugated with low behaviour diversity (*Clark & Melfi, 2012; Rose & Robert, 2013*) as that may develop into severe levels of ARBs (*Konjević et al., 2015*). Overall, these findings indicate that behavioural welfare measures (enclosure usage, behaviour diversity, and ARB) have strong interlinkages and vary across inter-individual differences (viz., personality, rearing-history, sex, and social grouping). Zoo managers must take a proactive approach to improve the welfare status (*Mendl et al., 2009*) of captive Asiatic lions. Since enrichment interventions are effective in bringing complexity to sterile enclosures (*Swaisgood & Shepherdson, 2005; Reading, Miller & Shepherdson, 2013*), tailored-enrichment interventions must be integrated with husbandry practices for Asiatic lions (*Powell, 1995; Cannon et al., 2016*). Studies also show that positive keeper-animal relationships can improve welfare (*Whitham & Wielebnowski, 2013*). Finally, regular behaviour monitoring of captive wild animals should be incorporated into the husbandry practices to improve welfare and prevent development of stereotypy (*Watters, Margulis & Atsalis, 2009*).

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Sitendu Goswami conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Praveen C. Tyagi and Pradeep K. Malik conceived and designed the experiments, authored or reviewed drafts of the paper, critical evaluations of the work, and approved the final draft.
- Pradeep K. Malik conceived and designed the experiments, authored or reviewed drafts of the paper, critical evaluations of the work, and approved the final draft.
- Shwetank J. Pandit, Riyazahmed F. Kadivar and Malcolm Fitzpatrick performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Samrat Mondol conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

For behavioural data collection, we obtained the appropriate approvals from Gujarat Forest Department (Permit no: WLP/28/A/1316-21/2015-16). All data were collected non-invasively and complies with the regulations of zoo animal welfare standards set by the Central Zoo Authority, Government of India.

Data Availability

The following information was supplied regarding data availability:

Details of the housing areas and conditions for the captive subjects are available in the Study Area section of the article. Related schematic diagrams are available in Fig. 1 and Fig. S1. All details related to the individual subjects, their personality and bold/shy behaviour details are available in Table 1. The ethogram used in this study is available in Table 2. The compiled behaviour data used for all analyses is available in Data S1. No additional data is required to replicate the results presented in this study.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.8425#supplemental-information.

REFERENCES

Ainsworth MS, Bowlby J. 1991. An ethological approach to personality development. *American Psychologist* 46:333–341 DOI 10.1037/0003-066X.46.4.333.

Allport FH, Allport GW. 1921. Personality traits: their classification and measurement. *The Journal of Abnormal Psychology and Social Psychology* 16:640 DOI 10.1037/h0069790.

Altmann J. 1974. Observational study of behaviour: sampling methods. *Behaviour* 49:227–267 DOI 10.1163/156853974X00534.

Amato KR, Van Belle S, Wilkinson B. 2013. A comparison of scan and focal sampling for the description of wild primate activity, diet and intragroup spatial relationships. *Folia Primatologica* 84:87–101 DOI 10.1159/000348305.

Banerjee K, Jhala YV, Chauhan KS, Dave CV. 2013. Living with lions: the economics of coexistence in the Gir forests, India. *PLOS ONE* 8:1–11 DOI 10.1371/journal.pone.0049457.

Banerjee K, Jhala YV, Pathak B. 2010. Demographic structure and abundance of Asiatic lions *Panthera leo persica* in Girnar Wildlife Sanctuary, Gujarat, India. *Oryx* 44:248–251 DOI 10.1017/S0030605309990949.

Bateson M, Matheson SM. 2007. Performance on a categorisation task suggests that removal of environmental enrichment induces pessimism in captive European starlings (*Sturnus vulgaris*). *Animal Welfare* 16:33–36.

Boissy A, Erhard HW. 2014. How studying interactions between animal emotions, cognition, and personality can contribute to improve farm animal welfare. In: *Genetics and the behavior of domestic animals.* Cambridge: Elsevier, 81–113 DOI 10.1016/B978-0-12-394586-0.00003-2.

Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C, Forkman B, Dimitrov I, Langbein J, Bakken M, Veissier I, Aubert A. 2007. Assessment of positive emotions in animals to improve their welfare. *Physiology & Behavior* 92:375–397 DOI 10.1016/j.physbeh.2007.02.003.

Bremner-Harrison S, Prodohl PA, Elwood RW. 2004. Behavioural trait assessment as a release criterion: boldness predicts early death in a reintroduction programme of captive-bred swift fox (*Vulpes velox*). *Animal Conservation* 7:313–320 DOI 10.1017/S1367943004001490.

Broom D. 1986. Indicators of poor welfare. *British Veterinary Journal* 142:524–526 DOI 10.1016/0007-1935(86)90109-0.

Broom DM. 1991. Animal welfare: concepts and measurement. *Journal of Animal Science* 69:4167–4175 DOI 10.2527/1991.69104167x.

Broom DM. 2011. A history of animal welfare science. *Acta Biotheoretica* 59:121–137 DOI 10.1007/s10441-011-9123-3.

Butterworth A, Mench JA, Wielebnowski N. 2011. Practical strategies to assess (and improve) welfare. *Animal Welfare* 200–214.

Cannon TH, Heistermann M, Hankison SJ, Hockings KJ, McLennan MR. 2016. Tailored enrichment strategies and stereotypic behavior in captive individually housed macaques (*Macaca spp.*). *Journal of Applied Animal Welfare Science* 19:171–182 DOI 10.1080/10888705.2015.1126786.

Carere C, Locurto C. 2011. Interaction between animal personality and animal cognition. *Current Zoology* 57:491–498 DOI 10.1093/czoolo/57.4.491.

Caro TM, Roper R, Young M, Dank GRR. 1979. Inter-observer reliability. *Behaviour* 69:303–315 DOI 10.1163/156853979X00520.

Carter AJ, Marshall HH, Heinsohn R, Cowlishaw G. 2013. Personality predicts decision making only when information is unreliable. *Animal Behaviour* 86:633–639 DOI 10.1016/j.anbehav.2013.07.009.

Casas P. 2019. funModeling: exploratory data analysis and data preparation tool-box. R package version 1.9.3. *Available at https://CRAN.R-project.org/package=funModeling*.

Chadwick C. 2014. Social behaviour and personality assessment as a tool for improving the management of cheetahs (*Acinonyx jubatus*) in captivity. Doctoral dissertation, University of Salford.

Chakrabarti S, Jhala YV. 2017. Selfish partners: resource partitioning in male coalitions of Asiatic lions. *Behavioral Ecology* 28:1532–1539 DOI 10.1093/beheco/arx118.

Clark FE, Melfi VA. 2012. Environmental enrichment for a mixed-species nocturnal mammal exhibit. *Zoo Biology* 31:397–413 DOI 10.1002/zoo.20380.

Clegg ILK. 2018. Cognitive bias in zoo animals: an optimistic outlook for welfare assessment. *Animals* 8:1–25 DOI 10.3390/ani8070104.

Coelho CM, De Azevedo CS, Young RJ. 2012. Behavioral responses of maned wolves (*Chrysocyon brachyurus, Canidae*) to different categories of environmental enrichment stimuli and their implications for successful reintroduction. *Zoo Biology* 31:453–469 DOI 10.1002/zoo.20410.

Cohen J. 1992. A power primer. *Psychological Bulletin* 112:155–159 DOI 10.1037/0033-2909.112.1.155.

Cole EF, Quinn JL. 2014. Shy birds play it safe: personality in captivity predicts risk responsiveness during reproduction in the wild. *Biology Letters* 10:1–5 DOI 10.1098/rsbl.2014.0178.

Cooper JJ, Nicol CJ. 1996. Stereotypic behaviour in wild caught and laboratory bred bank voles (Clethrionymus glareolus). *Animal Welfare* 5:245–257.

Corsetti S, Borruso S, Di Traglia M, Lai O, Alfieri L, Villavecchia A, Cariola G, Spaziani A, Natoli E. 2018. Bold personality makes domestic dogs entering a shelter less vulnerable to diseases. *PLOS ONE* 13(3):e0193794 DOI 10.1371/journal.pone.0193794.

Dawkins MS. 1990. From an animal's point of view: motivation, fitness, and animal welfare. *Behavioral and Brain Sciences* 13:1–9 DOI 10.1017/S0140525X00077104.

Dawkins MS. 2004. Using behaviour to assess animal welfare. *Animal Welfare* 13:3–7 DOI 10.1177/1091581812471490.

Engel GL. 1980. The clinical application of the biopsychosocial model. *American Journal of Psychiatry* 137:535–544 DOI 10.1176/ajp.137.5.535.

Fox RA, Millam JR. 2014. Personality traits of pair members predict pair compatibility and reproductive success in a socially monogamous parrot breeding in captivity. *Zoo Biology* 33:166–172 DOI 10.1002/zoo.21121.

Franks B, Higgins ET, Champagne FA. 2014. A theoretically based model of rat personality with implications for welfare. *PLOS ONE* 9(4):e95135 DOI 10.1371/journal.pone.0095135.

Fraser D. 1999. Animal ethics and animal welfare science: bridging the two cultures. *Applied Animal Behaviour Science* 65:171–189 DOI 10.1016/S0168-1591(99)00090-8.

Fraser D. 2009. Animal behaviour, animal welfare and the scientific study of affect. *Applied Animal Behaviour Science* 118:108–117 DOI 10.1016/j.applanim.2009.02.020.

Fraser D, Duncan IJ. 1998. Pleasures, pains and animal welfare: toward a natural history of affect. *Animal Welfare* 7:383–396.

Frost AJ, Winrow-giffen AA, Ashley PJ, Sneddon LU. 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal Society: Biological Sciences* 274:333–339 DOI 10.1098/rspb.2006.3751.

Funder DC. 1995. On the accuracy of personality judgment: a realistic approach. *Psychological Review* 102:652–670 DOI 10.1037/0033-295X.102.4.652.

Funder DC, Colvin CR. 1988. Friends and strangers: acquaintanceship, agreement, and the accuracy of personality judgment. *Journal of Personality and Social Psychology* 55:149–158 DOI 10.1037//0022-3514.55.1.149.

Gartner MC, Powell DM. 2011. Personality assessment in snow leopards (*Uncia uncia*). *Zoo Biology* 29:1–15 DOI 10.1002/zoo.20385.

Gartner MC, Powell DM, Weiss A. 2014. Personality structure in the domestic cat (*Felis silvestris catus*), Scottish wildcat (*Felis silvestris grampia*), clouded leopard (*Neofelis nebulosa*), snow leopard (*Uncia uncia*). *Journal of Comparative Psychology* 128:414–426 DOI 10.1037/a0037104.

Gartner MC, Powell DM, Weiss A. 2016. Comparison of subjective well-being and personality assessments in the clouded leopard (*Neofelis nebulosa*), snow leopard (*Panthera uncia*), and African lion (*Panthera leo*). *Journal of Applied Animal Welfare Science* 19:294–302 DOI 10.1080/10888705.2016.1141057.

Gilby IC, Pokempner AA, Wrangham RW. 2010. A direct comparison of scan and focal sampling methods for measuring wild chimpanzee feeding behaviour. *Folia Primatologica* 81:254–264 DOI 10.1159/000322354.

Gliem JA, Gliem RR. 2003. Calculating, interpreting, and reporting Cronbach's alpha reliability coefficient for Likert-type scales. In: *Midwest research-to-practice conference in adult, continuing, and community education.*

Goold C, Newberry RC. 2017. Modelling personality, plasticity and predictability in shelter dogs. *Royal Society Open Science* 4:170618–170618 DOI 10.1101/145367.

Gosling SD, John OP. 1999. Personality dimensions in nonhuman animals: a cross-species review. *Current Directions in Psychological Science* 8:69–75 DOI 10.1111/1467-8721.00017.

Gosling SD, Vazire S. 2002. Are we barking up the right tree? Evaluating a comparative approach to personality. *Journal of Research in Personality* 36:607–614 DOI 10.1016/S0092-6566(02)00511-1.

Griffin AS, Guillette LM, Healy SD. 2015. Cognition and personality: an analysis of an emerging field. *Trends in Ecology and Evolution* 30:207–214 DOI 10.1016/j.tree.2015.01.012.

Harding EJ, Paul ES, Mendl M. 2004. Cognitive bias and affective state. *Nature* 427:312 DOI 10.1038/427312a.

Haskell M, Wemelsfelder F, Mendl MT, Calvert S, Lawrence AB. 2018. The effect of substrate-enriched and substrate-impoverished housing environments on the diversity of behaviour in pigs. *Behaviour* 133:741–761.

Hediger H. 1968. *The psychology and behaviour of animals in zoos and circuses.* New York: Dover Publications.

Herrelko ES, Buchanan-Smith HM, Vick S. 2015. Perception of available space during chimpanzee introductions: number of accessible areas is more important than enclosure size. *Zoo Biology* 34:397–405 DOI 10.1002/zoo.21234.

Highfill L, Hanbury D, Kristiansen R, Kuczaj S, Watson S. 2010. Rating vs. coding in animal personality research. *Zoo Biology* 29:509–516 DOI 10.1002/zoo.20279.

Higley JD, Hasert MF, Suomi SJ, Linnoila M. 1991. Nonhuman primate model of alcohol abuse: effects of early experience, personality, and stress on alcohol consumption. *Proceedings of the National Academy of Sciences of the United States of America* 88:7261–7265 DOI 10.1073/pnas.88.16.7261.

Hill SP, Broom DM. 2009. Measuring zoo animal welfare: theory and practice. *Zoo Biology* 28:531–544 DOI 10.1002/zoo.20276.

Izzo GN, Bashaw MJ, Campbell JB. 2011. Enrichment and individual differences affect welfare indicators in squirrel monkeys (*Saimiri sciureus*). *Journal of Comparative Psychology* 125:347–352 DOI 10.1037/a0024294.

Japyassú HF, Malange J. 2014. Plasticity, stereotypy, intra-individual variability and personality: handle with care. *Behavioural Processes* 109:40–47 DOI 10.1016/j.beproc.2014.09.016.

Jhala YV, Chellam R, Qureshi Q, Meena V, Chauhan KS, Dave C, Banerjee K. 2006. Social organization and dispersal of Asiatic lions and ecological monitoring of Gir. Technical Report. Wildlife Institute of India, Dehradun, India.

Jhala YV, Mukherjee S, Shah N, Chauhan KS, Dave CV, Meena V, Banerjee K. 2009. Home range and habitat preference of female lions (*Panthera leo persica*) in Gir forests, India. *Biodiversity and Conservation* 18:3383–3394 DOI 10.1007/s10531-009-9648-9.

Joslin P. 1973. *The Asiatic Lion: a study of ecology and behaviour.* University of Edinburgh.

Kessel AL, Brent L. 1996. Space utilization by captive-born baboons (*Papio sp.*) before and after provision of structural enrichment. *Animal Welfare* 5:37–44.

Kistler C, Hegglin D, Würbel H, König B. 2010. Structural enrichment and enclosure use in an opportunistic carnivore: the red fox (*Vulpes vulpes*). *Animal Welfare* 19:391–400.

Konjević D, Bata I, Sindičić M, Njemirovskij V. 2015. Zoochosis as a cause of excessive dental wear in captive Siberian tiger-a case report. *Veterinarska Stanica* 46:59–63.

Koolhaas J, Korte S, De Boer S, Van Der Vegt B, Van Reenen C, Hopster H, De Jong I, Ruis M, Blokhuis H. 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews* 23:925–935 DOI 10.1016/S0149-7634(99)00026-3.

Korte SM, Olivier B, Koolhaas JM. 2007. A new animal welfare concept based on allostasis. *Physiology & Behavior* 92:422–428 DOI 10.1016/j.physbeh.2006.10.018.

Kroshko J, Clubb R, Harper L, Mellor E, Moehrenschlager A, Mason G. 2016. Stereotypic route tracing in captive Carnivora is predicted by species-typical home range sizes and hunting styles. *Animal Behaviour* 117:197–209 DOI 10.1016/j.anbehav.2016.05.010.

Lakens D. 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in Psychology* 4:1–12 DOI 10.3389/fpsyg.2013.00863.

Locurto C. 2006. Individual differences and animal personality. *Comparative Cognition & Behavior Reviews* 2:67–78 DOI 10.3819/ccbr.2008.20004.

Loehlin JC. 1992. *Genes and environment in personality development.* New York: Sage Publications, Inc.

Mallapur A, Qureshi Q, Chellam R. 2002. Enclosure design and space utilization by Indian leopards (Panthera *pardus*) in four zoos in southern India. *Animal Welfare* 5:111–124 DOI 10.1207/S15327604JAWS0502_02.

Martin P, Bateson PPG. 1993. *Measuring behaviour: an introductory guide.* Cambridge: Cambridge University Press.

Martin-Wintle MS, Shepherdson D, Zhang G, Huang Y, Luo B, Swaisgood RR. 2017. Do opposites attract? Effects of personality matching in breeding pairs of captive giant pandas on reproductive success. *Biological Conservation* 207:27–37 DOI 10.1016/j.biocon.2017.01.010.

Mason GJ, Latham NR. 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? *Animal Welfare* 13:57–69 DOI 10.2307/4493573.

Mason G, Mendl M. 2007. Why is there no simple way of measuring animal welfare? *Animal Welfare* 2:301–319.

Mason G, Rushen J. 2006. Stereotypic behaviour in captive animals: fundamentals and implications for welfare and beyond.

McMahon CR, Harcourt R, Bateson P, Hindell MA. 2013. Animal welfare and conservation, the debate we must have: a response to Draper and Bekoff (2012). *Biological Conservation* **158**:424 DOI 10.1016/j.biocon.2012.10.027.

Meehan CL, Mench JA. 2002. Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. *Applied Animal Behaviour Science* **79**:75–88 DOI 10.1016/S0168-1591(02)00118-1.

Meehan CL, Mench JA. 2007. The challenge of challenge: can problem solving opportunities enhance animal welfare? *Applied Animal Behaviour Science* **102**:246–261 DOI 10.1016/j.applanim.2006.05.031.

Meena V. 2008. *Reproductive strategy and behaviour of male Asiatic lions.* Dehradun: Forest Research Institute.

Meena V. 2009. Variation in social organisation of lions with particular reference to the Asiatic Lions *Panthera leo persica* (Carnivora: Felidae) of the Gir forest, India. *Africa* **1**:158–165.

Melotti L, Oostindjer M, Bolhuis JE, Held S, Mendl M. 2011. Coping personality type and environmental enrichment affect aggression at weaning in pigs. *Applied Animal Behaviour Science* **133**:144–153 DOI 10.1016/j.applanim.2011.05.018.

Mendl M, Burman OHP, Parker RMA, Paul ES. 2009. Cognitive bias as an indicator of animal emotion and welfare: emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science* **118**:161–181 DOI 10.1016/j.applanim.2009.02.023.

Miller LJ, Pisacane CB, Vicino GA. 2016. Relationship between behavioural diversity and faecal glucocorticoid metabolites: a case study with cheetahs (*Acinonyx jubatus*). *Animal Welfare* **25**:325–329 DOI 10.7120/09627286.25.3.325.

Mills D. 1998. Personality and individual differences in the horse, their significance, use and measurement. *Equine Veterinary Journal* **30**:10–13.

Moneta GB, Spada MM. 2009. Coping as a mediator of the relationships between trait intrinsic and extrinsic motivation and approaches to studying during academic exam preparation. *Personality and Individual Differences* **46**:664–669 DOI 10.1016/j.paid.2009.01.012.

Moorhouse TP, Dahlsjö CAL, Baker SE, Cruze NCD. 2015. The customer isn't always right—conservation and animal welfare implications of the increasing demand for wildlife tourism. *PLOS ONE* **10**:10 DOI 10.1371/journal.pone.0138939.

Morton FB, Lee PC, Buchanan-Smith HM. 2013. Taking personality selection bias seriously in animal cognition research: a case study in capuchin monkeys (*Sapajus apella*). *Animal Cognition* **16**:677–684 DOI 10.1007/s10071-013-0603-5.

Muehlmann AM, Lewis MH. 2012. Abnormal repetitive behaviours: shared phenomenology and pathophysiology. *Journal of Intellectual Disability Research* **56**:427–440 DOI 10.1111/j.1365-2788.2011.01519.

Murphy LB. 1977. Responses of domestic fowl to novel food and objects. *Applied Animal Ethology* **3**:335–349 DOI 10.1016/0304-3762(77)90058-X.

Neal Webb SJ, Hau J, Schapiro SJ. 2018. Captive chimpanzee (*Pan troglodytes*) behavior as a function of space per animal and enclosure type. *American Journal of Primatology* **80(1)**:e22947 DOI 10.1002/ajp.22947.

Owen MA, Swaisgood RR, Blumstein DT. 2017. Contextual influences on animal decision-making: significance for behavior-based wildlife conservation and management. *Integrative Zoology* 12:32–48 DOI 10.1111/1749-4877.12235.

Panksepp J. 2011. The basic emotional circuits of mammalian brains: do animals have affective lives? *Neuroscience and Biobehavioral Reviews* 35:1791–1804 DOI 10.1016/j.neubiorev.2011.08.003.

Pant SC. 2015. 14th Lion population estimation report—2015.

Pastorino GQ, Soares NRP, Mazzola S, Preziosi R. 2016. Personality assessment and feline—keepers' relationship in lions (*Panthera leo*). *International Journal of Health Animal Science & Food Safety* III:1–2.

Pastorino GQ, Viau A, Curone G, Pearce-kelly P, Faustini M, Vigo D, Mazzola SM, Preziosi R. 2017. Role of personality in behavioral responses to new environments in captive Asiatic lions (*Panthera leo persica*). *Veterinary Medicine International* DOI 10.1155/2017/6585380.

Plowman AB. 2003. A note on a modification of the spread of participation index allowing for unequal zones. *Applied Animal Behaviour Science* 83:331–336 DOI 10.1016/S0168-1591(03)00142-4.

Powell D. 1995. Preliminary evaluation of environmental enrichment techniques for African lions (*Panthera leo*). *Animal Welfare* 4:361–370.

Powell D, Svoke JT. 2008. Novel environmental enrichment may provide a tool for rapid assessment of animal personality: a case study with giant pandas (Ailuropoda melanoleuca). *Journal of Applied Animal Welfare Science* 11:301–318 DOI 10.1080/10888700802329673.

Rabin LA. 2003. Maintaining behavioural diversity in captivity for conservation: natural behaviour management. *Animal Welfare* 12:85–94.

Razal CB, Pisacane CB, Miller LJ. 2016. Multifaceted approach to personality assessment in cheetahs (*Acinonyx jubatus*). *Animal Behaviour* 3:22–31 DOI 10.12966/abc.03.01.2016.

Reading RP, Miller B, Shepherdson D. 2013. The value of enrichment to reintroduction success. *Zoo Biology* 32:332–341 DOI 10.1002/zoo.21054.

Rose P, Robert R. 2013. Evaluating the activity patterns and enclosure usage of a little-studied zoo species, the sitatunga (*Tragelaphus spekii*). *Journal of Zoo and Aquarium Research* 1:14–19.

Ross SR, Shender MA. 2016. Daily travel distances of zoo-housed chimpanzees and gorillas: implications for welfare assessments and space requirements. *Primates* 57:395–401 DOI 10.1007/s10329-016-0530-6.

RStudio. 2015. RStudio: integrated development environment for R. Boston: RStudio Inc.

Schoenecker B, Heller KE, Freimanis T. 2000. Development of stereotypies and polydipsia in wild caught bank voles (Clethrionomys glareolus) and their laboratory-bred offspring. Is polydipsia a symptom of diabetes mellitus? *Applied Animal Behaviour Science* 68:349–357 DOI 10.1016/S0168-1591(00)00108-8.

Schreck CB. 2010. Stress and fish reproduction: the roles of allostasis and hormesis. *General and Comparative Endocrinology* **165**:549–556 DOI 10.1016/j.ygcen.2009.07.004.

Sheperdson D, Carlstead K, Wielebnowski N. 2004. Cross-institutional assessment of stress response in zoo animals using longitudinal monitoring of faecal corticoids and behaviour. *Animal Welfare* **13**:105–113.

Sih A, Bell A, Johnson JCC. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology & Evolution* **19**:372–378 DOI 10.1016/j.tree.2004.04.009.

Skrzypczak N. 2016. Personality traits in the Atlantic spotted dolphin (Stenella frontalis): syndromes and predictors of neophilia. ProQuest dissertations and theses, 55.

Sneddon LU, Braithwaite VA, Gentle MJ. 2003. Animal studies repository novel object test: examining nociception and fear in the rainbow trout. *The Journal of Pain* **4**:431–440 DOI 10.1067/S1526-5900(03)00717-X.

Spiezio C, Valsecchi V, Sandri C, Regaiolli B. 2018. Investigating individual and social behaviour of the Northern bald ibis (*Geronticus eremita*): behavioural variety and welfare. *PeerJ* **6**:e5436 DOI 10.7717/peerj.5436.

Spinu V, Grolemund G, Wickham H. 2018. lubridate: make dealing with dates a little easier. R package version 1.7.4. *Available at http://www.jstatsoft.org/v40/i03/*.

Spruijt BM, Bos R Van den, Pijlman FT. 2001. A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. *Applied Animal Behaviour Science* **72**:145–171 DOI 10.1016/S0168-1591(00)00204-5.

Srivastav A. 2014. International Studbook of Asiatic lion (*Panthera leo persica*). WII. Dehradun.

Stamps J, Groothuis TGG. 2010. The development of animal personality: relevance, concepts and perspectives. *Biological Reviews* **85**:301–325 DOI 10.1111/j.1469-185X.2009.00103.x.

Stanton LA, Sullivan MS, Fazio JM. 2015. A standardized ethogram for the Felidae: a tool for behavioral researchers. *Applied Animal Behaviour Science* **173**:3–16 DOI 10.1016/j.applanim.2015.04.001.

Swaisgood RR, Shepherdson DJ. 2005. Scientific approaches to enrichment and stereotypies in zoo Animals: what's been done and where should we go next? *Zoo Biology* **518**:499–518 DOI 10.1002/zoo.

Szokalski MS, Litchfield CA, Foster WK. 2013. What can zookeepers tell us about interacting with big cats in captivity? *Zoo Biology* **32**:142–151 DOI 10.1002/zoo.21040.

Tan HM, Ong SM, Langat G, Bahaman AR, Sharma RSK, Sumita S. 2013. The influence of enclosure design on diurnal activity and stereotypic behaviour in captive Malayan Sun bears (*Helarctos malayanus*). *Research in Veterinary Science* **94**:228–239 DOI 10.1016/j.rvsc.2012.09.024.

Traylor-Holzer K, Fritz P. 1985. Utilization of space by adult and juvenile groups of captive chimpanzees (*Pan troglodytes*). *Zoo Biology* **4**:115–127 DOI 10.1002/zoo.1430040205.

Van der Harst JE, Spruijt BM. 2007. Tools to measure and improve animal welfare: reward-related behaviour. *Animal Welfare* **16**:67–73.

Volpato GL, Giaquinto PC, De Castilho MF, Barreto RE, De Freitas EG. 2009. Animal welfare: from concepts to reality. *Oecologia Australis* 13:05–15 DOI 10.4257/oeco.2009.1301.01.

Watanabe S. 2007. How animal psychology contributes to animal welfare. *Applied Animal Behaviour Science* 106:193–202 DOI 10.1016/j.applanim.2007.01.003.

Watters JV. 2009. Toward a predictive theory for environmental enrichment. *Zoo Biology* 28:609–622 DOI 10.1002/zoo.20284.

Watters JV, Margulis SW, Atsalis S. 2009. Behavioral monitoring in zoos and aquariums: a tool for guiding husbandry and directing research. *Zoo Biology* 28:35–48 DOI 10.1002/zoo.20207.

Watters JV, Powell DM. 2012. Measuring animal personality for use in population management in zoos: suggested methods and rationale. *Zoo Biology* 31:1–12 DOI 10.1002/zoo.20379.

Wemelsfelder F. 1997. The scientific validity of subjective concepts in models of animal welfare. *Applied Animal Behaviour Science* 53:75–88.

Wemelsfelder F, Haskell M, Mendl MT, Calvert S, Lawrence AB. 2000. Diversity of behaviour during novel object tests is reduced in pigs housed in substrate-impoverished conditions. *Animal Behaviour* 60(3):385–394 DOI 10.1006/anbe.2000.1466.

Whitham J, Miller L. 2016. Using technology to monitor and improve zoo animal welfare. *Animal Welfare* 25:395–409 DOI 10.7120/09627286.25.4.395.

Whitham JC, Wielebnowski N. 2013. New directions for zoo animal welfare science. *Applied Animal Behaviour Science* 147:247–260 DOI 10.1016/j.applanim.2013.02.004.

Wickham H. 2016. ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics* 3:180–185.

Wickham H. 2017. Tidyverse: easily install and load 'tidyverse' packages. R package version 1. *Available at https://CRAN.R-project.org/package=tidyverse*.

Wilson DS, Clark AB, Coleman K, Dearstyne T. 1994. Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution* 9:442–446 DOI 10.1016/0169-5347(94)90134-1.

Wilson DS, Coleman K, Clark AB, Biederman L. 1993. Shy-bold continuum in pumpkinseed sunfish (Lepomis gibbosus): an ecological study of a psychological trait. *Journal of Comparative Psychology* 107:250–260 DOI 10.1037/0735-7036.107.3.250.

Wilson M, Kelling A, Poline L, Bloomsmith M, Maple T. 2003. Post-occupancy evaluation of zoo Atlanta's Giant Panda Conservation Center: staff and visitor reactions. *Zoo Biology* 22(4):365–382 DOI 10.1002/zoo.10102.

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Effects of a combined enrichment intervention on the behavioural and physiological welfare of captive Asiatic lions (*Panthera leo persica*)

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ABSTRACT

The endangered Asiatic lion (*Panthera leo persica*) is currently distributed as a single wild population of 670 individuals and ~400 captive animals globally. Although the captive lions are major hope for the species' long-term conservation through repatriation, their welfare status and management practises need research attention. To this end, we tested the efficacy of feeding, sensory and manipulable enrichment interventions on the welfare of Asiatic lions at the conservation breeding centre of Sakkarbaug Zoological Garden, Gujarat. We adopted a holistic approach by measuring physiological and behavioural responses of 35 captive Asiatic lions, divided into control (N = 16) and test (N = 19) groups. The test subjects approached feeding devices first and used manipulable devices for longer duration. Manipulable devices were used homogenously with two significant time peaks, but sensory devices were used sporadically throughout the day with no discernible peak usage. The control subjects remained unchanged in all welfare parameters compared to their pre-treatment levels. However, post-enrichment behavioural assessments showed higher behaviour diversity (95 % increase from the baseline period), reduced enclosure zone bias (40.25 % reduction) and aberrant repetitive behaviours (80.68 %) in test samples. Similarly, faecal corticosterone measures showed lower stress levels in test samples (58 % decrease), confirming significant improvement in all welfare indices than control groups. These results have universal applicability to assess welfare indices of other captive species in Indian zoos. We hope that the results will encourage zoo managers and regulatory agencies to improve animal welfare practices.

1. Introduction

Since the early Palaeolithic depictions of the "lowenmensch" by the Aurignacians (Chauvet et al., 1996), lions have been heralded across several cultures as emblems of man's relationship with nature (McCall, 1973). Once ubiquitous across south-western Asia between Syria and parts of eastern India (Ball, 1880; Joslin, 1984), asiatic lions were hunted to extinction from large parts of their range by the late 19th century (Jhala et al., 2019; MacKenzie, 2017; Storey, 1991). During the 1860s, the Asiatic lion population in Gir forests, India was struggling to survive. Timely conservation measures along with a hunting ban were instrumental to the recovery of the species. Sakkarbaug zoo, founded in 1863, played a definitive role in the conservation of Asiatic lions by treating injured and diseased individuals and maintaining a viable captive stock for future repatriation. Subsequent affirmative conservation actions including an ex-situ conservation breeding programme (Smith, 1984) led to the species revival from the edge of extinction. Today the extant population of more than 500 wild Asiatic lions (Gujarat Forest Department, 2015) inhabit fragmented habitats scattered over a human dominated landscape in Gujarat (Gogoi et al., 2020), Western India, along with approximately 400 Asiatic lions spread across zoos in several countries, with more than 60 % of the captive population residing in Indian zoos and conservation breeding centres (Srivastav et al., 2018). The survival and proliferation of this species relies on repatriation to insulate the extant population from future stochastic extinction events (Jhala et al., 2019). Although the conservation breeding programme has been successful in maintaining a genetically diverse stock, the welfare status and incumbent management practices for the animals has not received adequate research focus (Pastorino et al., 2017; Goswami et al., 2020).

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Conservation breeding programmes for endangered species should be designed to promote species-typical behaviours and cognitive plasticity for better post-release fitness (Rabin, 2003). The deleterious impacts of sterile captive environments manifest with loss of species-typical behaviours and increase in psychosomatic disorders in animals (Broom, 2011; Dawkins, 2004; Fraser, 1999; Rabin, 2003). Therefore, welfare-based management practices are vital for any successful conservation breeding programme (Swaisgood, 2010). The benefits of an individual-centric welfare evaluation (Joslin, 1984) complemented with targeted enrichment interventions has been successfully demonstrated for several captive animals viz., ursids (Carlstead et al., 1991; McGowan et al., 2010), felids (Powell, 1995; Suárez et al., 2017), canids (Cloutier and Packard, 2014; Leonard, 2008), equids (Bulens et al., 2013), small mammals (Clark and Melfi, 2012; Vargas and Anderson, 1999), reptiles (DeGregorio et al., 2017), and amphibians (Michaels et al., 2014). Apart from improving welfare, enrichment interventions have been shown to play an important positive role in increasing post-release fitness in several species (Brown et al., 2003; Rabin, 2003; Reading et al., 2013). Earlier study on welfare status of captive Asiatic lions reported that individual variations (personality and rearing history) are associated with differential welfare outcomes in lions housed under similar captive environments (Goswami et al., 2020), necessitating individually tailored husbandry regimen for the animals. This study focuses on identifying the areas for improvement of the current husbandry and management practices for Asiatic lions.

Feeding, sensory and manipulable enrichments have been shown to improve the welfare of captive felids (Powell, 1995; Van Metter et al., 2008). We tested the efficacy of a combined enrichment intervention on the welfare of captive Asiatic lions at Sakkarbaug Zoological Garden (SZG). We measured several behavioural (species-typical behaviour diversity, enclosure usage and aberrant repetitive behaviours) and physiological (faecal corticosterone metabolites) welfare indices as a response to enrichment interventions. This is the first controlled trial study to incorporate both behavioural and physiological tools to measure the welfare status change in response to enrichment interventions of Asiatic lions housed at a conservation breeding centre. We hope that our findings assist in the improvement of incumbent husbandry and management practices for the species.

2. Methods

2.1. Study area

We conducted this study at the Asiatic lion conservation breeding centre at SZG. The conservation breeding programme of Asiatic lions was initiated in 1958 with nine founders and has since proliferated to house more than 47 individuals housed at SZG with several breeding pairs in other participating zoos across the world. Since SZG houses both captive-born and wild-rescued individuals, it holds intrinsic value for the conservation of Asiatic lions. During the study period, the off-display conservation breeding facility housed 47 individuals in 20 large naturalistic enclosures. A schematic map of the facility is provided in Supplementary Fig. 1.

2.2. Subjects and housing

The Asiatic lion conservation breeding center at SZG has 21 enclosures spread across two blocks (block A = 15, block-B = 6). Enclosures at Block-A and Block-B are located 500 m apart and provide identical housing and husbandry conditions to the subjects. All enclosures provided adequate space (above 400m^2/animal in general with some having 1500 m^2/animal) for the animals. The naturalistic enclosures provided subjects with sufficient natural vegetation cover, simulating the habitat of Asiatic lions while protecting subjects from visitor disturbance. Enclosures were well covered with trees and shrubs, which provided subjects with a good combination of shade and sun throughout the day. Fresh drinking water was made available inside retiring cells adjoining the paddock area. Subjects were allowed free access to the paddock area and retiring cubicles throughout the day and night. However, these enclosures offered little in terms of novelty and cognitive enrichment. An earlier study with the same Asiatic lion individuals showed low enclosure space utilization, low behaviour diversity, and a high incidence of aberrant repetitive behaviours (Goswami et al., 2020). Due to logistical constraints, we randomly selected 15 enclosures (block-A = 12, block-B = 3) housing 35 Asiatic lions in pair (1:1 and 0:2) (N = 9 enclosures) or heterosexual (1:2) (N = 6 enclosures) configurations (Supplementary Table 1). Some of the subjects were wild-rescued (N = 19, Male = 11, Female = 8), while the rest were born in captivity (N = 16, Male = 3, Female = 13). Subjects were primarily adult animals (N = 31) with a few sub-adults (N = 4) and were randomly assigned to test and control groups to ensure uniformity of treatment. The test group consisted 19 subjects (Male = 7, Female = 12) the control group consisted 16 subjects (Male = 7, Female = 9), respectively. Due to random selection of these enclosures, some of the control enclosures were adjacent to test enclosure. To account for any external stimuli from influencing behaviour observations we provided visual barriers for all adjacently situated enclosures during the study period. All subjects were housed in the same enclosure with the same enclosure mates for at least a year and were accustomed to identical enclosure and management practices.

A group of 10 animal keepers and contractual workers carried out all husbandry work for the lions. Keepers typically reported for duty at 0700 hours and cleaned all leftover food from retiring cells during 0730–1100 hours. The leftover food items were weighed to measure the food consumption by each individual. The keepers would provide the daily ration of buffalo meat to the subjects between 1700–1800 hours at the feeding cubicle, where each lion was fed separately. After confirming that each lion had consumed their ration, keepers opened the gate to the paddock area from the retiring cubicle. After the lions had been fed, the gate to the enclosure was kept open giving the animals a choice to stay inside or move out to the paddock area. Keepers were also tasked with behavioural monitoring of the subjects and recorded the commencement and cessation of mating events between subjects. The zoo managers and the animal keepers met regularly to ascertain the welfare need of the captive animals based on subjective evaluations. The approach to welfare assessment was preventative and based solely on incidental keeper observations hence difficult to quantify and address. We expected to observe the effect of novel enrichment devices on the behavioural and physiological welfare indices of these captive animals.

2.3. Study design

We hypothesized that enrichment interventions would lead to significant improvement in behavioural (Goswami et al., 2020) and physiological welfare measures for all test subjects in contrast to the controls. While it is possible that sex and social grouping of the study individuals could have some influence on the types of interactions during enrichment interventions, earlier study with the same individuals showed that sex and social vs. paired grouping did not have any bearing on the welfare indices (Goswami et al., 2020). We divided the subjects into control (N = 16, eight enclosures) and treatment (N = 19, seven enclosures) groups by a blind draw of enclosures for enrichment interventions. Block A had 12 enclosures (seven treatment and five control) whereas block B had three enclosures (all controls) (Supplementary Fig. 1). We conducted the study in two phases: baseline and post-enrichment. During the baseline period, we measured behaviour for 14 days/subject and collected two fresh faecal samples/subject/week. This was followed by a one-week exposure to the enrichment intervention to get test subjects accustomed to the new devices. During the testing period (14 days) we recorded the behaviour of test subjects as a response to the daily enrichment intervention. Apart from the enrichment interventions, housing and husbandry conditions remained unchanged for both groups throughout the course of the study.

2.4. Data collection

2.4.1. Behaviour observations

Two observers (viz., first author and field assistant) collected all behaviour data, which necessitated accounting for inter-observer reliability. We created a detailed ethogram of the study subjects from one month of *ad-libitum* behaviour sampling and compared the behaviour data recorded from the same subject by both observers to ensure consistency (Goswami et al., 2020). We commenced data collection only after inter-observer reliability levels remained consistent across three consecutive sessions (Cronbach's alpha >0.9). We simultaneously video recorded all behaviour sessions to aid in data entry and reduce errors. We recorded behaviour during 0500–1100, 1200–1800, and 2200-0500 hours. We used instantaneous scan sampling (Altmann, 1974) at 1-minute intervals to record all behavioural states and all occurrences of behavioural events (Goswami et al., 2020). During every six-hour data collection period, we recorded four hours of behaviour data with 30 min rest for observers after every hour. Data for the observer-resting period was later tabulated from the video recordings. We collected 195 ± 12.3 h of behaviour data/subject during the study period.

We measured three behavioural welfare indices: species-typical behaviour diversity (Wemelsfelder et al., 2000), spread of participation index (Plowman, 2003) and aberrant repetitive behaviours or stereotypy (Mason and Latham, 2004) to measure the welfare of Asiatic lions (Goswami et al., 2020). We compared the behavioural welfare indices of all test and control subjects across the baseline and enrichment period. Baseline information for diversity of behaviour repertoire in control and test subjects was collected using Shannon-Weiner diversity index (SWI) (Goswami et al., 2020). We measured enclosure usage patterns of subjects based on the spread of participation index (SPI) (Plowman, 2003) to understand if the enclosures provided enough complexity to meet their welfare requirement (Plowman, 2003; Ross and Shender, 2016). We divided each enclosure (N = 15) into ten zones, i.e., three primary zones (proximal, medial, and distal), which were subdivided into three secondary zones (left, middle, right), and the tenth zone was the area adjacent to the entrance to feeding cubicles (Goswami et al., 2020). We measured the proportion of aberrant repetitive behaviours (ARBs) or stereotypy as an indicator of poor welfare and compared their prevalence between control and test subjects across treatments.

2.4.2. Physiological measures

We measured faecal corticosterone metabolites of all subjects to contrast the physiological impacts of the enrichment interventions on control and test subjects. Faecal corticosterone is a reliable physiological indicator of stress and compromised welfare in captive felids (Ruskell et al., 2015; Schildkraut, 2016; Vaz et al., 2017; Young et al., 2004). We collected two fresh faecal samples/week from each subject (both control and test) during the entire study period (pre-enrichment and post-enrichment).

One day prior to the faecal sample collection, we cleaned all faecal materials from the enclosures. On the day of faecal sample collection, subjects were monitored all night and the location of faeces was visually matched with the subject. We rejected and removed samples where multiple individuals defecated at the same spot leading to confusion. We collected all fresh faecal samples using the dry sampling approach (Biswas et al., 2019) and stored in −20 °C freezer onsite and later transported to the Wildlife Institute of India in dry ice. The samples were stored in the laboratory at −20 °C freezer until further processing. To control for effects of moisture and diet we pulverized the frozen samples before lyophilizing (#FD-5, Allied Frost, New Delhi, India) them for 72 h prior to hormone extraction (Mondol et al., 2020). Subsequently, we sieved lyophilized samples through a 0.5 mm stainless steel mesh to obtain homogenized faecal powder. We thoroughly mixed the dried faecal powder and extracted hormone by pulse-vortexing 0.1 g of powder in 15 mL of 70 % ethanol, followed by centrifugation at 2200 rpm for 20 min (Mondol et al., 2020; Wasser et al., 2010). We collected and stored the hormone extracts in 2 mL cryochill vials (1:15 dilution) and stored in −20 °C freezer till further analyses.

We used corticosterone EIA kit (#K014, Arbor Assays, MI, USA) for corticosterone metabolite estimation in faecal hormone extracts. Sample extracts were air-dried inside an incubator and resuspended in assay buffer as per required dilutions. Each sample was assayed in duplicate using kit protocol and the optical density was measured at 450 nm using an ELISA plate reader (#GMB-580, Genetix Biotech Asia Pvt. Ltd., New Delhi, India). Hormone metabolite concentration is interpolated using four parametric logistic (4 PL) regression function in GraphPad prism software version 5 (GraphPad Software, California, USA). Cross-reactivities of the antibody are listed in Supplementary Table 2.

We tested for parallelism and accuracy to validate the corticosterone assay. We used dilutions of pooled extracts from a random combination of male and female samples (N = 20) to assess reliable quantification of corticosterone at different concentrations and find optimal dilutions for final assays (at 50 % binding). We plotted the relative dose against percent bound hormones for the pools and the standards and generated best-fit curve using 4 PL regression, where parallel slopes indicate similar immunoreactivity at different concentrations. For accuracy test, we spiked corticosterone standards with equal volumes of diluted faecal extract of known concentration (dilution level close to 50 % bound from parallelism test) and assayed with standards. We plotted the results as regression lines using observed and expected concentrations to show that faecal contaminants were not interfering with assay accuracy at the tested dilution. We calculated inter- and intra- assay coefficients of variation using repeated measures of same-pooled extract.

2.4.3. Enrichment interventions

Enrichment interventions add cognitive complexity to enclosures and provide animals the opportunity to express species-typical behaviours (Mellen and Shepherdson, 1997; Skibiel et al., 2007). We used three types of enrichment devices: manipulable (Mellen and Shepherdson, 1997; Powell, 1995), sensory (Skibiel et al., 2007), and feed (Powell, 1995; Skibiel et al., 2007) (Table 1). Manipulable enrichments included hanging lion-sam balls, burlap bags, wooden perches/ platforms, wooden planks etc., whereas the sensory enrichments included olfactory augmentations such as scent trails made from the blood of buffalo, urine of unknown conspecifics, and dung of other prey species (Sambar, *Rusa unicolor*) etc. We also installed sensory enrichment (tactile) by wrapping rough coir rope on the bark of trees inside enclosures to promote auto-grooming and scent-marking behaviours in the subjects. Nutritional enrichment has been shown to garner the highest amount of attention in captive animals and can be useful for addressing neophobia to enrichment devices in shy individuals (Powell, 1995; Resende et al., 2009; Skibiel et al., 2007). We provided a range of nutritional enrichment devices, which included frozen dressed chicken, buffalo tails that were suspended by natural fibre ropes from trees at different parts of the enclosure. While manipulable and sensory enrichment devices were quasi-permanent and required little daily care, nutritional enrichment devices needed to be replenished every day. We installed and replenished enrichment devices between 0600–0700 hours at four enclosures every day. Keepers would arrive early and confine the subjects inside retiring cells with food rewards, while the enrichment interventions were placed at designated enclosures. We furnished all enclosure zones with the same combination of manipulable, sensory and nutritional enrichment.

To calculate enrichment device preferences, we collated all scans during which Asiatic lions interacted with the enrichment devices. We also recorded the type of behaviours that were performed by the subjects while interacting with the enrichment devices viz., exploration, social play, aggression, foraging and fear (Table 2). We counted the different types of behaviours performed per enrichment device by all subjects.

Table 1
Description of enrichment devices used in this study at enclosures.

Sl. no	Enrichment device	Enrichment type	Description
1	Platform	Manipulable	A log platform made to enable subjects to access vantage points within the enclosure
2	Sam ball	Manipulable	A hard-plastic ball with no holes or perforations
3	Log branches	Manipulable	Tree logs sections with bark suspended from trees
4	Lion bite ball	Manipulable	A hard-plastic ball with a rattle inside suspended by a bungee rope with canvas sheet that acts as a bite
5	Buffalo shank	Feeding	Buffalo shank suspended from a zipline
6	Chicken carcass	Feeding	Frozen Chicken carcass suspended from a tree by natural fibers
7	Buffalo tail	Feeding	Skinned buffalo tail suspended from tree branches
8	Buffalo blood	Feeding	Frozen buffalo blood cubes, provided on a clean concrete platform
9	Star anise oil mixed with garlic	Sensory	Sprayed on tree trunks or used to create scent trails to other enrichment devices
10	Prey dung	Sensory	Sambar (*Rusa unicolor*) dung smeared on tree trunks or mixed with enclosure soil at certain zones
11	Conspecific urine	Sensory	Earth drenched in the urine conspecifics from nearby enclosures smeared on tree trunks, or mixed with enclosure substrate at certain zones

Table 2
Behaviours observed that are associated with different enrichment devices.

Sl. no	Behaviour	Description	Category
1	Aggressive Growl	Baring teeth with short vocalizations directed at conspecifics or humans, while interacting with enrichment	Aggression
2	Charge	Run up to a conspecific at a rapid short charge as a result of being challenged while suing an enrichment device	Aggression
3	Strike	Hit conspecific with one paw, while defending an enrichment device	Aggression
4	Bite	Bite conspecific, while asserting control over an enrichment device	Aggression
5	Allogrooming	Lick conspecific, while interacting with enrichment device	Social
6	Nuzzling	Rub noses with conspecifics with soft grunts, and inviting them to play with enrichment device	Social
7	Roll	Rolling in the dirt with conspecific while interacting with a enrichment device	Social
8	Rub	Rub body or neck against the enrichment device	Explore
9	Sniffing	Raise head and sniff near a sensory enrichment	Explore
10	Flehmen	Bare teeth and sniff the air near an enrichment device	Explore
11	Scent mark	Spray urine or scratch the tree next to an enrichment device	Explore
12	Digging	Dig near an enrichment device	Explore
13	Scratching	Scratch the trees where enrichment devices are installed	Explore
14	Chase	Chase away conspecifics trying to use or steal enrichment device successfully or unsuccessfully. Does not result in an overtly aggressive interaction	Chase
15	Run	Run around the enclosure with the enrichment device in mouth. May or May not be chased by conspecific	Play
16	Stalk	Stalk an enrichment device	Play
17	Play	Kick around an enrichment device	Play
18	Drag	Drag enrichment device along the enclosure	Play
19	Bite	Bite enrichment device	Play
20	Eat	Eat a food based or sensory enrichment device	Forage
21	Lick	Lick enrichment devices intently	Forage
22	Neophobia	Approach enrichment device slowly and run away	Fear
23	Fearful growl	Growling at enrichment device, ears back and cowering	Fear
24	Circling	Warily circling around the enrichment device	Fear

2.5. *Data analysis*

We analysed all data in R v3.6.2 (R Core Team, 2020) with R studio (RStudio, 2020). We used packages "tidyverse" (Wickham et al., 2019), "psych" (Revelle, 2019) and "dplyr (Wickham et al., 2020),"ggpubr" (Kassambara, 2020), "sur" (Harel, 2020) and "ggplot2" (Wickham, 2011) to calculate statistical outputs and create graphical summaries of the findings. We conducted tests for normality and ensured that variances were similar between groups to select appropriate parametrical and non-parametrical statistical analysis. We used unpaired *t*-test to compare the welfare indices between test and control groups during baseline and post-enrichment interventions. We used paired *t*-tests, to compare the differences in welfare indices within test and control groups and measured the amount of time spent by test subjects on different types of enrichment devices. Additionally, we calculated the effect size (Cohen, 1992, p. 199) of the enrichment intervention for the test group, which is scale-independent and gives a measure of the magnitude of difference between baseline and post-treatment conditions.

3. Results

3.1. *Enrichment preferences of study subjects*

We collected a total of 288,120 scans from all subjects (N = 35) during the baseline period. During 14 days of enrichment intervention, we recorded 138,083 scans from control subjects (N = 16), and 165,098 scans from test subjects (N = 19). We found test subjects using enrichment devices across 109,238 scans. The results of the hourly percentages of enrichment use and enrichment-directed behaviours performed by test subjects are presented in Fig. 1. The test subjects used manipulable enrichment devices for longer durations (56.8 %) compared to all other types viz., sensory (12.99 %) and feeding (30.20 %) (Table 3). It was challenging to measure the usage levels of sensory enrichments accurately and hence these values may have been under-reported in our results. When subjects were released inside the enclosures, they approached the feed-based enrichment devices first followed by manipulable and sensory devices. This trend continued throughout the intervention period. Feed-based enrichment devices were extensively used during the first two hours of the day between 0700–0900 hours (Fig. 1, Table 4) and garnered little or no interest after they were depleted. On the other hand, manipulable and sensory enrichment device usage peaked twice daily at 0700 and 1600 h (Fig. 1, Table 4) with continued usage throughout the day.

In terms of enrichment directed behaviours, we found that the peak of aggression (16.87 %), chase (22.04 %), exploratory (16.14 %) and forage (20.04 %) behaviours in test subjects occurred between 0700–1100 hours (Table 4). The next peak of enrichment-directed behaviours occurred between 1300–1700 hours constituting mostly of play (57.94 %) and exploratory behaviours (25.25 %) (Table 4). Early in the day (0700–0900 hours), when subjects were actively exploring around the enclosure for enrichment devices, aggressive dominant and displacement behaviours were common but subsided as the day progressed (Fig. 1, Table 4). We found that manipulable devices (11.51 %) were more commonly associated with aggressive behaviours compared to food-based enrichment (2.94 %) (Table 3). We also observed that

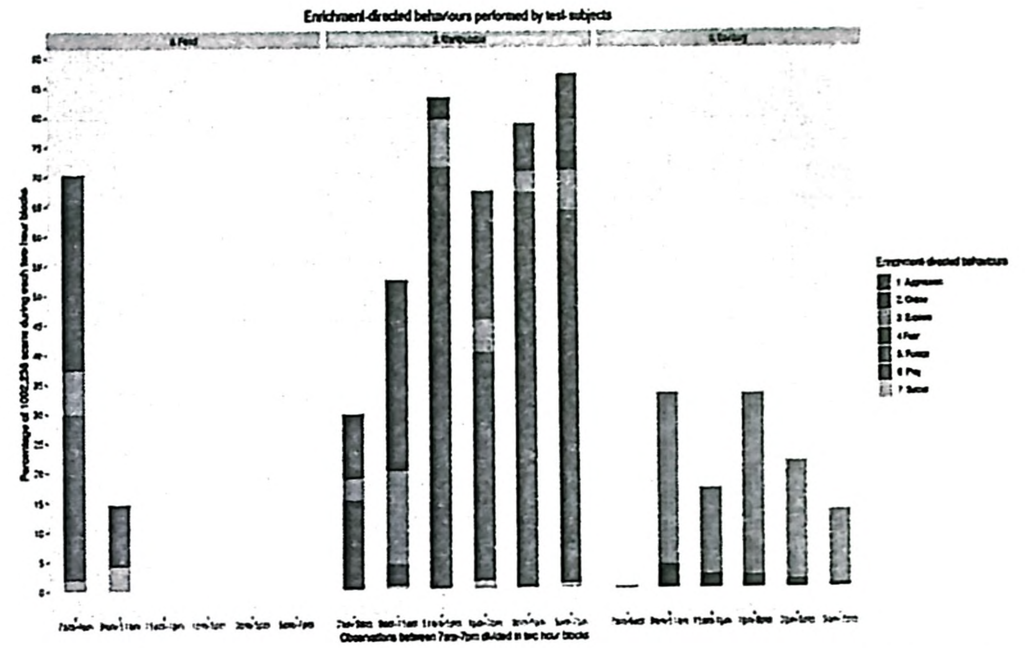

Fig. 1. Temporal pattern of enrichment device use and enrichment-directed behaviours by Asiatic lions on a. Feeding, b. Manipulable, and c. Sensory enrichment devices. Primary X-axis represents the hours of the observation period from 0700- 1900 h divided in two-hour blocks. The primary Y-axis represents the percentage of scans during each two-hour block spent performing a certain enrichment-directed behaviour. Enrichment-directed behaviours are represented as coloured stacked bars on the vertical axis (1:7), while enrichment devices associated with said behaviours are represented on separate panels.

Table 3
Enrichment device usage with behaviours performed (N = 109,238 scans).

Enrichment device	Feed (%)	Manipulable (%)	Sensory (%)
Aggression	2.94	11.51	0
Chase	11.54	1.89	0
Explore	3.05	5.95	11.77
Fear	0	1.96	0
Forage	11.48	0	0
Play	0	35.16	1.21
Social	1.16	0.35	0
Total	30.20	56.8	12.99

positive social behaviours were more commonly associated with food-based enrichment devices (1.16 %) rather than the manipulable devices (0.35 %) (Table 3). Subjects usually monopolized the manipulable enrichment devices and showed high fidelity towards specific items. The feed-based enrichment devices were associated with the highest proportion of chase behaviour (11.54 %), resulting in one animal monopolizing a device and chasing away others (Table 3). Some feed-based enrichment devices were consumed within minutes (e.g. dressed chicken) while others required more processing time (e.g. buffalo tail, frozen blood cubes). Once the feed enrichment devices were completely utilized, the lions concentrated on the manipulable and sensory enrichments and engaged in social and exploratory behaviours throughout the later parts of the day (Table 3, Fig. 1). The manipulable enrichment devices were associated with the largest variety of species-typical behaviours, which included 35.16 % play behaviour (Table 3) and were more commonly associated with aggressive interactions (11.51 %) compared to all other enrichment types. Aggressive behaviours occurred when enclosure mates tried to steal or use a certain manipulable device from the lion that was playing with it. Since we installed more enrichment devices than subjects in every enclosure, displaced individuals had the opportunity to interact with other devices when displaced by conspecifics. Sensory enrichment devices contributed primarily to exploratory behaviours (11.7 %) that led to increased enclosure usage.

However, it is important to point out that the true impacts of different types of enrichment devices cannot be assessed merely through the measuring the amount of time the animal spent with it, but by comparing the welfare indices of test subjects with control and their own baseline status. The results clearly demonstrate that the presence of different types of enrichment devices was instrumental in mitigating monopolization of resources by dominant individuals.

Table 4
Hourly percentages of enrichment usage and enrichment-directed behaviours by test subjects during the interventions.

Enrichment usage during each two-hour block						
Enrichment	0700–0900 (N = 44,845)	0900–1100 (N = 10,240)	1100–1300 (N = 13,514)	1300–1500 (N = 9,674)	1500–1700 (N = 14,928)	1700–1900 (N = 16,037)
Feed	70.22	14.64	0	0	0	0
Manipulable	29.54	52.33	83.09	67.20	78.73	87.02
Sensory	0.22	33.01	16.90	32.79	21.26	12.97
Enrichment-directed behaviours performed during each two-hour block						
Aggression	`14.13	41.19	0	21.66	7.02	13.06
Chase	29.62	1.65	3.76	0	1.13	3.16
Explore	11.07	44.86	22.65	36.28	23.51	19.09
Fear	0.79	3.48	0	5.53	2.39	3.34
Forage	27.97	0	0	0	0	0
Play	14.50	4.02	73.33	35.19	65.71	60.52
Social	1.89	4.78	0.23	1.32	0.21	0.79

3.2. Behavioural welfare indices

During pre-enrichment period, behaviour diversity levels of control subjects (M = 0.9, SD = 0.3) were similar to that of the test subjects (M = 0.83, SD = 0.35). After enrichment interventions, the behaviour diversity of control subjects remained unchanged (M = 0.77, SD = 0.35, t (30) = 1.11, p = 0.29) while that of the test subjects increased significantly (M = 1.62, SD = 0.18, t(36) = -8.8, p < 0.001, Cohen's d = 2.83) (Table 5, Fig. 2a).

During the baseline, we observed high SPI values in test (M = 0.77, SD = 0.12) and control groups (M = 0.82, SD = 0.09) indicating enclosure zone use bias. However, after enrichment intervention the SPI values decreased significantly indicating, enclosure usage homogeneity (M = 0.46, SD = 0.08) for test subjects (t(36) = 9.37, p < 0.0001, Cohen's d = 3.03), while it remained unchanged for control subjects (M = 0.8, SD = 0.13, t(30) = 0.5, p = 0.61) (Table 5, Fig. 2b).

During baseline, test (M = 12.32, SD = 6.03), and control subjects (M = 12.8, SD = 6) showed similar levels of aberrant repetitive behaviours. After enrichment interventions, we observed a significant decrease in ARBs in test subjects (M = 2.38, SD = 2.46, t (36) = 6.65, p < 0.0001, Cohen's d = 2.15) while the levels remained unchanged for the control group (M = 13.31, SD = 6, t(30) = 0.23, p = 0.81) (Table 5, Fig. 2c). Table 5 represents the comparison of welfare indices within control and test groups across pre-enrichment and post-enrichment phases. Fig. 1 represents the comparison of welfare indices between control and test groups across pre-enrichment and post-enrichment phases.

3.3. Faecal corticosterone level

Parallelism and accuracy tests for corticosterone metabolites showed reliable measures from lion faeces across different concentration ranges. Serial dilutions of faecal extracts paralleled the standard curves (Fig. S2a). There were no differences between slopes of standard and pooled extract curves for corticosterone (F(1,10) = 2.06, P = 0.182), and the curves were significantly different in their elevation (F(1,11) = 66.25, P = 0.0001). Accuracy tests produced slopes of 0.92 at working dilution of 1:60 (Fig. S2b), suggesting that faecal extracts did not interfere with their metabolite measurement precisions. Intra-assay coefficient of variation (CV) was 9.8 %, whereas inter-assay CV was 8.99 % (Supplementary Table 2).

We compared the average faecal corticosterone levels of each subject across baseline and post-enrichment phases. Compared to baseline levels (M = 7.74 μg/gm, SD = 3.2), the faecal corticosterone measures of test subjects decreased significantly (M = 2.46 μg/gm, SD = 0.85, t(36) = 5.33, p < 0.0001, Cohen's d = 2.22), while it remained unchanged from baseline (M = 7.1 μg/gm, SD = 4.08) for control subjects (M = 6.96 μg/gm, SD = 3.93, t(30) = 0.093, p = 0.92) (Table 5, Fig. 2d). These results confirm that the enrichment interventions led to significant improvement in behavioural and physiological welfare indices of test subjects as compared to the control group during pre-enrichment period.

4. Discussion

Animal welfare is not limited to the prevention of cruelty and amelioration of symptoms of stress, but also accords the opportunity to express species-typical behaviour patterns (Broom, 2011). The National Zoo Policy of India (MoEF,India, 1998) espouses animal welfare with captive animal management, yet welfare-centric management protocols remain divorced from husbandry practices across several zoos. Although few and far between, welfare research from Indian zoos report a prevalence of stereotypic behaviours (Goswami et al., 2020; Mallapur et al., 2007; Sanyal, 1892; Vaz et al., 2017) and elevated faecal corticosterone levels (Vaz et al., 2017) across several captive felids, and prescribe specie-appropriate enclosure design and enrichment interventions as remedial measures (Goswami et al., 2020; Mallapur et al., 2007; Vaz et al., 2017). Enrichment interventions have been shown to improve welfare conditions in several captive felids including African lions (Mellen and Shepherdson, 1997; Powell, 1995; Skibiel et al., 2007), yet enrichment protocols are seldom incorporated in the daily management of captive Asiatic lions at Indian zoos. The Asiatic lion conservation breeding programme has successfully maintained a viable captive population insulated from stochastic ecological events. Most research pertaining to captive Asiatic lions investigate their genetic and demographic status (Bagatharia et al., 2013; Pastorino et al., 2017) with only a handful mentioning their welfare (Goswami et al., 2020; Mallapur et al., 2002; Pastorino et al., 2017; Vaz et al., 2017). Our study is the first to assess the effects of enrichment protocols on the welfare of captive Asiatic lions.

Welfare encompasses both internal and external conditions affecting an animal and hence must be measured using a multifarious approach. As highlighted by Miller et al. (2020) the focus on negative welfare indicators has led to zoos adhering to minimum husbandry guidelines that attempt to suppress symptoms of poor welfare rather than trying to improve existing conditions. In this study, we measured two traditional negative welfare parameters like ARB (Mason and Latham, 2004) and faecal corticosterone levels (Schildkraut, 2016; Vaz et al., 2017) along with two positive welfare indicators (Miller et al., 2020) viz., spread of participation index (SPI) (Cabana et al., 2018; Powell, 1995) and behaviour diversity (Pastorino et al., 2017). Previous studies on captive African lions that establish the positive effects of enrichment interventions have primarily relied on behavioural welfare indices (Martinez-Macipe et al., 2015; Ncube and Ndagurwa, 2010; Powell, 1995; Regaiolli et al., 2019; Van Metter et al., 2008). Our study is the first to showcase both the behavioural and physiological impacts of enrichment interventions in captive Asiatic lions housed in a conservation breeding programme. The welfare evaluation framework used in this study can be

Table 5
Summary statistics of welfare indices within control and test groups.

Welfare indices	Pre-enrichment	Post-enrichment	t-value*	Significance (p-value)	Effect size (Cohen's d)
Control group (N = 16)					
Behaviour diversity (SWI)	0.9 ± 0.31	0.77 ± 0.35	1.11	0.29	0.39
Spread of participation index (SPI)	0.82 ± 0.09	0.8 ± 0.13	0.51	0.61	0.17
Aberrant repetitive behaviours (ARB)	12.81 ± 6	13.31 ± 6.07	0.23	0.81	0.08
Faecal corticosterone measure	7.1 ± 4.08	6.96 ± 3.93	0.09	0.92	0.03
Test group (N = 19)					
Behaviour diversity (SWI)	0.83 ± 0.35	1.62 ± 0.18	−8.8	0.0001	2.83
Spread of participation index (SPI)	0.77 ± 0.12	0.46 ± 0.08	9.37	0.0001	3.03
Aberrant repetitive behaviours (ARB)	12.32 ± 6.03	2.38 ± 2.46	6.65	0.0001	2.15
Faecal corticosterone measure	7.74 ± 3.25	2.46 ± 0.85	6.85	0.0001	2.22

* For control group t(30) and test group t(36).

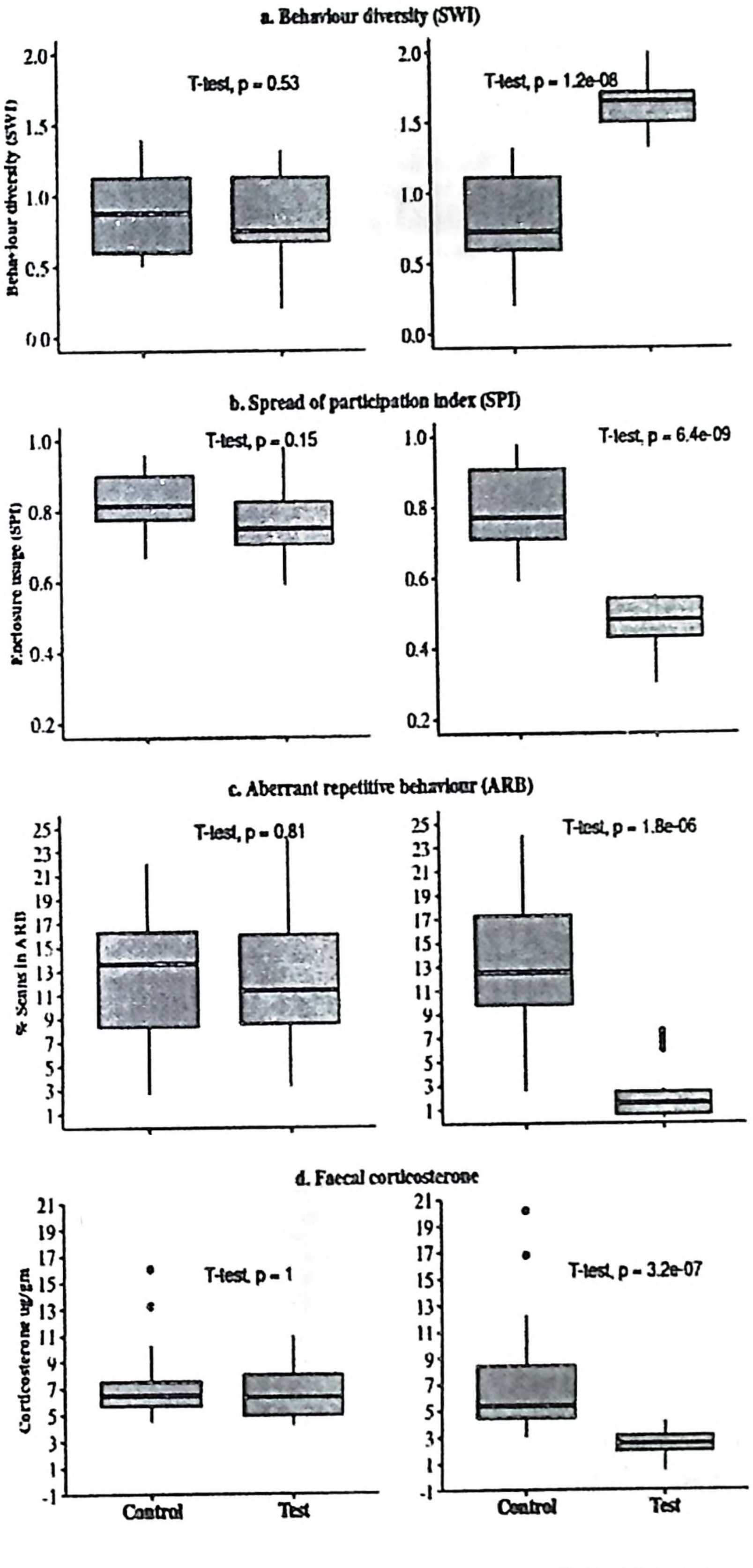

Fig. 2. Boxplots comparing the welfare indices of test and control subjects across pre-enrichment and post-enrichment conditions for a. Behaviour diversity. b. Spread of participation index, c. Aberrant repetitive behaviours (ARB), d. Faecal corticosterone levels.

applied universally (for other species) to measure the impacts of goal-oriented enrichment interventions.

While previous studies have tested the welfare impacts of food (Powell, 1995; Van Metter et al., 2008), manipulable (Powell, 1995), sensory (Martínez-Macipe et al., 2015; Regaiolli et al., 2019) and social stimulation (Leonard, 2008; Ncube and Ndagurwa, 2010) individually. This is the first control trial study to test the efficacy of a combined enrichment strategy on the welfare of Asiatic lions. This has allowed us to understand the temporal usage pattern of enrichment devices in Asiatic lions, which can prove useful for tailoring enrichment strategies. For example, individual test subjects showed high fidelity towards manipulable enrichment devices, which were also associated with the highest amount of conspecific-directed aggression. Therefore, to reduce stress and aggressive behaviours we recommend providing more manipulable devices than the number of animals in an enclosure. Upon release, subjects rushed to the feeding enrichment devices, therefore to prevent aggression and injury, it is important to spread such devices far apart, which will reduce chances of monopolization and food-based aggression. While sensory enrichment devices are designed to encourage exploratory behaviours in lions, the feed-based enrichment devices create a positive association for these novel objects and reduce neophobia. Finally, we found that manipulable enrichment devices allow subjects to interact and exert control over the captive environment while engaging in species-typical behaviours. Our combined enrichment strategy brought novelty in a captive environment and presented animals with the choice to express species-typical behaviour patterns. All enrichment devices used in this study can be locally sourced or fabricated with minimal effort and require less than thirty minutes to install or replenish with a team of three people.

Post-enrichment intervention, we recorded a significant improvement in the welfare indices of test subjects compared to the control subjects. Subjects that would normally stay near the retiring cells and remain inactive all day started exploring and utilizing different parts of the enclosure and followed by a significant increase in behaviour diversity levels. Faecal corticosterone levels of test subjects decreased significantly from the baseline, while it remained unchanged for control subjects, showing the inter-linkages between behavioural and physiological indices of welfare. Our findings agree with existing research that associate enrichment interventions with increased behavioural diversity (Rabin, 2003; Wemelsfelder et al., 2000), lowered SPI (Nogueira et al., 2004; Rose and Robert, 2013; Traylor-Holzer and Fritz, 1985), reduction of ARB (Clark and Melfi, 2012; Mallapur et al., 2007; Vaz et al., 2017), and reduction of stress levels (Hutchinson et al., 2012; Marcon et al., 2018; Mitra and Sapolsky, 2009; Nazar and Marin, 2011). However, it is important to point out that due to logistic constraints (sample size, time etc.) we did not address the variable responses of subjects with different personality types on enrichment interventions. Earlier work on captive Asiatic lions indicated that lions with different personality traits are likely to react differently to novel enrichment devices (Goswami et al., 2020). We suggest that future studies should focus on addressing impacts of such individual variations on differential responses and enrichment preferences with larger sample size. We studied the largest captive population of Asiatic lions, but the methodologies of this study can be scaled for smaller zoos housing fewer animals. Although we used at least three types of enrichment devices per intervention at multiple enclosure zones for experimental uniformity, at a smaller scale, zoo managers can try one enrichment device type per enclosure and rotate them with a new one every week. Our results conclusively show that enrichment interventions can lead to better behavioural and physiological welfare for Asiatic lions. We have delineated a practical but holistic approach to enrichment interventions that can be implemented and tested with little effort and manpower. With this study, we hope to empower and enable zoo managers and regulatory agencies to mandate the incorporation of enrichment interventions into daily husbandry regimen and proactively improve the welfare status of the animals under their care. We sincerely hope that the results of this study contribute to the improvement in animal welfare practices across ex-situ institutions at Indian zoos and conservation breeding programs.

5. Conclusion

The success of conservation breeding program depends on repatriating endangered animals to reclaimed habitats, hence housing the animals in appropriate welfare conditions while persevering behaviour diversity is of utmost importance. The Asiatic lion conservation breeding program has successfully maintained a genetically diverse stock of the species in captivity that has proliferated to an appreciable number. With the incorporation of welfare-centric management practices, the Asiatic lions housed in the program can be ideal representatives of the species and great candidates for future reintroduction programmes. Until that day of repatriation, we should strive to create a cognitively enriching environment that preserves the species-specific traits of the Asiatic lions. Like the endangered Asiatic lion, the Indian ex-situ institutions run conservation breeding programmes for several endemic species. So far, the welfare status of animals housed under conservation breeding programmes has received little research attention. We hope this study encourages zoo managers and regulators to incorporate enrichment interventions with animal management protocols.

Ethics statement

This study was conducted in accordance with CZA norms on the welfare of animals housed in conservation breeding centres. We did not conduct any invasive sampling procedures on the subjects for experimental setup and data collection. All necessary precautions were taken to ensure that enrichment interventions were integrated with the daily husbandry regimens of the test subjects.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.applanim.2021.105222.

References

Altmann, J., 1974. Observational study of behavior: sampling methods. Behaviour 49, 227–267.

Bagatharia, S.B., Joshi, M.N., Pandya, R.V., Pandit, A.S., Patel, R.P., Desai, S.M., Sharma, A., Panchal, O., Jasmani, F.P., Saxena, A.K., 2013. Complete mitogenome of Asiatic lion resolves phylogenetic status within Panthera. BMC Genomics 14, 572. https://doi.org/10.1186/1471-2164-14-572.

Ball, V., 1980. Observations on lion-breeding in the Gardens of the royal zoological society of Ireland. Trans. Royal Irish Acad. 28, 723–756.

Biswas, S., Bhatt, S., Paul, S., Modi, S., Ghosh, T., Habib, B., Nigam, P., Talukdar, G., Pandav, B., Mondol, S., 2019. A practive faeces collection protocol for multidisciplinary research in wildlife science. Curr. Sci. 116, 1878–1885. https://doi.org/10.18520/cs/v116/i11/1878-1885.

Broom, D.M., 2011. A history of animal welfare science. Acta Biotheor. 59, 121–137. https://doi.org/10.1007/s10441-011-9123-3.

Brown, C., Davidson, T., Laland, K., 2003. Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. J. Fish Biol. 63, 187–196.

Bulens, A., Van Beirendonck, S., Van Thielen, J., Driessen, B., 2013. The enriching effect of non-commercial items in stabled horses. Appl. Anim. Behav. Sci. 143, 46–51.

Cabana, F., Maguire, R., Hsu, C.-D., Plowman, A., 2018. Identification of possible nutritional and stress risk factors in the development of marmoset wasting syndrome. Zoo Biol. 37, 98–106. https://doi.org/10.1002/zoo.21398.
Carlstead, K., Seidensticker, J., Baldwin, R., Park, N.Z., 1991. Environmental enrichment for zoo bears. Zoo Biol. 16, 3–16.
Chauvet, J.-M., Brunel Deschamps, E., Hillaire, C., 1996. Dawn of Art: the Chauvet Cave: the Oldest Known Paintings in the World.
Clark, F.E., Melfi, V.A., 2012. Environmental enrichment for a mixed-species nocturnal mammal exhibit. Zoo Biol. 31, 397–413. https://doi.org/10.1002/zoo.20380.
Cloutier, T.L., Packard, J.M., 2014. Enrichment options for African painted dogs (*Lycaon pictus*). Zoo Biol. 33, 475–480.
Cohen, J., 1992. A power primer. Psychol. Bull. 112, 155.
Dawkins, M.S., 2004. Using behaviour to assess animal welfare. Anim. Welf. 13, 3–7.
DeGregorio, B.A., Sperry, J.H., Tuberville, T.D., Weatherhead, P.J., 2017. Translocating ratsnakes: does enrichment offset negative effects of time in captivity? Wildl. Res. 44, 438–448.
Fraser, D., 1999. Animal ethics and animal welfare science: bridging the two cultures. Appl. Anim. Behav. Sci. 65, 171–189.
Gogoi, K., Kumar, U., Banerjee, K., Jhala, Y.V., 2020. Spatially explicit density and its determinants for Asiatic lions in the Gir forests. PLoS One 15, e0228374.
Goswami, S., Tyagi, P.C., Malik, P.K., Pandit, S.J., Kadivar, R.F., Fitzpatrick, M., Mondol, S., 2020. Effects of personality and rearing-history on the welfare of captive Asiatic lions (*Panthera leo persica*). PeerJ 8, e8425. https://doi.org/10.7717/peerj.8425.
Gujarat Forest Department, Sr., 2015. 14th Lion Population Estimation Report – 2015. Gujarat Forest Department, Sasan Gir, Junagadh, India.
Harel, D., 2020. ez: Companion to "Statistics Using R: An Integrative Approach.
Hutchinson, K.M., McLaughlin, K.J., Wright, R.L., Ortiz, J.B., Anouti, D.P., Mika, A., Diamond, D.M., Conrad, C.D., 2012. Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity. Neurobiol. Learn. Mem. 97, 250–260.
Jhala, Y.V., Banerjee, K., Chakrabarti, S., Basu, P., Singh, K., Dave, C., Gogoi, K., 2019. Asiatic lion: ecology, economics and politics of conservation. Front. Ecol. Evol. 7, 312.
Joslin, P., 1984. The environmental limitations and future of the Asiatic lion. J. Bombay Nat. History Soc. Bombay 81, 648–664.
Kassambara, A., 2020. ggpubr: "ggplot2" Based Publication Ready Plots.
Leonard, D.A., 2008. An Evaluation of Environmental Enrichment for Two Highly Social and Endangered Canid Species, the African Wild Dog (*Lycaon pictus*) and Mexican Wolf (*Canis lupus baileyi*). Saint Louis University.
MacKenzie, J.M., 2017. The Empire of Nature: Hunting, Conservation and British Imperialism.
Mallapur, A., Qureshi, Q., Chellam, R., 2002. Enclosure design and space utilization by Indian leopards (*Panthera pardus*) in four zoos in Southern India. Anim. Welf. 5, 111–124.
Mallapur, A., Waran, N., Sinha, A., 2007. A note on enrichment for captive lion-tailed macaques (*Macaca silenus*). Appl. Anim. Behav. Sci. 108, 191–195. https://doi.org/10.1016/j.applanim.2006.11.020.
Marcon, M., Mocelin, R., Benvenutti, R., Costa, T., Herrmann, A.P., de Oliveira, D.L., Koakoski, G., Barcellos, L.J., Piato, A., 2018. Environmental enrichment modulates the response to chronic stress in zebrafish. J. Exp. Biol. 221.
Martinez-Macipe, M., Lafont-Lecuelle, C., Manteca, X., Pageat, P., Cozzi, A., 2015. Evaluation of an innovative approach for sensory enrichment in zoos: semiochemical stimulation for captive lions (*Panthera leo*). Anim. Welf. 24, 455–461.
Mason, G.J., Latham, N.R., 2004. Can't stop, won't stop: is stereotypy a reliable animal welfare indicator? Anim. Welf. 13, 57–69. https://doi.org/10.1017/1962728600014275.
McCall, D.F., 1973. The prevalence of lions: kings, deities and feline symbolism in Africa and elsewhere. Paideuma 130–145.
McGowan, R.T.S., Robbins, C.T., Alldredge, J.R., Newberry, R.C., 2010. Contrafreeloading in grizzly bears: implications for captive foraging enrichment. Zoo Biol. 29, 484–502.
Mellen, J.D., Shepherdson, D.J., 1997. Environmental enrichment for felids: an integrated approach. Int. Zoo Yearb. 35, 191–197.
Michaels, C.J., Downie, J.R., Campbell-Palmer, R., 2014. The importance of enrichment for advancing amphibian welfare and conservation goals. Amphib. Reptile Conserv. 8, 7–23.
Miller, L.J., Vicino, G.A., Sheftel, J., Lauderdale, L.K., 2020. Behavioral diversity as a potential Indicator of positive animal welfare. Animals 10, 1211. https://doi.org/10.3390/ani10071211.
Mitra, R., Sapolsky, R.M., 2009. Effects of enrichment predominate over those of chronic stress on fear-related behavior in male rats. Stress 12, 305–312.
MoEF, India, 1998. National Zoo Policy.
Mondol, S., Booth, R.K., Wasser, S.K., 2020. Fecal stress, nutrition and reproductive hormones for monitoring environmental impacts on tigers (*Panthera tigris*). Conserv. Physiol. 8 coz091.
Nazar, F.N., Marin, R.H., 2011. Chronic stress and environmental enrichment as opposite factors affecting the immune response in Japanese quail (*Coturnix coturnix japonica*). Stress 14, 166–172.
Ncube, S., Ndagurwa, H.G.T., 2010. Influence of social upbringing on the activity pattern of captive lion (*Panthera leo*) cubs: benefits of behavior enrichment. Curr. Zool. 56, 389–394.
Nogueira, S.S., Bernardi, L.G., Nogueira-Filho, S.L., 2004. A note on comparative enclosure facility usage by wild and captive-born capybaras (*Hydrochoerus hydrochaeris*). Appl. Anim. Behav. Sci. 89, 139–143.
Pastorino, G.Q., Viau, A., Curone, G., Pearce-Kelly, P., Faustini, M., Vigo, D., Mazzola, S.M., Preziosi, R., 2017. Role of personality in behavioral responses to new environments in captive Asiatic lions (*Panthera leo persica*). Vet. Med. Int. 2017.
Plowman, A., 2003. A Note on a Modification of the Spread of Participation Index Allowing for Unequal Zones. https://doi.org/10.1016/S0168-1591(03)00142-4.
Powell, D., 1995. Preliminary evaluation of environmental enrichment techniques for African lions (*Panthera leo*). Anim. Welf. 4, 361–370.
R Core Team, 2020. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rabin, L.A., 2003. Maintaining behavioural diversity in captivity for conservation: natural behaviour management. Anim. Welf. 12, 85–94.
Reading, R.P., Miller, B., Shepherdson, D., 2013. The value of enrichment to reintroduction success. Zoo Biol. 32, 332–341. https://doi.org/10.1002/zoo.21054.
Regaiolli, B., Rizzo, A., Ottolini, G., Miletto Petrazzini, M.E., Spiezio, C., Agrillo, C., 2019. Motion illusions as environmental enrichment for zoo animals: a preliminary investigation on lions (Panthera leo). Front. Psychol. 10, 2220.
Resende, L.S., Remy, G.L., Ramos Jr., V de A., Andriolo, A., 2009. The influence of feeding enrichment on the behavior of small felids (Carnivora: Felidae) in captivity. Zoologia (Curitiba) 26, 601–605.
Revelle, W., 2019. psych: Procedures for Psychological, Psychometric, and Personality Research. Evanston, Illinois.
Rose, P., Robert, R., 2013. Evaluating the activity patterns and enclosure usage of a little-studied zoo species, the sitatunga (*Tragelaphus spekii*). J. Zoo Aquar. Res. 1, 14–19.
Ross, S.R., Shender, M.A., 2016. Daily travel distances of zoo-housed chimpanzees and gorillas: implications for welfare assessments and space requirements. Primates 57, 395–401. https://doi.org/10.1007/s10329-016-0530-x.
RStudio, 2020. RStudio: An Integrated Development Environment for R.
Ruskell, A.D., Meiers, S.T., Jenkins, S.E., Santymire, R.M., 2015. Effect of bungee-carcass enrichment on behavior and fecal glucocorticoid metabolites in two species of zoo-housed felids. Zoo Biol. 34, 170–177. https://doi.org/10.1002/zoo.21192.
Sanyal, R.B., 1892. A Handbook of the Management of Animals in Captivity in Lower Bengal. Printed at the Bengal Secretariat Press.
Schilikrant, R., 2017. Characterisation of Positive Welfare Indicators in Captive African Lions (*Panthera leo*) (Master's Thesis). University of Sydney.
Skibiel, A.L., Trevino, H.S., Naugher, K., 2007. Comparison of several types of enrichment for captive felids. Zoo Biology: Published affiliation Am. Zoo Aquarium Assoc. 26, 371–381.
Smith, G.L., 1984. International Studbook: Indian Lion. *Panthera leo persica* (Meyer, 1826). Knoxville Zoological Park.
Srivastav, A., Nigam, P., Begum, N., 2018. Indian National Studbook of Asiatic Lion (*Panthera leo persica*).
Storey, W.K., 1991. Big cats and imperialism: lion and tiger hunting in Kenya and Northern India, 1898-1930. J. World Hist. 2, 135–173.
Suárez, P., Recuerda, P., Arias-de-Reyna, L., 2017. Behaviour and welfare: the visitor effect in captive felids. Anim. Welf. 26, 25–34.
Swaisgood, R.R., 2010. The conservation-welfare nexus in reintroduction programmes: a role for sensory ecology. Anim. Welf. 19, 125–137.
Traylor-Holzer, K., Fritz, P., 1985. Utilization of space by adult and juvenile groups of captive chimpanzees (*Pan troglodytes*). Zoo Biol. 4, 115–127. https://doi.org/10.1002/zoo.1430040205.
Van Metter, J.E., Harriger, M.D., Bolen, R.H., 2008. Environmental enrichment utilizing stimulus objects for African lions (*Panthera leo leo*) and Sumatran tigers (*Panthera tigris sumatrae*). Bios 79, 7–16.
Vargas, A., Anderson, S.H., 1999. Effects of experience and cage enrichment on predatory skills of black-footed ferrets (*Mustela nigripes*). J. Mammal. 80, 263–269.
Vaz, J., Narayan, E.J., Dileep Kumar, R., Thenmozhi, K., Thiyagesan, K., Baskaran, N., 2017. Prevalence and determinants of stereotypic behaviours and physiological stress among tigers and leopards in Indian zoos. PLoS One 12, e0174711.
Wasser, S.K., Azkarate, J.C., Booth, R.K., Hayward, L., Hunt, K., Ayres, K., Vynne, C., Gobush, K., Canales-Espinosa, D., Rodríguez-Luna, E., 2010. Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. Gen. Comp. Endocrinol. 168, 1–7.
Wemelsfelder, F., Haskell, M., Mendl, M.T., Calvert, S., Lawrence, A.B., 2000. Diversity of behaviour during Novel Object Tests Is Reduced in Pigs Housed in Substrate-impoverished Conditions.
Wickham, H., 2011. ggplot2. Wiley Interdisciplinary Reviews: Computational Statistics 3, pp. 180–185.
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., 2019. Welcome to the tidyverse. J. Open Source Softw. 4, 1686.
Wickham, H., Henry, L., Müller, K., 2020. dplyr: A Grammar of Data Manipulation.
Young, K.M., Walker, S.L., Lanthier, C., Waddell, W.T., Monfort, S.L., Brown, J.L., 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoid analyses. Gen. Comp. Endocrinol. 137, 148–165.