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A case study: resocialization of two sibling Striped hyenas (*Hyaena hyaena*) in a wildlife park in northeastern Italy

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To Tommaso,
wishing him to find his own way and,
once grown up,
to realize the dreams he made as a child.

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ABSTRACT

The concept of zoo is changed over the years and, nowadays, the role of the modern zoos can be summarized in four keywords: conservation, education, research, and recreation. The interest in the topic of animal welfare has grown a lot, and researchers are looking for ways to meet the five freedoms in this context and how to assess it. In this regard, behaviour, physiology and emotional state are considered as main indicators.

The case study described in this work stems from the requirement, within a zoological garden, to resocialize two sibling Striped hyenas, born and raised in captivity, that have expressed an antagonist behaviour towards each other since the age of three years. This forced the staff of the zoo to manage the hyenas allowing the access to the outdoor enclosure to a single animal every other day and this off-exhibit management might have compromised their welfare status over the years. It was assumed that, as adverse behaviours emerged upon reaching sexual maturity, this could be among the potential causes. Thus, prior to resocialization, the first step was to try to suppress the sexual grounds of the hyenas through the insertion of a hormonal implant (Deslorelin). Direct behavioural observations were carried out before the implant, although originally planned also during and after the resocialization attempts. They were carried out using continuous behavioural recordings applied by one assessor during five 30-minutes intervals in the morning and five 30-minutes intervals in the evening. Four morning and four evening intervals were used to record individual behaviours of each hyena in each indoor and outdoor location. Social behaviours were assessed during the first and last 30-minutes intervals before the exit and after the entering when both hyenas were indoors. Biological samples (stool and blood samples) were collected throughout the study and analyzed in order to examine Cortisol (HC), Dehydroepiandrosterone (DHEA), Estradiol (E2) and Testosterone (TST) concentrations. Moreover, video recordings were used to assess the spatial use of the exhibits by both hyenas and to evaluate their emotional states in the different locations using the Qualitative Behavioural Assessment (QBA) ex-situ. Striped hyena's biology and its management in captivity was deepen using information available in literature and experts' advice from different zoos around the world.

Results of the quantitative behavioural observations of both individual and social behaviours confirmed the state of poor welfare when hyenas were indoors and QBA turned out to be a valid method to interpret the difference in terms of response of the two hyenas to the environment. Although the implant had worked (due to the hormonal values evidenced by the analysis of fecal samples collected in the pre- and post-implantation), the socialization did not occur. The possible reasons behind the fail in the resocialization attempts came from the gathered knowledge on the species biology and the similar difficulties shared by other zoos pointing out how the application of the five freedoms in the zoo context still finds many controversial issues. Indeed, main difficulties in zoos are meeting the different needs (visitors, owners, keepers, animals, authorities, etc.) considering the vastness of species usually hosted and lack of access to detailed information about their biology and needs. Considering other situations inside zoological parks hosting Striped hyenas all over the world, it seems that the in-group management of this species is rather complicated, given their distinctly solitary nature. Thus, the best solution suggested is a permanent separation of the hyenas. This could be done through the relocation of one of the animals in a new exhibit, previously adapted to the needs of this species, in order to allow a daily access to an outdoor enclosure and improve their welfare status.

RIASSUNTO

Nel corso degli anni, il concetto di zoo è molto cambiato ed oggi il suo ruolo può essere riassunto in: conservazione, educazione, ricerca e svago. Sebbene il tema del benessere animale sia cresciuto di importanza, la sua applicazione nel contesto dello zoo trova ancora molte difficoltà, tra cui capire come rispettare le cinque libertà e definire metodi validi per la sua misurazione. A questo proposito, gli indicatori principali risultano essere comportamento, parametri fisiologici e stato emotivo. Il caso studio descritto in questo lavoro ha l'obbiettivo risocializzare due esemplari di Iene striate (Hyaena hyaena), nate e cresciute in cattività presenti in un parco faunistico del nord Italia. Le iene sono fratello e sorella e, dall'età di tre anni, hanno iniziato a manifestare un comportamento aggressivo tra loro; ciò ha costretto lo staff del parco a gestire i due animali tenendoli separati all'interno dello stesso recinto, consentendo l'accesso all'esterno ad un animale alla volta, solo a giorni alterni. Con il passare degli anni, questa situazione ha compromesso ulteriormente il loro stato di benessere. Essendo i comportamenti avversi emersi al raggiungimento della maturità sessuale, si ipotizzava che questa potesse essere una delle cause scatenanti. Il primo passo è stato, quindi, indurre una sterilizzazione reversibile, attraverso l'utilizzo di un impianto ormonale a base di Deslorelin, al fine di sopprimere la componente sessuale prima di tentare la risocializzazione o trovare soluzioni alternative. Le osservazioni comportamentali dirette sono state fatte nella settimana precedente l'inserimento dell'impianto, tuttavia al momento della pianificazione dello studio erano previste delle osservazioni anche durante e dopo i tentativi di risocializzazione. L'osservatore ha eseguito delle misurazioni comportamentali continue, divise in cinque intervalli da 30 minuti ciascuno, ripetuti al mattino e alla sera. Sono stati osservati i comportamenti individuali delle due iene, sia nel recinto interno che in quello esterno. Invece, nei primi e negli ultimi 30 minuti di ogni giornata (prima dell'uscita mattutina e dopo il rientro serale) le iene sono state osservate insieme (ciascuna nel proprio box), per individuare eventuali comportamenti sociali. Durante lo studio sono stati raccolti ed analizzati campioni di feci e di sangue per determinare le concentrazioni di Cortisolo (HC), Deidroepiandrosterone (DHEA), Estradiolo (E2) e Testosterone (TST). Inoltre, sono state utilizzate delle telecamere per riprendere le iene e valutare l'utilizzo dello spazio all'interno

dei recinti (dentro e fuori). In un secondo momento, gli stessi video (frammenti di circa un minuto) sono stati utilizzati per l'analisi qualitativa del comportamento applicando il metodo QBA per stabilire come cambiava lo stato emotivo degli animali per l'effetto della tesi (iena × ambiente interno o esterno). Approfondimenti sulla biologia e sulla gestione in cattività della Iena striata sono stati ottenuti tramite ricerca bibliografica e la richiesta di consigli di gestori di 50 zoo a livello mondiale e di esperti. I risultati dell'analisi quantitativa del comportamento sia individuale che sociale hanno confermato che gli animali manifestavano uno stato di scarso benessere quando si trovavano nel recinto interno. Inoltre, il metodo QBA si è rivelato efficace nell'interpretazione delle risposte delle iene in relazione all'ambiente, evidenziando differenze tra le due iene. Dati i valori degli ormoni estratti dai campioni biologici nel pre- e post- impianto, è stato assunto che il contraccettivo stesse avendo un effetto. Tuttavia, ciò non ha avuto ripercussioni sul comportamento dei due animali, che infatti hanno mantenuto un atteggiamento ostile e la risocializzazione non è potuta avvenire. Confrontando le informazioni presenti in letteratura e altre situazioni all'interno di parchi zoologici che ospitano Iene striate in tutto il mondo, pare che la gestione in gruppo di questa specie sia alquanto complicata, vista la loro indole spiccatamente solitaria. Questa potrebbe essere una possibile spiegazione al fallimento del tentativo di risocializzazione. Tuttavia, per il personale dei parchi, dovendo far fronte a diverse esigenze e data la vastità del numero di specie solitamente ospitate, risulta difficile avere delle nozioni dettagliate sulla biologia di ciascuna. Quindi può accadere che il management non sia sempre ottimale e che non vengano correttamente applicate le cinque libertà. La gestione non adeguata degli animali può portare allo sviluppo di comportamenti anomali, come risposta a situazioni stressanti o fabbisogni non rispettati. Assume quindi particolare rilevanza la necessità di avere un personale correttamente formato e di studi preliminari sulla biologia delle specie presenti. Al fine di migliorare questa condizione, la soluzione suggerita è stata quella di riadattare un recinto già presente nel parco e trasferire lì uno dei due animali, separandoli definitivamente ma migliorando il loro stato di benessere consentendo ad entrambi l'accesso giornaliero all' esterno.

1. INTRODUCTION

1.1. Brief history of zoos

Collection of animal has a long history, dating back at least 4.000 years.
Before about 1800, collections of exotic animals were usually referred to as “menageries” and the zoo in the form which we are now familiar with is a relatively recent phenomenon. There is evidence of ancient menageries in Egypt, Mesopotamia, China and Greece (2000-2500 BC), where animals were kept in captivity for education and contemplation, while the Romans kept wild animals for fighting against gladiators in the arenas, just for public entertainment. That was the reason why many animals became rare or even locally extinct, in Roman times. It is reported that also the Aztecs and Incas (South America) used to keep exotic animals.
Very few records of zoos are reported during the Dark Ages and Medieval times. Some examples are collections in Bagdad, Cairo, Istanbul, China, and Western Europe.
In the thirteen centuries, the Holy Roman Emperor Frederick II established a major new zoo in Europe. This was in Palermo, Italy, but then he set up permanent zoos in other Italian cities and contributed to the development of zoos elsewhere in Europe, due to his habit of sending wild animals as gifts to other European heads of State (notably, to his brother in law, England’s Henry III).
The Tower of London and Versailles are two examples of European zoos in the seventeen centuries: a private royal collection, conceived to king’s and his court entertainment, rarely open to the public. They were emblems of state, and the animal houses were often elaborate and lavish.
From the sixteen to the eighteen centuries there are numerous records of wild animals being transported in wagons to the towns and villages of Europe, and that permitted to see these kinds of animals also by ordinary people.
Gradually, through the eighteen and nineteen centuries, thanks to a wider public interest in natural history and a greater inclination towards exploration and travels, zoo’s audience changed, and they became public places, rather than private and exclusive menageries.

That led to the birth of the “modern” zoological garden, with Jardin des Plantes (Paris), Tiergarten Schonbrumm (Vienna) and Regnent’s Park Zoo (London) as World’s leaders.

By comparison with Europe, the history of North America zoos only stretches back to a little over a century ago. Before that, there were circuses, traveling menageries and some private collections, but none of them was associated with royalty. Twentieth-century zoos in the USA were usually civic institutions and did not develop links to museums or universities, unlike the major “new” zoos in Europe did.

Naturalistic zoo design was not prompted by a scientist or architect, but by a German animal dealer and trainer, Carl Hagenbeck, whose conception of the zoo was without bars and based on images of natural habitats (“panoramas”). He developed Hamburg Tierpark (Germany), which was the first permanent zoo with concrete and cement rocks and gorges, based on real geological formations. They reproduced landscapes to house animals, such as African or Polar Panorama.

The 1920s and 1930s saw the start, in some zoos, of a depressing move away from Haganbeck-inspired naturalistic exhibit, towards the so-called “Disinfectant Era”. It consisted of the introduction of easy to clean cages with tiled walls, smooth concrete floors, plate glass viewing windows and steel doors. These exhibits had detrimental effects on animal welfare by promoting high levels of stress and fear, stereotypic behaviors, boredom and depression in species-specific ways.

Organized opposition from a new generation of animal rights and animal welfare organizations started to rise. Many zoos, in response to the increasing criticism and declining visitor numbers, reinvented themselves as conservation organizations.

The result was that in the second part of the twentieth century, zoo exhibits became more naturalistic, with a generation of bio-parks, ecosystem exhibits, wildlife parks and safari parks that do not focus only on a single species, but rather on animals in their natural environments (Hosey et al., 2013).

At the start of the twenty-first century, zoos are meant to entertain and educate the public but have a strong emphasis on scientific research and species conservation. There is a trend toward giving animals more space and recreating natural habitats, in order to respect their behavioural necessities and with a special attention to animal welfare.

Zoos are usually regulated and inspected by the government, by a wide and complex range of legislation, and non-legislative agreements and guidelines, operating at international,

national and sub-national levels. However, not all countries have specific zoo legislation. Most zoos do not operate in isolation, but belong to regional, national and international associations, which aim is to promote best practices and to coordinate their activities.

1.2. The role of modern zoos

Modern zoos are no longer only places that keep a few animals for people to go and look at. They are scientifically run and governmentally regulated institutions, which have a significant role to play. Many zoos describe their role in terms of four keywords: conservation, education, research, and recreation. Moreover, the knowledge about the needs of animals is increased and there is an improvement in the ways of maintaining them in captivity.

Conservation is concerned with trying to maintain biodiversity, and it is needed in response to the human impact on the environment. Habitat destruction and hunting have already resulted in the extinction of many species, and many more will follow in the next couple of decades. Not only terrestrial habitats are under treats, but also lakes, rivers, and marine habitats are in danger due to pollution and drainage schemes. The introduction of invasive non-native or exotic species and global warming are another major contributor to the extinction of many species (Hosey et al., 2013).

So that, information, education and research programs become essential, and zoos are effective means to spread those messages to the public. There are associations that inspire and engage the many visitors to take action for the conservation of species and nature: for example, the World Association of Zoos and Aquariums (WAZA), is dedicated to the care and conservation of animals and their habitats around the world. It promotes cooperation between leading zoos, aquariums, national and regional associations, as well as with leading wildlife experts, academics, and universities. Its members tackle global issues such as the illegal wildlife trade, coral-reef restoration, marine litter, sustainable palm oil, and climate change. The Italian Union of Zoos and Aquarium (UIZA) strongly supports the education and scientific research programs within Italy and Europe. It claims that the zoo is the place where people can understand the naturalistic culture and be sensitized to environmental problems and nature conservation. Moreover, thanks to the attraction created by animals, they can be a vehicle to talk about scientific, biological and socio-economic issues related to nature.

To attract more visitors, zoos house not only endangered species, but also species that are attractive for people, but not at risk of extinction, called “empathic species”. Also, new births bring zoos to the attention of a wide public, highlighting the good results of the reproduction programs (La Cauza, 2014).

Of course, with the economic contribution of the visitors, it is also possible to support the park and the research projects. In Italy visitor contribution is the unique form of sustainment. For many years, research conducted on zoo animals tended to be concerned primarily with anatomy and taxonomy but there is a huge potential in zoos to undertake behavioural, genetic and physiological research that contributes to the *ex situ* and *in situ* conservation (Hosey et al., 2013). The first concerns the preservation of animal biodiversity within structures such as zoos, especially for highly endangered species, which natural habitat is extremely threatened, and their reintroduction back into the wild when possible; the second occurs directly in the natural environment of the species, through the creation of animal sanctuaries, not accessible to the public, where the animals (that are already present or reintroduced from zoological gardens) are protected (La Cauza, 2014).

Nowadays, the animals present in zoological gardens are mostly born and raised in captivity. Illegal trade and the capture of wild animals is prohibited and strongly opposed. Animals may be move from a zoo to another, but they are not under trading relationships; rather these exchanges are based on reproductive or space needs (for example, births that are surplus or to make breeding pairs). The reproduction of the captive animals is controlled, with special attention to avoid inbreeding. Zoos can also house animals from seizure operations. Of course, all this is strictly regulated, but the legislative framework varies considerably from country to country and from region to region.

1.3. Welfare of zoo animals

Animal welfare science is the study of an animal’s quality of life and it takes on many different meanings, depending on the context. In this case, the definition considered is the state of an animal as regards its attempts to cope with its environment, and it ranges from very poor to very good (Broom, 1986).

Coping consists of the ability of an organism to tolerate and respond to a range of stimulations, as also its ability to make adaptations, which depend on genetic and environmental influences.

Animal welfare is peculiar to an individual and goes beyond species-specific characteristics mostly related to dietary needs, hearing sensitivity, and thermoregulatory needs. This means that animals are very different from each other because they experience the world differently and have different needs, even when they are exposed to the same conditions. They endow with personality, which is defined as a consistent reaction to different environmental variables, resulting from a synthesis of the animal's genetic make-up and its past experiences (Hosey et al., 2013). This is evident in the case of zoo animals. These animals come from very heterogeneous backgrounds, which may vary greatly in their previous life experiences, and this can influence their ability to cope with certain challenges (Hill and Broom, 2009).

Much of the work in the field of animal welfare has been conducted on farm animals, because of the financial interest in commercial farming, the large number of animals involved in food production and the relatively small number of species. In contrast, zoos house a larger number of animal species, with smaller numbers of individuals. Differences in zoo animals and the controversial needs to which zoos respond lead to limited numbers of rigorous scientific studies on the effects of many husbandry practices on animal welfare and lack of validated welfare assessment methods.

In modern wild animal keeping, individual welfare should be previously ensured (the ideal should be to work toward good welfare in a proactive way, and not wait until problems emerge) and a useful way to do research in this field is to focus on these fundamental considerations:

- it is necessary to know the needs, for both behaviour and resources, of zoo animal species, and find appropriate conditions to help them in meeting these needs;
- try to assess how animals respond to our efforts to improve welfare, and what opportunities they have in different settings, or with different sex, age, and so on;

- evaluate how individual animals differ from one another in strategies to meet their needs, and in their responses to environmental stimuli (including their responses to efforts to improve their welfare), by giving animals choices in their environment;
- give to zoo opportunities that promote the conservation and the education of the public, through a better representation of wild animal species and their habitats (Hill and Broom, 2009).

Studies should not consider only how to achieve good welfare, but also how welfare could be improved. One way of achieving this is through appropriate exhibit design and animal management. Every challenge that disrupts the individual homeostasis and reduces its fitness is defined as "stress", although there are stimulations that may bring benefits. That is why a scientific approach to these issues is important, as it is effective collaboration and communication between zoos, universities and field sites (Hill and Broom, 2009).

Nowadays, zoos shall endeavor to comply with high standards of animal welfare and husbandry (they have to exceed the minimum legal requirements), and a genuine and strong commitment to wildlife conservation besides complying with local, national and international legislation.

1.3.1. The five freedoms in the zoo context

The assessment of animal welfare was based on checking whether the five freedoms are respected. Although revised and updated several times (Mellor, 2016), the concepts behind the originally stated five freedoms in the Brambell Report (1965) in relation to farm animals' welfare are still valid and commonly accepted (Table 1-1). From them derived the five principles of animal care and management, which are the foundations of the standards set also for modern zoo practice. In 2012, the Farm Animal Council (FAWC) stated that these freedoms are ideal states rather than standards for acceptable welfare, and they are seen as a logical and comprehensive guide for effective animal well-being assessment and management. The concept of the five freedoms was then expanded towards that of the Five Domains, in which physio-functional states, mental states and behavioural states are differentiated underling the single freedom of living a life worth living (Mellor, 2016).

Freedoms	Provisions
1. Freedom from thirst, hunger and malnutrition	By providing ready access to fresh water and a diet to maintain full health and vigour. Food and water must be present and available according to the physiological and ethological needs of the animal and respecting hygiene standards.
2. Freedom from discomfort and exposure	By providing an appropriate environment including shelter and a comfortable resting area. it is related to the appropriateness for the captive species being kept but minimizing the possibility to escape. It also includes health, safety and hygiene considerations.
3. Freedom from pain, injury, and disease	By disease prevention or rapid diagnosis and prompt treatment. It reaffirms the need to minimize other kinds of health hazard and keep comprehensive records of the animals and their health.
4. Freedom from fear and distress	By ensuring conditions and treatment which avoid mental suffering. It includes statements about the provision of appropriate physical and social environments; it involves also a reference to zoo visitors and the provision of facilities for animals to avoid human contact if they so choose.
5. Freedom to express normal behaviour	By providing sufficient space, proper facilities and company of other subjects of the same species (captive breeding and avoidance of producing between-taxa hybrids assume importance). Physical and social environments have to promote behaviours similar to those seen in the wild.

Table 1-1: The five freedoms and five provisions for promoting animal welfare.

Along with the implementation of the five freedoms, zoos need to meet also other standards related to aspects of zoo management that not concerned primarily with animal care, including transportation and movement of live animals, conservation and education measures, public safety in the zoo, animals' identification and records keeping, public facilities, staff and training.

1.4. Assessing animal welfare

Scientific methods for assessing animal welfare are complex and multi-disciplinary.
To collect objective data, researchers are developing standardized repeatable methodologies and indicators. However, it is hard to standardize and validate animal welfare indicators making them feasible, and reliable. Welfare indicators can be divided into resource-based and animal-based measures. The resource-based are variables that are not measured in the animals but in their environment. Examples of resource-based indicators are the size and design of the enclosures where animals are kept, ambient temperatures and humidity, water provision or environmental enrichment. Animal-based indicators include all those variables that are assessed by direct observations of animals or measured directly in animals, such as changes in behaviour, appearance, health and physiological parameters.
However, choosing which measures can be used to evaluate animal welfare is not easy, and each measure should be first validated in order to evaluate specifically what they are aimed at (e.g. an animal-based measure of fearfulness evaluates the state of fear and not of response to stimuli). Currently there are several biomarkers and animal-based measures being developed and applied from farm to other categories of animals based on data collected in different contexts and during positive physiological and social situations in particular (Hosey et al., 2013). A lot of attention is also give to indicators of positive welfare (e.g. play behaviour) and to positive emotional state.

1.4.1. Behaviour

Animal behaviour consists of a series of behavioural patterns, which are considered as "normal"; any deviations from this are defined as "abnormal" behaviour, and it is correlated to poor welfare. However, it is difficult to define the value of the deviation to be considered abnormal. Normal behaviour should occur in healthy animals whose behavioural repertoires have developed under conditions that offer uninhibited opportunities and are appropriate to the animals' needs for behaviour and resources. This leads to some considerations. First of all, a deep knowledge of animal behaviour is necessary to discriminate between what is normal and what is abnormal. The behavioural repertoire of a single species is huge, and such knowledge is currently lacking for many species, especially in the zoo contest. When information is not available for a given species, predictions about their needs can be based to a certain extent on the knowledge of those of similar species, or of species occupying similar ecological niches, about which more may be known (Hill and Broom, 2009). Another consideration is that universal welfare indicators do not exist, because animals have a variety of ways of responding to those conditions, and it's all about the individual. Moreover, behavioural indicators of welfare need to be interpreted carefully: not only can animals hide signs of poor welfare in their behaviour, but also, they vary in their behavioural responses to stimuli. It is therefore important to associate different indicators together (including behavioural patterns, frequencies, physiological measures, and contexts) when we evaluate the meaning of a specific behaviour.

The zoo context implies that it is important to study not only a species behavioural repertoire in the wild but also that in captivity: patterns should be drawn up for individuals living in good wild and captive conditions, as a guide for comparison. Keepers have here an essential role because they are in close contact with the animals and can evaluate or just report their attempt to adapt to certain aspects of life in captivity.

Another method to monitor animals' behaviour could be the use of technology. A recent study tested an automatic behaviour recognition system to assess the quality of life of dogs housed in kennels (Barnard et al., 2016). They used a software able to automatically cluster frequently observed temporal and spatial patterns of movement without any pre-set ethogram. This 3D framework was designed to be invariant to the dog's shape and size and could be extended to zoo quadrupeds in artificial housing. The algorithm ensured high accuracy and standardization; minimal effort is required in long term recordings and the automated video was highly correlated to human observations. In the future, it may be a good help in recognizing not only ethograms but also stereotypes.

The difficulty in keeping captive animals is to understand how their behaviour varies from that in the wild, and then meet their needs, while fulfill needs may lead to the development of abnormal behaviour, such as stereotypic or injurious behaviour, which indicate poor welfare. A way to prevent this is represented by enrichments, which are changes that stimulate a wide variety of behaviours in case of environmental deprivations, in order to improve the animal’s physical fitness and mental well-being (Hosey et al., 2013). However, enrichment efforts are not always successful (sometimes they are even pejorative); thus, it is important to assess carefully their efficacy.

Usually, the captive environment may not look naturalistic when compared with the wild, but the functionality of the wild environment and the opportunities for a normal range of behaviour can be mimicked to a degree in captivity (Hill and Broom, 2009). Just as the behavioural repertoires of animals can vary between different communities or populations in the wild, so too can captive animals exhibit novel behaviour patterns that are not usually seen among their wild conspecifics, but this does not necessarily mean that the behaviour is ‘‘abnormal”. Novel behaviour patterns that develop in zoos are adaptations to the captive environment and might be beneficial to the animals. An example is playing behaviour in adult animals that can occur at higher rates in captivity compared with the wild, because the importance of other behaviours, such as antipredator tactics, may be reduced in zoo environment (Hill and Broom, 2009). Another example regarding to the need to have more available space to walk inside the exhibit, is a study on Zoo Elephants (Holdgate et al., 2016), which demonstrate that, while in the wild walking is an effective means for meeting social or nutritional needs, in zoo settings, social and nutritional needs are addressed through management practices and therefore, the functional need for walking is reduced and no associations were found between walking and welfare indicators.

1.4.2. Physiology

Physiological indicators are correlated with the emotional state of the animal, and therefore, with its welfare state. Examples of physiological indicators are changes in the heart and respiratory rates, body temperature, adrenal response, neurotransmitters and carcass characteristics. The monitoring of these parameters requires invasive methods, such as blood samples. In the zoo context, it is very difficult to collect this kind of data because usually anesthesia is needed, and measurements are not very accurate. Exceptions could occur when the animals are trained for medical purpose (that is accustomed to being handled) or by using a wide range of newly developed technology.

A recent study (Whitham and Miller, 2016) discussed the use of technology for automatic recording of behaviour and tracking of physiological indicators of welfare. Because zoos care for exotic, endangered animals, they considered approaches that involved minimal restraint and handling rather than those that require surgical procedures or other invasive treatments. They focused primarily on tools that can be applied in naturalistic settings (such as accelerometers, radio frequency identification systems, bioacoustics system) and discussed the use of non-invasive methods for investigating physiological welfare indicators (thermography, tracking measures of heart rate). Other difficulties within the zoo are related to the wildlife park's needs, to the availability of keepers in collaborating with the researcher and to the attitude of the researcher itself (Hill and Broom, 2009).

A commonly-used physiological measure of welfare in zoo animals and one that can be obtained non-invasively is concentration of adrenal hormones: measuring activity in the sympathetic-adrenal medullary system and the hypothalamic-pituitary-adrenocortical (HPA) system. Measurement of fecal glucocorticoids, or their metabolites, can be useful to studies of welfare in the zoo environment, especially in assessing short-term responses to stressors, for a variety of species, although it must be remembered that, while the HPA axis is one of the main mediators of the endocrine response to stress, it also responds to other types of stimulation that are beneficial and may require activity (Hill and Broom, 2009).

1.4.3. Emotional state

An animal's body language can reveal important aspects of its physical and mental health, and therefore welfare. Welfare in this context encompasses more than just the absence of suffering: it concerns the quality of an animal's entire relationship with its environment and how it lives its life. The question is whether the qualitative perception of animals as sentient beings provides an authentic, legitimate perspective that could potentially receive scientific support, or whether it reflects an anthropomorphic projection of human values. This approach can be useful for primates and socially sophisticated mammals (such as wolves, dogs, and elephants), but would increase our emotional and moral sensitivity to the plight of captive animals. To provide scientific support to perceptions of animal body language it is particularly important to adopt a "whole animal" perspective and judge the details of posture and behaviour taking into consideration the entire animal's interaction with its surroundings. Wemelsfelder (2007) proposed a qualitative behavioural assessment method. Qualitative Behavioural Assessment (QBA) is an integrated measure that quantifies the aspects of an animal's body language and therefore compares them objectively, by translating in numbers that can then be analyzed statistically. It is not necessarily what the animal is doing, but how it is doing it. Because body language is dynamic, QBA allows the capture of subtle changes in an animal's body language that can be important for welfare assessment and may otherwise be overlooked when individual behaviours are isolated and quantified. QBA relies on observer assessments of the body language of animals (viewed in live or as video) using a set of descriptive terms (e.g. anxious, calm, etc.). These terms are usually a set of a fixed list (FL) determined after consultation with experts or generated by observers who watch the preview of a small number of clips and then generate their list of descriptive terms (a process called free choice profiling: FCP). The QBA is the only measure that captures positive aspects of animal welfare: including aspects of a positive mental state, rather than merely avoiding negative experiences, which is becoming an increasingly important component of any welfare assessment scheme index as it is likely to promote animal wellbeing and greater biological function. As such, QBA has been included as the 12th Criteria in the animal welfare assessment of farm animals developed through the European 6th FP Welfare Quality Project that has been carried out from 2004 to 2009.

1.5. Main challenges for achieving good animal welfare in zoo and the off-exhibit animal issues

Enclosure design and husbandry of captive animals have to meet the needs of many stakeholders: animals, keepers, and visitors. Other needs include reducing environmental impact and promoting sustainability, in compliance with building regulations. Animals should have the facilities and opportunities to ensure that the five freedoms are fulfilled. Usually, little is known about most of the species housed, so housing and husbandry regimes are created through the past experiences of other zoos or a generalization between the basic needs of similar species. Several elements that need to be taken into consideration while designing enclosures are the necessity of adequate space and access to resources, social vs individual housing and the addition of furnishing and enrichments. Types of enrichments could be: passive (physical exhibit elements), active (staff activated elements) or animal activated (includes on-display and off-displayed animal areas) (Rowe and Sherwen, 2017).

Besides different species-specific needs, there are also individual differences that set one animal apart from another (possibility to isolate) and affect how that animal functions within its environment. These differences include the animal's sex, age, reproductive status, size, social rank, temperament, health, past experience, and other factors that contribute to its individuality (Hosey et al., 2013).

The design of a zoo animal enclosure must consider (Rowe and Sherwen, 2017):

- species;
- natural habitat and ecology;
- natural biology and behaviour;
- number of animals housed;
- individual animal personalities.

Keepers' needs are to ensure that the animals in their care can have good welfare: this means that they should have good training or familiarized with biology, ethology and animal husbandry. To help keepers in achieving their daily duties, some important and influential details will facilitate husbandry and that should be considered in the enclosure design. These are: easy to clean exhibit; consideration of how staff can move animals safely

within the enclosure; providing easy access into the enclosure for staff and, where necessary, vehicles (Hosey et al., 2013).
Several studies have been done to understand the needs and the motivations of zoo visitors. For example, Morgan et al. (1999) describe zoo visitors in relation to their primary motivation and social orientation, highlighting that zoological parks are considered to be the most important source of contact between people and animals in modern society and that educational, recreational, intrinsic, and altruistic reasons are statistically significant. Another study (Rayan and Saward, 2004) carried out on a sample of 359 visitors in the Hamilton Zoo (New Zealand), reports that zoo represents an opportunity for family-based trips, however visitors are not interested in acquiring detailed information about wildlife and give more importance to the viewing of animals than to the recognition that animals might require "private places". The result serves to adapt the design of exhibits to their demands, recognizing the role of education and entertainment of the zoo, but also public's political and financial support.
Visitors consistently want to see naturalistic enclosure and active animals (playing, climbing, and eating). The aesthetic of an enclosure, the level of activity displayed and the appearance of an animal, not whether it has enrichments in its enclosure, are used by visitors as indicators of animal well-being. However, is important to consider that, usually, visitors have little or no knowledge of this topic. Sometimes some conflicts of interest do occur, such as visitors want to be able to view the animals easily, whereas the animals want to be able to hide, or visitors want animals to have large complex enclosures, whereas keepers need to be able to have easy access to them for service (Hosey et al., 2013). Resolving these conflicts is a challenge that often requires to compromise some aspects, but always has as its main aim the animal welfare.
Over the past few decades, zoo enclosure changed from the first-generation hard barren, to soft complex second and third generations. This led towards naturalistic enclosures, which are giving the impression of a greener environment but require additional planting and removal of bars or other harsh-looking structures within the enclosure (Hosey et al., 2013). An unobstructed view of the animal by the public is no longer a top priority in recent trends in zoo designs: use of vertical space, natural substrate composition and ground cover, appropriate flora, and feeding tactics which encourage foraging behaviour (where

applicable), are all measures which have been used to develop enriched environments for captive animals (Kohn, 1994).

There are three types of naturalistic enclosure: realistic, which reproduces the animals' wild habitat, including land formation and living plants; modified, which simulate the animals' wild habitat by using available materials to substitute for the real ones; and naturalistic, in which no attempt is made to duplicate the animals' wild habitat and natural material is used in a stylistic way (Hosey et al., 2013). The next step is the concept of immersion exhibit, which emphasized the human-animal contact (with experiences such as walk-through exhibits or touch situations).

Animal welfare regulations specify that the basic housing requirements are adequate space, shelter, and protection from inclement weather, compatibility with natural habitat conditions, the safety of the animal and the public; they are being amended as the database of available information expands (Kohn, 1994).

But there are still a few problems in the present era, which mainly concern animal husbandries, such as animal nutrition and sanitation. Animal compatibility is another husbandry issue which may be explosive, particularly in the light of current trends for group/social housing. Establishing and maintaining a compatible social structure in captivity is very hard because the change of any kind within a group - through the birth, death, temporary removal and subsequent reintroduction of a group member or changing in the age - can lead to social aggression and dangerous behaviour (Kohn, 1994).

In the future, that is way all zoos may need to employ not only highly skilled handlers and veterinarians, but also an animal behaviourist.

1.6. Striped Hyena Biology

- Scientific Classification

The scientific classification of Striped hyena is reported in Table 1-2:

Kingdom	Phylum	Class	Order	Suborder	Family	Genus	Species
Animalia	*Chordata*	*Mammalia*	*Carnivora*	*Feliformia*	*Hyaenidae*	*Hyaena*	*H. hyaena*

Table 1-2: Scientific classification of Striped hyena.

The family of Hyaenidae is composed by: Aardwolf (*Proteles cristatus*), Striped hyena (*Hyaena hyaena)*, Brown hyena (*Hyaena brunnea)* and Spotted hyena (*Crocuta Crocuta)* (Figure 1-1).

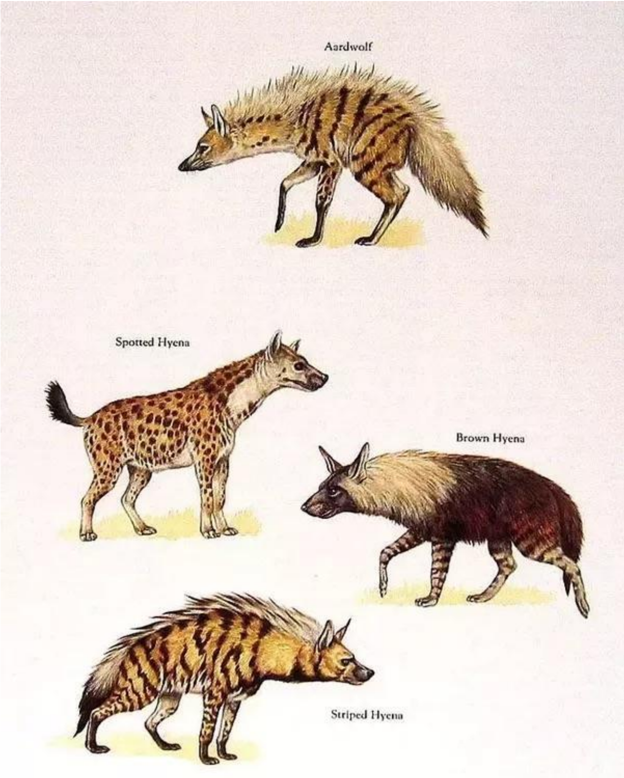


Figure 1-1: Four components of Hyaenidae family. On the top: Aardwolf [Proteles cristatus - Sparrman, 1783]; left: Spotted hyena [Crocuta crocuta - Erxleben, 1777]; right: Brown hyena [Hyaena (Parahyaena) brunnea - Thunberg, 1820]; on the lower: Striped hyena [Hyaena (Hyaena) hyaena - Linnaeus, 1758]. Picture taken from: https://www.reddit.com/r/hyenas/comments/9gzhw6/the_hyaenidae_family/

There are currently 5 subspecies of Striped hyena in the wild (*Hyaena hyaena barbara, Hyaena hyaena dubbah, Hyaena hyaena hyaena, Hyaena hyaena sultana* and *Hyaena hyaena syriaca*). In Europe, the captive population is currently divided as:

- Generic Striped hyena (*Hyaena hyaena*.) 54 animals in 25 zoos
- Eastern African Striped hyena (*Hyaena hyaena dubbah*) 10 animals in 6 zoos
- Syrian Striped hyena (*Hyaena hyaena syriaca*) 3 animals in 1 zoo
- Arabian Striped hyena (*Hyaena hyaena sultana*) 31 animals in 7 zoos.

One subspecies (*Hyaena hyaena sultana*) have been cleverly kept separately from the population creating a subpopulation in the European captive hyenas (Houssaye, 2018).

- Habitat and distribution

Striped hyenas are found in the open savannahs, grasslands, arid mountainous regions and scrub woodlands of West, North and East Africa (from Morocco and Senegal to Egypt and then Sudan, Ethiopia, Somalia, Uganda, Kenya, and finally central Tanzania) and West to South Asia; they may have recently expanded into Nepal (Figure 1-2).
The distribution of the Striped hyena is now patchy in most places suggesting that it occurs in many small isolated populations (Mills and Hofer, 1998).

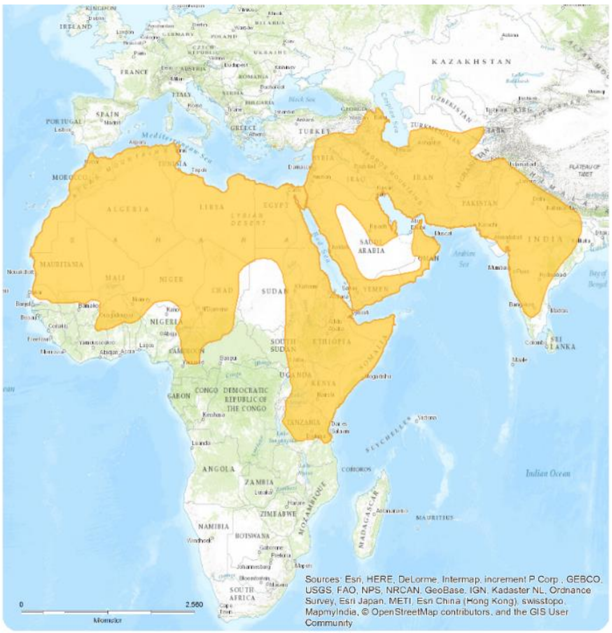


Figure 1-2: Main habitat zones of Striped hyenas.
Figure taken from the website:
https://www.iucnredlist.org/species/10274/45195080#geographic-range

- Physical description

This medium-sized, dog-like animal has the coat generally pale grey or beige. On the flanks there are five to nine indistinct vertical stripes and rows of diffuse spots; on the fore and hind legs, there are clearer black transverse and horizontal stripes. The mane has grey hairs at the base and black at the extremity. The snout and ears are pointed and dark, while the cheeks are much lighter. The tip of the tail is black with white undercoat. A specimen of Striped hyena is reported in Figure 1-3.

Figure 1-3: Example of full-growth specimen of Striped hyena. Picture taken from the website: https://www.lecornelle.it/animali-schede/iena-striata/

The Striped hyena has a massive and short torso, supported by long and thin legs. The hind legs are considerably shorter than the fore ones. Their neck is long but largely motionless. The ears are very big while the eyes are small. The masticatory muscles are well developed. The skull, with a very high sagittal crest, a shortened facial region, and a rounded frontal bone, is typical of this genus. It differs both from that of the Brown Hyena And from that of the Spotted hyena, due to the smaller dimensions and the slightly less massive structure.

- Longevity

In the wild, Striped hyenas can live up to 12 years, while in captivity they can reach even 23 years (Alvaro, 2016).

- Sexual dimorphism

An evident sexual dimorphism in body measurements and weight is absent: body weight is about 35 kg and the height at the withers is 60-80 cm per adult animal.
Genital appearance in juvenile Striped hyena is transiently masculinized in females, and feminized in males, and the existence of these traits does not seem to support evolutionary models of masculinization in Spotted hyena which rely on masculinization originating within that species (Wagner, 2006). Striped hyena females do not have the enlarged clitoris and the false scrotal sac, which is typical in Spotted hyena*'s* female genitalia.
They have 3 pairs of udders.
Males have a big pocket of naked skin located near the anal opening, in theirs opened large anal glands, located above the anus. Between the openings of the anal glands and above these are present sebaceous glands.

- Female genitalia

Young female genitalia (Figure 1-4) presents the following components a) prominent, dark, hairless labia-like folds develop anterior to the vagina; b) vagina; c) prominent labia-like protrusion with the urogenital opening on the posterior side; d) anus.

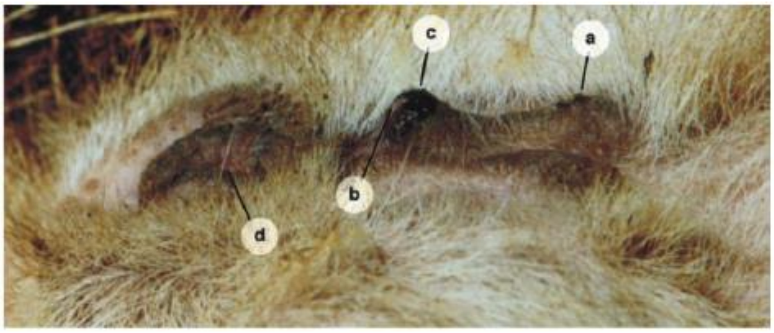


Figure 1-4: Detail of young hyena female genitalia.
Picture taken from: "Behavioural ecology of the Striped hyena (Hyaena hyaena)" (Wagner, 2006)

In the adult females (Figure 1-5) the labia-like protrusion becomes far less prominent, the opening of the urogenital canal moves perpendicular to the body, and the swelling of the labial folds diminishes completely, with only a patch of dark skin remaining.

Figure 1-5: Detail of adult hyena female genitalia.
Pictures taken by the observer at the Parco Faunistico Valcorba.

- Male genitalia

Young male genitalia (Figure 1-6) are composed by a) smooth, hairless, pre-scrotal skin folds apparent lateral to the raphae at the junction between the scrotum (b) and penis (c); d) anus.

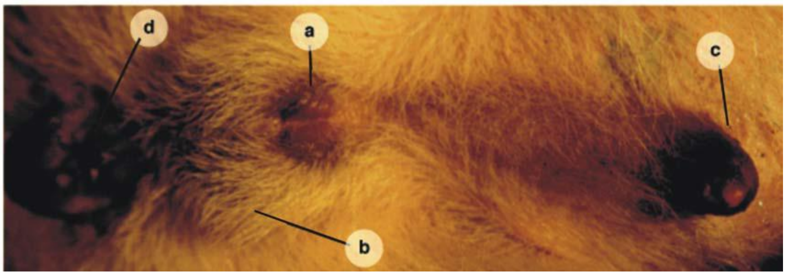


Figure 1-6: Details of young hyena male genitalia.
Picture taken from: "Behavioural ecology of the Striped hyena (Hyaena hyaena)" (Wagner, 2006)

In adult males (Figure 1-7), the swelling of the skin folds is reduced and becomes partially integrated into the raphae at the base of the testes (Wagner, 2006).

Figure 1-7: Details of adult hyena male genitalia.
Pictures taken by the observer at the Parco Faunistico Valcorba.

- Communication

In Striped hyena, smell is the sense through which they socialize or manifest their authority on similar individuals; they recognize themselves by sniffing face, neck and anal glands. This species is not as noisy as the Spotted Hyena And its vocalizations are limited to a sort of laughter and a howling.

- Territoriality

In general, food type and size are correlated with spacing patterns in carnivores. Whereas food abundance confines group size, the distribution of food resources determines territory size. Female behaviour reflects an attempt to meet the demands of the environment, while male behaviour is adapted to maximize mating opportunities; this explains the reason why home-ranges of the male are larger (Wagner, 2006).
The Striped hyena is not a particularly territorial animal and the territories belonging to several individuals often overlap. The home range is long-lasting and is marked regularly.
When marking the territory, the Striped hyena use the secretion of their anal pocket (known as "Hyena Butter") to mark grass, stems, stones, tree trunks and other objects (Figure 1-8).

Figure 1-8: Hyena marking the territory. Picture taken from the website: https://www.videoblocks.com/video/striped-hyena-hyaena-hyaena-scent-marking-b4ysbbk5fjfb1o401.

- Reproduction

Sexual maturity starts at two or three years of age.

Females are polyestrous: oestrum lasts one day and oestrous cycles last between 40-50 days. This means that males will have several occasions to mate with the female throughout the year. More males (usually two) contend one female, defending the territory within which she lives.

The Striped hyena is tendentially monogamous and in each pair the male helps the female to settle in a den and provide food after the birth. Juvenile males may help their mothers with cubs; however, scientists believe the father plays no part in cub rearing (Wagner, 2006).

- Management of the cubs

Striped hyena delivers and grows its cubs in potholes of the ground, called "den".

The gestation lasts 90-91 days and the delivery is preceded by an intense digging behaviour of the female. The litter size is one to five cubs; at birth, the puppies have light grey fur and evident black striations: they are blind, and the ears are closed (Alvaro, 2016). In (Figure 1-9) Striped hyena's puppies are shown.


Figure 1-9: Striped hyena's puppies. Pictures taken from the website: https://www.reddit.com/r/aww/comments/1evrm3/striped_hyena_pups/

This contrasts with what happens in the puppies of the Spotted hyena, which born almost fully developed.

The females are very protective and refuse the male if he gets too close; post-partum oestrum occurs 20 to 21 days after litter's birth. The cubs leave the den at 1 month and they are weaned at 2 months. The average interval between two litters is 14 months.

- The den

The Striped hyenas are nocturnal hunters. They only leave the den when the sun is fully set and return there at dawn to avoid periods of extreme temperature, or to keep out of the sight of predators. They dig their dens or occupy those of other species; examples of hyena's den are shown in Figure 1-10.


Figure 1-10: Hyena's dens. Pictures taken from the website: http://sujanluxury.com/blog/2016/04/26/oh-lakkad-bagga-the-striped-hyenas/.

- Diet

Striped hyenas are omnivorous scavengers, feeding on carrion, although they will occasionally kill small- and medium-size mammals and other small vertebrates. They also eat insects, some fruits, and human refuse (MPALA, 2018).

- Relationship with other predators/competition

The main predators of the Striped Hyena Are men and large carnivores, such as cheetah, leopard, panther, lion, or tiger. In the Middle East and Central Asia, the main competitor of the Striped hyena is the wolf. It has been observed that this species is submissive towards the largest Spotted hyena.
When attacked, the Striped hyena deflects the rectum and sprays a pungent liquid from the anal glands. Despite having the habit of faking themselves dead when they feel threatened, they bravely face even larger predators in food disputes.

- Social behaviour

It is considered “grouping” when two or more individuals of either sex share the same defended space. “Associating” simply implies close physical proximity. “Sociality” is a case of grouping that is generally more permanent; it implies frequent direct interaction and cooperation, and individuals remain associated over long periods of time.
Although three of the four species have been well studied, descriptions of Striped hyena ecology have been based on very limited observations. Based on these studies, Striped hyenas share spatial grouping, but group members are solitary: association and cooperation within social groups are very low and there are largely independent movements and solitary foraging and feeding within a shared home-range (Wagner, 2006). However, there are some exceptions. In Central Asia, they form monogamous breeding pairs and previous litter cubs may remain with the pair. Groups of up to 7 animals were seen in Libya. In Israel, they are mostly solitary but sometimes they gather in groups around food sources. In Central Kenya, there are groups composed of one female and about three males, which may or not be relatives. The members of the group occupy the same territory but meet only for rest or for different social interactions. Females mate not only with males of the same group but also with males of other groups (Alvaro, 2016).
The essentially polyandrous spatial organization of Striped hyenas, combined with little direct social interaction, is unique among the Carnivora.
During the manifestations of aggression between conspecifics, the back hair of the mane is erected (Figure 1-11).

Figure 1-11: A Striped hyena with back hair erected during an aggressive manifestation.
Picture taken from the website: http://it.nextews.com/fb2116c4/

Lowered ears indicate a tendency to bite. While fighting, the Striped hyenas try to bite the opponent's throat or paws, but never on the mane, which acts as a signaling device.

A frightened animal erects the mane totally, but not the hair of the tail. An aggressive animal erects the mane and the fur on the back; when also the tail is raised and its hairs are bristly, it means that the aggressive attitude is very high. Tail lowered between the hind legs is observed when the animal escapes (Alvaro, 2016).

During meetings, Striped hyenas investigate and lick the mid-back region where the mid-dorsal crest is situated. Greetings also involve sniffing of the nose and extruded anal pouch, and repeated pawing of the throat of the greeting partner; a greetings interaction between two Striped hyenas is shown in Figure 1-12. During some social interactions and mating, the anus can be extroverted even for a length of 5 cm.

Figure 1-12: A greeting interaction between two Striped hyenas.
Picture taken from the website: https://thereaderwiki.com/en/Striped_hyena

- Conservation

The International Union for Conservation of Nature (IUCN) lists Striped Hyena As a species of lower risk (near-threatened; Figure 1-13) (AbiSaid and Dloniak, 2015).

Figure 1-13: Striped hyena risk assessment by IUCN (International Union for Conservation of Nature).

The global population is estimated under 10.000 mature individuals. Such an assessment of the current population trends of the Striped hyena is complicated by several problems: they are nocturnal, solitary, occur at low densities often in rugged country, sightings are infrequent, and surveys difficult to carry out. Moreover, in areas where the range of the Striped hyena overlaps with that of the Spotted Hyena And the Aardwolf, few people acknowledge or recognize a difference between the Hyaenidae species (Mills and Hofer, 1998). Density is estimated as greater than 0.02 individual/km^2; for comparison, Spotted hyenas in the same ecosystem exceed 1 individual/km^2 (Wagner, 2006).

Outside of protected areas, Striped hyenas are very vulnerable to human-induced habitat degradation. Prejudices against hyenas reach back centuries. Striped hyenas tend to raid human croplands for fruits and vegetables, is considered to be a predator of livestock, and in some areas have been reported raiding human graves. Thus, the species is heavily persecuted (including destruction or blockage of dens, poisoning carcasses, or the use of the fire to chase animals out of dens). There is also illegal trade in skins and heads, and some of their body parts are also used in traditional medicine.

But the reasons for the apparent decline in population do not include only persecution (especially by poisoning), but also the decreasing of a natural and domestic source of carrion (due to declines in the populations of other large carnivores and their prey, and changes in livestock practices). It may come close to meeting a continuing decline of 10% over the next three generations (AbiSaid and Dloniak, 2015).

1.7. Striped hyena management in captivity

Striped hyenas should be kept individually. Keeping them in temporary groups is possible when cubs are young and not sexually mature. However, this possibility varies due to the capacities of both indoor and outdoor enclosures and the individual behaviour of each animal. If housed in pairs, compatibility could be a problem, especially when adult animals are introduced. As a result, many compatible pairs were developed from littermates and inbreeding was observed in some captive collections.

If male and female don't match, this can lead to stress and injuries. Anyway, visual contact between two incompatible animals is strongly recommended. If the coupling is allowed, the pair could be put together only when the female is in oestrum.

It is always suggested to separate animals during the night or at least for feeding.

Often, in captivity, is necessary to control animals' population and Striped hyenas are concerned by this control. The main reasons are to avoid inbreeding and because placing captive born young in others zoo is not easy. Nonreversible contraceptive methods are ovariohysterectomy of females and male's castration; reversible methods are hormonal implants and physical separation. The social structure can be impacted by contraception: it can lead to a change of dominance, the rejection of an individual or the improvement of conflicts between individuals (Mills and Hofer, 1998).

When new individual needs to be introduced, it is important to follow several steps. Animals should be kept separated but should have olfactory and auditory exposure to each other. Then, should be given visual contact with each other: if during this process the animals display symptoms associated with stress (e.g., pacing, diarrhea, excessive vocalization) for more than three hours, the introduction should return to the previous step. If animals are not already in an adjacent fence, in this phase of the reintroduction they should be moved in closer contact. The actual introduction (full tactile exposure) should take place in the largest enclosure available. Preferably, the enclosure should be familiar to the least dominant animal and include ample run-arounds. Joining of two animals is also possible inside their indoor box: the advantage is a quicker possibility of separating them if things go wrong; the disadvantage is little space available to run. Keepers have to be present during first meetings and be able to separate animals if they show excessive discomfort or if they start fighting.

2. AIM

The aim of this project was to resocialize two siblings Striped hyenas (*Hyaena hyaena*) that live in a wildlife park in northeastern Italy, in order to improve their welfare status by allowing a daily access to the outdoor enclosure for both of them. That was not done at the time of the outset of the study because the two animals had demonstrated abhorrent attitude towards each other for years, forcing them to live apart.

The rationale behind this case study was that the aggressiveness of the two animals, and the consequent stressful management conditions adopted by the zoo staff, could have been caused by sexual grounds since it started at sexual maturity. Thus, the first attempt was to eliminate this factor by putting a hormonal subcutaneous implant in both hyenas, causing a temporary and reversible suppression in the synthesis of the sexual hormones, before their rapprochement in close proximity. The final goal was to resocialize them or find alternative solutions.

3. MATERIALS AND METHODS

3.1. Zoo and animals

The Striped hyenas subject of this study live in a wildlife park, the “Parco Faunistico Valcorba”, which is located in Stroppare di Pozzonovo, Padua, in northeastern Italy.
Parco Faunistico Valcorba (Figure 3-1), houses about 330 animals belonging to 80 different species and extends over 200.000 square meters with meadows, trees (there are about 22.000 plants), walkways, and ponds.

Figure 3-1: Official logo of Parco Faunistico Valcorba; website: http://www.parcovalcorba.com/.

The park originates from a traditional zoo, Monte Lonzina Zoo, which was intended only for animal display. The setting up period lasted ten years (from 1990 to 2000) and had the purpose of creating a new environment, with larger and more comfortable exhibits for the animals. Nowadays the animals have wide naturalistic exhibits, designed according to their individual and specific needs.
Valcorba park is a member of Italian Union of Zoos and Aquariums (UIZA,
Figure 3-2a) and its working group of educators (Eduzoo,
Figure 3-2b), promotes several campaigns and awareness-raising projects. Since October 2018 the park has joined the prestigious European Association of Zoo and Aquarium (EAZA, Figure 3-2c) and has taken part in different campaigns proposed by them. For example, it is the only Italian zoo that host the North China leopard (*Panthera pardus japonensis*), whose population ex situ is managed by EAZA’s European Endangered Species Programmes (EEP).

a)

UIZA (Unione Italiana Giardini Zoologici e Acquari) logo.
Web-site:
https://uiza.org/

b)

Eduzoo logo.
Web-site:
https://uiza.org/eduzoo/

c)

EAZA (European Association of Zoo and Aquarium) logo.
Web-site:
https://www.eaza.net/

Figure 3-2: Official logos of UIZA, EDUZOO and EAZA associations.

It participated in international breeding programs and conservation of endangered species, both in EEP and European Studbooks (ESB), and worked for years in support to the Convention on International Trade in Endangered Species of wild fauna and flora (CITES) National Forest organism by hosting seized animals.

Some of the conservation projects supported are: “Let’s save the moon bears!”, “Save the Caissara”, “Reason for hope”, in collaboration with different associations and zoos as Animal Asia Foundation, Park Zoo Punta Verde, Brazilian organization Instituto de Pesquisas Ecológicas, Parco Natura Viva and the Waldrapp team, among others.

It annually welcomes students from various Italian Universities and allows them to improve or complete their training through internships or research projects.

The two Striped hyenas (Figure 3-3a and b) are brother and sister and come from Le Cornelle wildlife park, Valbrembo (BG), Italy. They were born on November 20th, 2010 and have been moved to the Valcorba Park on April 28th, 2011, at the age of 5 months. They were hand reared. Prior to movement, they have been vaccinated with canine heptavalent and feline trivalent vaccine and treated against rabies.

a) Female hyena: Hyena A b) Male hyena: Hyena B

Figure 3-3: Female (a) and male (b) hyenas in the outdoor exhibit. Picture taken at the Parco Faunistico Valcorba by the observer.

For the first three years, they shared the same exhibit, which consists of a big outdoor space (Figure 3-4) and a smaller indoor area, made of three fenced pens (Figure 3-5), to allow the two hyenas to stay inside during the nighttime.

The keepers report no problems during these first three years, but all at once the animals started showing abhorrent behaviour and attacking each other, with bites to the neck and ears, pursuits, and strong vocalizations. At the outset of these behaviours, keepers report that it was very difficult to separate them, but fortunately, they did not suffer from major injuries. The animals have been living separated ever since, and the few attempts to put them together have repeatedly failed. The animals can go outside every other day during the daytime and spend overnight indoors in two separate pens. While indoor, they may see each other and have a visible and olfactory contact, but there is an empty pen between them. The same building hosts also the indoor pens of the leopards (physically separated by full walls but with fence doors for the access of the keeper). All leopards are allowed outdoor during the daytime so the hyena remaining indoors every other day stays alone.

The indoor housing allows its exposure to the natural light (photoperiod preserved) through roof windows. This situation was stressful for the animals; in fact, they developed stereotypies and bizarre behaviour. In which case, assistance has been asked.

Figure 3-4: Outdoor exhibit of the hyenas' enclosure. Picture taken at the Parco Faunistico Valcolba by the observer, from one of 3 observation points (the so called "mandrilli window").

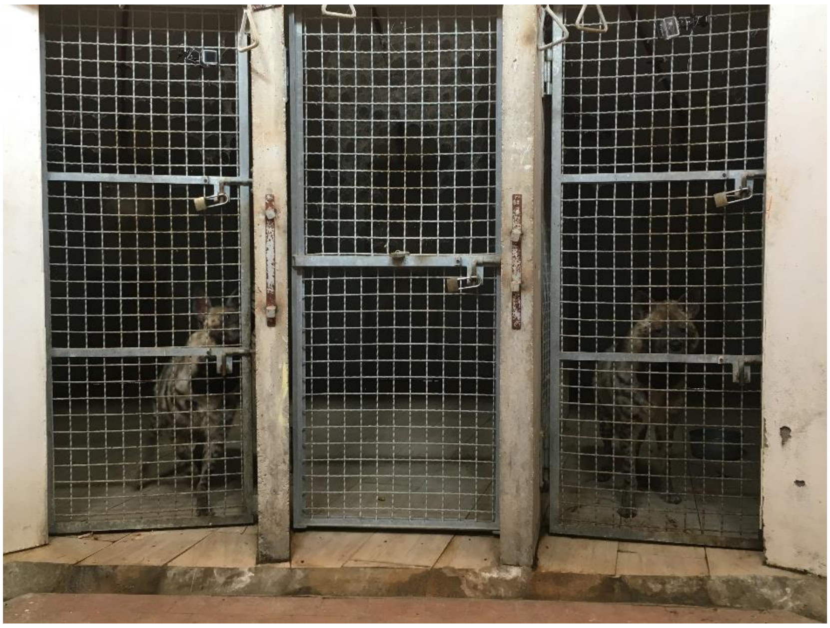


Figure 3-5: Indoor area of the hyenas' enclosure, made of three fenced pens, to allow the two hyenas to stay inside during the nighttime. Hyena A (female) is that on the left, Hyena B (male) is that on the right. Picture taken at Parco Faunistico Valcolba by the observer.

3.2. Case study

Given the situation arisen in the park, a case study was set up. Case studies include a selection of variables on which to focus, a data collection procedure, and a method of analysis of the observations and impressions. Frequently, they are exploratory in nature when the phenomenon or process is not clearly understood and enable researchers to record unexpected findings as they emerge. The data of case studies are often written summations of important observations, relationship, and inferences. Interviews, expertise, observations and test results may also serve as a data-generating device. Generally, a portion of the study is devoted to a preliminary research process, based on bibliographical research and comparison with other similarly conducted studies (Waalen, 1991).

Initially, the setting up of the study considered different options, but experimental overtures were not feasible for different reasons; thus, a case study approach was decided and variables on which to focus were selected. Among the different hypothesis behind the above described behaviours and aggressive attitudes of the two hyenas, one of the most accredited was a possible link with the sexual/hormonal condition. After a first definition of the actual management of the two animals and its effect on their welfare status, the sexual component was temporarily suppressed trying a hormonal sterilization through a subcutaneous implant, put in both animals. This was followed by two attempts of resocialization.

3.3. Data collection

3.3.1. Direct behavioural data collection

Demeanours of the two hyenas were assessed by one observer who has been previously familiarized with a list of potential behaviours and behavioural observation strategies (La Cauza, 2014) (ANNEX A: BEHAVIOURS). Four days of behavioural observation sessions were organized prior to the hormonal implant. The hyenas have been observed in both indoors and outdoors situations to analyze their individual and potential social behaviours, from May 28th to 31st, 2018 (Table 3-1).

Every day the observation sessions were carried out starting early in the morning, recording behaviours for 30 minutes (from 8.30 to 9.00 am), when both hyenas were indoors in their respective pens, before one of them was allowed outside. At that hour the park was still close to the visitors and the leopards sharing the building with the hyenas during the night were already outdoor. The observations of the two hyenas together indoors were also done for 30 minutes (from 6.00 to 6.30 pm) after the closure of the park, when the hyena that spent the day outdoors was closed back indoors. The individual observations of each animal were performed for two hours (four 30 minutes bouts) from 9.00 to 11.00 am, and other two hours (four 30 minutes bouts) from 4.00 to 6.00 pm.

During the 30 minutes of observation, the observer recorded how many times the animals manifested a specific behaviour or attitude applying the continuous recording method (Martin and Bateson, 2007). Data have been collected using scoring sheets in which date, identification of the hyena (A: female hyena; B: male hyena), starting and ending time of the continuous behavioural assessment and each specific behaviour per observation time point were indicated.

	28th May 2018	**29th May 2018**	**30th May 2018**	**31st May 2018**
8.30-9.00 am	A and B inside	A and B inside	A and B inside	A and B inside
9.00-11.00 am	A inside	B inside	B outside	A outside
4.00-6.00 pm	A inside	B inside	B outside	A outside
6.00-6.30 pm	A and B inside	A and B inside	A and B inside	A and B inside

Table 3-1: Daily schedule of the observation sessions.

The behaviours considered for social observations were:

NORMAL AND AFFILIATIVE

- Attentive behaviour: the individual observes and pays attention to the conspecific;
- Maintenance: the individual performs those actions designed to satisfy his physiological needs (urinating, defecating, eating and bathing);
- Autogrooming: the individual performs those self-care actions, such as cleaning and scratching;

- Territoriality: the individual marks the territory through his scent glands or rubbing himself on the ground or against objects;
- Exploration: the individual olfactory and visually explores the environment, also through interaction with objects;
- Locomotion: the individual moves in different ways from one area of the environment to another;
- Social: when they greet each other, they lick the median region of the back, smell their nose, protrude their anal pocket or touch their throats with their paws;
- Reproductive: the individual is mating. The male bites the skin of the female's neck during intercourse. During some social interactions and mating, the anus can be everted;
- Inactivity (passive rest): the individual rests with the other animal present;
- Vocalizations: “laughter” or howl;

COMPETITIVE

- Dominance: the individual imposes himself on a conspecific sometimes with bites to the throat or legs, but never on the mane. During the manifestations of aggressiveness between conspecifics, the black hairs of the mane are erected and act as a signaling device;
- Submission: the individual extends showing the belly or shakes the snout below the dominant individual;

ABNORMAL

- Stereotype: normal behaviour performed with high frequency, abnormal behaviour for the species, normal behaviour but not aimed at the specific function (considered only if this behaviour is maintained for at least 5 minutes);

UNSEEN

- No data collected, because the subject was not visible, or his behaviour could not be correctly interpreted.

The behaviours considered for individual observations were:

NORMAL

- Attentive behaviour: the individual observes and pays attention to what happens in the environment or to a specific direction;

- Maintenance: the individual performs those actions designed to satisfy his physiological needs (urinating, defecating, eating and bathing);
- Autogrooming: the individual performs those self-care actions, such as cleaning and scratching
- Territoriality: the individual marks the territory through his scent glands or rubbing himself on the ground or against objects;
- Exploration: the individual olfactory and visually explores the environment, also through interaction with objects;
- Locomotion: the individual moves in different ways from one area of the environment to another;
- Inactivity (passive rest): the individual rest alone;
- Vocalizations: “laughter” or howl;

ABNORMAL

- Stereotype: normal behaviour performed with high frequency, abnormal behaviour for the species, normal behaviour but not aimed at the specific function (considered only if this behaviour is maintained for at least 5 minutes);

UNSEEN

- No data collected, because the subject was not visible, or his behaviour could not be correctly interpreted.

3.3.2. Video recordings

Five cameras (GoPro®) have been used to record the hyenas in order to assess their behaviour and spatial use of the different areas of the outdoor enclosure in particular (Figure 3-6) on the same days of direct behavioural observations (May 28th to May 31st). They were collocated on the doors of the inner pens and in three different places in the outdoor enclosure to evaluate if the two hyenas were using same or different spots of the enclosure in the same time intervals of the day.

Figure 3-6: One of the cameras (GoPro) used to record the hyenas. Picture taken at Parco Faunistico Valcolba by the observer.

3.3.3. Clinical visit, hormonal implant and blood sampling

On June 1st, 2018, a team of veterinarians came to the zoo to visit the hyenas, check their gender and put the subcutaneous hormonal implant. Animals were anesthetized one at the time (Hyena B and then A) using Ketamine, Medetomidine and Midazolam, administered through a blowgun. The drug doses were calculated on an estimated weight of 40 kg. The efficacy of the anesthesia was controlled by keeping monitored respiratory and heart rate, temperature, diastolic, systolic and average pressure and the presence of the palpebral reflex (Figure 3-7). The presence of the microchip and correspondence of the identification code were verified with a reader. Gender assessment was done through a visual and ultrasound examination: no testicular abnormalities were detected in Hyena B (which was considered sexually mature and active), while in Hyena A it was not possible to perform a complete ultrasound examination at the genital apparatus, because her parameters required an early awakening from anaesthesia. The subcutaneous hormonal implant (Suprelorin®: Virbac Animal Health) was put in the dorsal-lumbar region of the left hip, after shearing and disinfecting this zone (Figure 3-8). The implants contained 4.7 mg of Deslorelin in slow-release. Blood samples were collected only once because the collection necessarily required sedation of the animals. They were taken from the Saphenous vein using test tubes without anticoagulant and destined for hormonal research. Plasma was separated from the 2 ml of blood and it was refrigerated during transport to the storage unit at -20°C until analysis. A pesticide treatment with Ivermectin was done. Finally, hyenas were weighed by lifting them with a blanket connected to a scale (Figure 3-9). Alcohol was shed on the paws to help lowering body temperature and facilitate awakening.

All these operations lasted about forty minutes per each Hyena And they easily recovered after the administration of Atipamezole. Animals were checked by the veterinary team two hours later and by the park vet in the evening.

Figure 3-7: Clinic visit and monitoring of one of the hyenas. Picture taken at Parco Faunistico Valcolba by the observer.

Figure 3-8: The subcutaneous hormonal implant (Suprelorin®: Virbac Animal Health) and its inserting in the dorsal-lumbar region of the left hip. Picture taken at Parco Faunistico Valcolba by the observer.

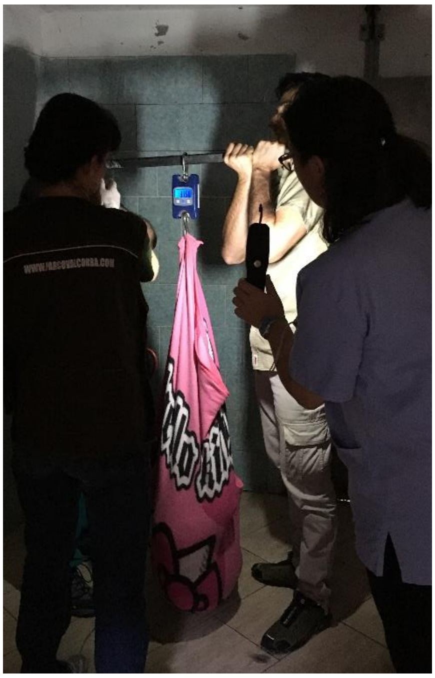


Figure 3-9: Weighing of the hyenas by lifting them with a blanket connected to a scale. Picture taken at Parco Faunistico Valcolba by the observer.

3.3.4. Biological samples collection and analysis

Feces of Hyena A and B were collected three (day -4, -3, and -2) and two times (day -4 and -2) respectively before the implant was inserted (day 0). Although planned in the setting out of the study, no stool samples were collected on June 1st, neither the first few days afterwards. However, throughout the month of July, keepers were instructed to collect stool samples two times a week, for each hyena. Stool samples were placed in plastic boxes and stored at -20°C, until processed for hormonal determinations.

Samples were collected by the keepers without a fixed daily interval depending on their availability and on the basis of presence/absence of hyenas inside the fence. During the day it was possible to collect only the feces in the pen of the hyena that was outdoors (if feces were present).

The biological matrices must be submitted to an extraction procedure with an organic solvent before measuring the steroid hormones Testosterone (TST), Estradiol (E2), Cortisol (HC), and Dehydroepiandrosterone (DHEA). Extractive procedures depend on hormone and matrix (in this case plasma and stool). The steroid extraction was performed from wet and dry stool samples following the double extraction technique described for dry samples, with some modifications (Brown et al., 1994). Fecal samples were added with 90% ethanol. The mixture was carefully shaken, boiled and vortexed; this procedure is repeated twice. The ethanolic supernatants were collected in conic glass tube and the remaining part was dried under N_2 flow. The dry extracts were dissolved in absolute ethanol (100% ethanol), divided in aliquots, dried again under N_2 flow and dissolved in absolute methanol. A summary of sample volume, solvent volume, and analytic procedure is described in Table 3-2. This extraction technique was also described by Vernocchi et al. (2018) for an evaluation of fecal sexual steroids' changes in two female cheetahs.

HORMON	MATRIX	VOLUME	SOLVENT VOLUME	ANALYTIC PROCEDURE
TST	Stool	200 mg	90% Ethanol 5 ml + 5 ml 100% Ethanol 1 ml Methanol 250 µl	Loaded: 20 µl/well
E2	Stool	200 mg	90% Ethanol 5 ml + 5 ml 100% Ethanol 1 ml Methanol 250 µl	Loaded: 20 µl/well
HC	Stool	200 mg	90% Ethanol 5 ml + 5 ml 100% Ethanol 1 ml Methanol 500 µl	Loaded: 10 µl/well
DHEA	Stool	200 mg	90% Ethanol 5 ml + 5 ml 100% Ethanol 1 ml Methanol 250 µl	Loaded: 20 µl/well

Table 3-2: Summary of sample volume, solvent volume, and analytic procedure of the fecal steroid extraction.

Plasma samples and the organic solvent (diethyl ether) were put in extraction tubes. Tubes were mixed, vortexed and then freeze, due to separate the liquid phase from the extract. The extract was subsequently dry under nitrogen (N_2) flow, washed with diethyl ether (to bring to the bottom steroids left on the walls of the test tubes) and led to dry a second time. The dried extract was than resolubilized in the buffer for RIA analysis. A summary of sample volume, solvent volume, and analytic procedure is described in Table 3-3.

HORMON	MATRIX	VOLUME	SOLVENT VOLUME	ANALYTIC PROCEDURE
TST	Plasma	200 µl	Diethyl ether 8 ml	Took: 1000 µl Loaded: 50 µl/well
E2	Plasma	200 µl	Diethyl ether 8 ml	Took: 1000 µl Loaded: 200 µl/well
HC	Plasma	200 µl	Diethyl ether 8 ml	Took: 1000 µl Loaded: 50 µl/well
DHEA	Plasma	200 µl	Diethyl ether 8 ml	Took: 1000 µl Loaded: 50 µl/well

Table 3-3: Summary of sample volume, solvent volume, and analytic procedure of the plasma steroid extraction.

TST, HC, E2 and DHEA were measured in fecal and plasma extracts by solid-phase microtiter radioimmunoassays (RIA). This method is based on the competition between the analyte (steroid hormone) to be quantified, and a tracer labelled with a radioactive isotope (usually ^3H), to bind the same specific antibody, which is present in the system in less quantity than the antigens. The greater the amount of analyte is present in the sample, the less will be the amount of radioactive tracer able to bind the antibodies placed in the well.

Hormone analyses in plasma extracts were performed by published procedures. Fecal estrogens, progestins (Biancani et al., 2009) and Cortisol (Biancani et al., 2017) were analysed by methods validated for fecal samples. DHEA was analysed by a method validated for extracts different from blood plasma (Placci et al. 2019; validated for horsehair extracts). The RIA method used for TST was described by Mucignat et al. (2014) and was validated for fecal extract samples by performing a parallelism test for the occasion.

3.3.5. Qualitative behavioural assessment

A randomly selected sample of 7 video clips was extracted from the video recordings carried out during the observation days. Each video clip had to show the hyenas' whole body and face and ears for at least 10 seconds. The videos were chosen in order to have both hyenas (A and B) in each location (indoor and outdoor) and together. Duration of each video clip is reported in Table 3-4.

VIDEO	VIDEO FRAGMENT (min)	HYENA	LOCATION
1	From 7:00 to7:43	B	Outer fence
2	From 7:37 to 8:09	B	Outer fence
3	From 12:10 to 13:10	A	Inner fence
4	From 2:00 to 3:00	B	Inner fence
5	From 1:57 to 2:57	A	Outer fence
6	From 1:40 to 2:40	A and B	Inner fence
7	From 1:12 to 2:18	A and B	Inner fence

Table 3-4: Identification and description of the video fragments selected for testing the QBA.

The 7 video clips were used for QBA ex-situ carried out during a lecture on QBA. The group of observers was a convenience sample composed of one Veterinary student, an expert in animal welfare, and thirteen students in their second year of Animal Care of the University of Padua. None of the observers had experience with observing captive hyena's behaviour and the observations were collected anonymously.

The observers were familiarized with the scoring sheets and the fixed term list (Wemelsfelder, 2007). They were then asked to watch each video clip and to score on a visual analog scale 12 cm long, the point between "minimum" = 0 and "maximum" = 12 corresponding to their evaluation of each term. The terms used were: *active; relaxed; uncomfortable; calm; content; tense; enjoying; indifferent; frustrated; friendly; bored; positively occupied; inquisitive; irritable; nervous; boisterous; uneasy; sociable; happy; distressed* (ANNEX B: QBA FOR VIDEOS).

3.3.6. Literature review and collection of experiences

Literature research was done by consulting books present in the library of the University of Padua, different websites and scientific journals retrieved using Web of science – Thomson ReutersTM (WOS) All Databases (Web of Science Core Collection and all Citation Indexes thicked in the search setting) within the entire timespan from year 1985 – present, Google Scholar and other.

To get information and support for changing the actual status and management of the Striped hyenas in captivity, a list of more than 100 European and non-European zoos that host this species was selected. The list was made in collaboration with the veterinarian of Valcorba Park. Only those where the Striped hyenas were currently present (alive) were contacted by email; this criterion of selection reduced the list to 50 zoos.

After explaining the aim of this project with detailed descriptions on the housing of the hyenas at the Valcorba Park and on the steps carried out so far in the attempt to resocialize them, they were asked to provide their advice and expertise on Striped hyenas' behaviour and management.

The feedbacks were submitted to text analysis to extract meaningful numeric indices from unstructured text using Text mining analysis and representing it visually with Word Cloud. Word clouds (also known as "Text Clouds" or "Tag Clouds") work in a simple way: the more a specific word appears in a source of textual data, the bigger and bolder it appears in the word cloud (Boostlab, 2014). There are some available software solutions, in this case we used "WordClouds.com".

3.4. Data management and statistical analysis of data

Data collected were reported in Excel® spreadsheets. Frequencies of individual and social quantitative behaviours were expressed as number of events performed per each observation interval of 30 minutes with the individual animal as experimental unit. Data were first submitted to descriptive statistics and tested for normality. Not normally distributed data regarding individual and social behaviours were submitted to non parametric one-way analysis to evaluate the effect of the thesis (hyena(s) × location (DF=4)) applying the Bonferroni adjustment. Normally distributed data regarding fecal sample hormonal concentrations were submitted to analysis of variance using SAS/STAT (version 9.3. SAS Institute Inc, Cary, NC, USA) applying a generalized linear model that considered the effect of the hyena, the pre- and post- implant sampling time interval and the interaction of the two main effects.

Qualitative behavioural assessment data (measured from 0 to 120 mm) were analyzed using a Principal Component Analysis (PCA). The analysis was based on a correlation matrix, without rotation, and two components were extracted (after pre-exploration of an unlimited number of components, selection of eigenvalues >1 adjusted by the explained variances to avoid under/over factoring). The PCA scores were submitted to analysis of variance using a generalized linear model that considered the effect of the hyena(s) in each location (DF = 4). The value of $P<0.05$ was the minimum threshold of statistical significance.

4. RESULTS AND DISCUSSION

Overall the two hyenas did not show any visible clinical sign of disease during the study and they had a good body condition and good condition of the fur (that was thick and clean). At day 0 their respective body weights resulted 37.5 kg for Hyena B and 37 kg for Hyena A.

4.1. Quantitative behavioural analysis

Results regarding quantitative individual behaviours highlighted that the effect of the thesis (hyena × location) had a significant effect (P < 0.05) on the following behaviours: Attentive behaviour, Maintenance, Autogrooming, and Locomotion. No significant effect was demonstrated for Territoriality, Exploration and Inactivity. Vocalizations and Stereotype were not submitted to non parametric statistical test because either too rare or occurring only indoors with similar frequencies among animals. Results were provided as box plots representing individual behaviours according to Hyena and location (Figure 4-1).

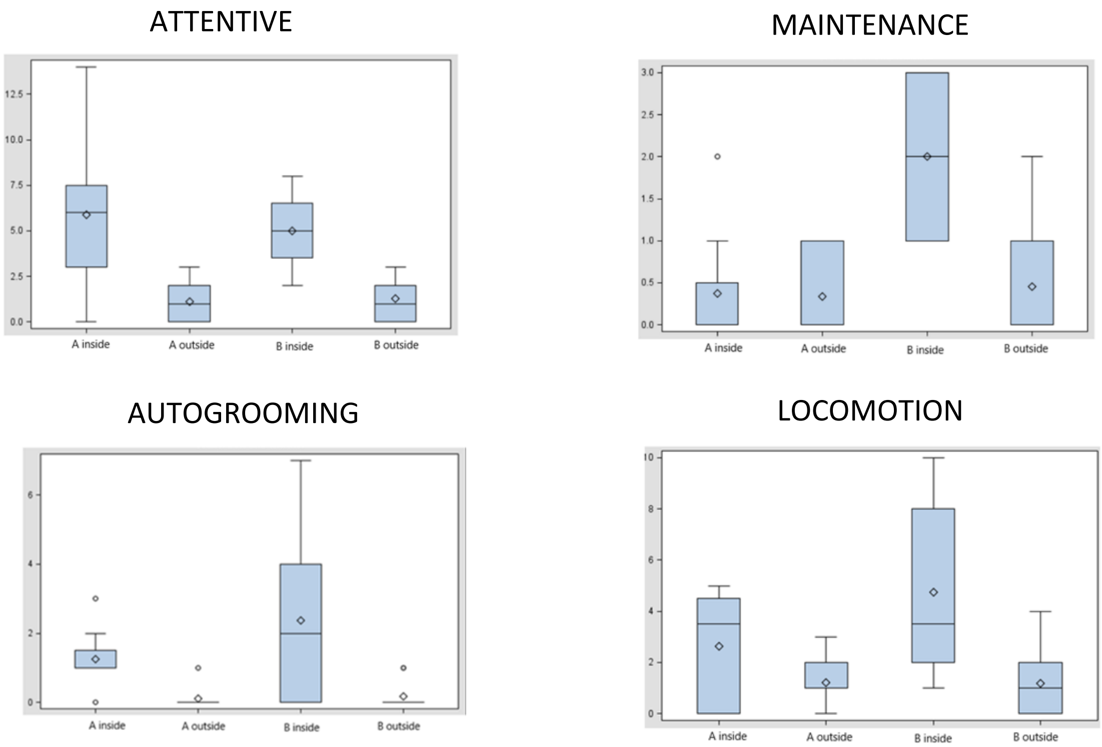


Figure 4-1: Box plots representing individual behaviours according to Hyena and location.

When indoors, both hyenas spent several minutes walking in the area, sniffing and licking around the perimeter of the fence. It seemed that the male hyena did more locomotion than the female (Figure 4-2). Sometimes they flipped and marked their water bowls with the anal glands everted: this behaviour was registered at the time in which the other hyena was coming back to the indoor pen. Another way of marking was by rolling on the floor.

Especially when the hyena in the outdoor enclosure was approaching the guillotine, the other hyena showed attentive behaviour both to the observer and to the surrounding environment. In a specific case, Hyena B was observed going through the guillotine walking slowly and with light steps, the fur on the back erected and vocalizing (it was a sort of bark/growl). This data was important because they rarely vocalize.

Hyena B seemed to manifest more maintenance activities (urinating, defecating, lying down) than Hyena A. Specifically, Hyena B did more autogrooming compared to Hyena A, licking fur, genitals, and limbs. These actions were performed always in the same areas of the enclosure (Figure 4-3).

The most noticeable observation was that both hyenas showed stereotypes when indoors (2.9 ± 1.81 and 2.3 ± 1.67 events lasting ≥ 5 minutes each in 30 minutes, respectively for Hyeana A and B). They consisted in going back and forth the sides of the fence: Hyena A used to go back and forth the right wall that defined the empty enclosure, in the direction of that was the Hyena B; Hyena B used to walk in front of the guillotine and along the right and left walls of the fence (Figure 4-3).

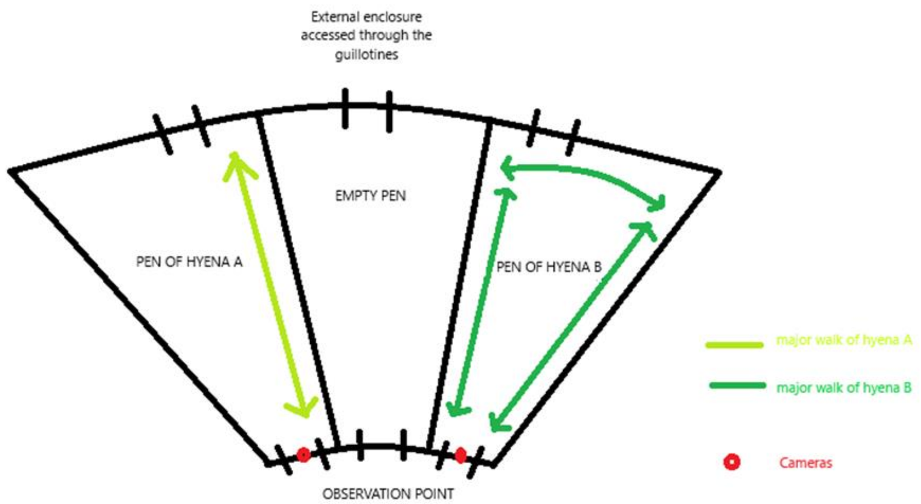
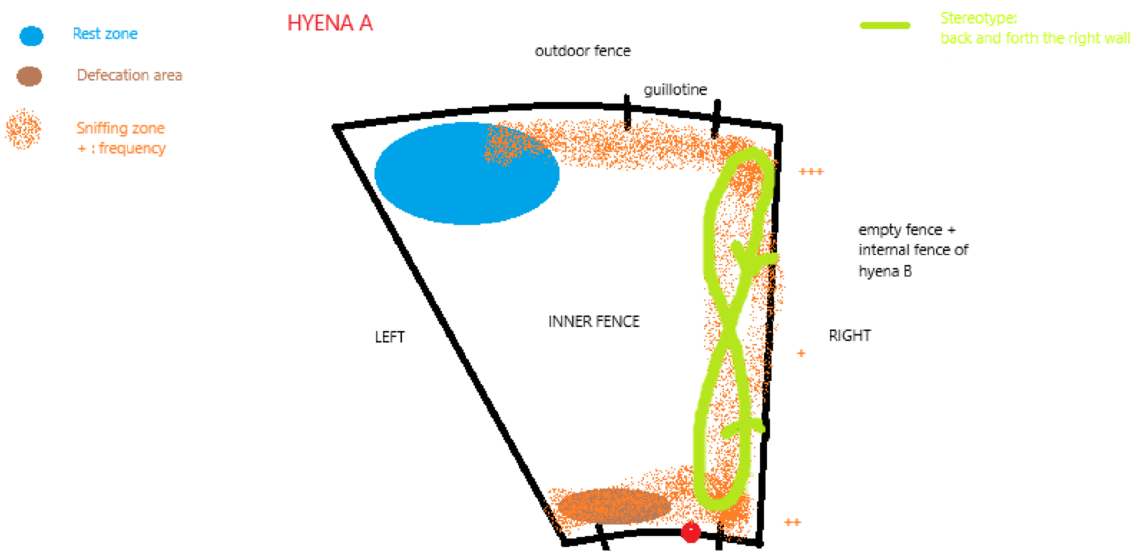
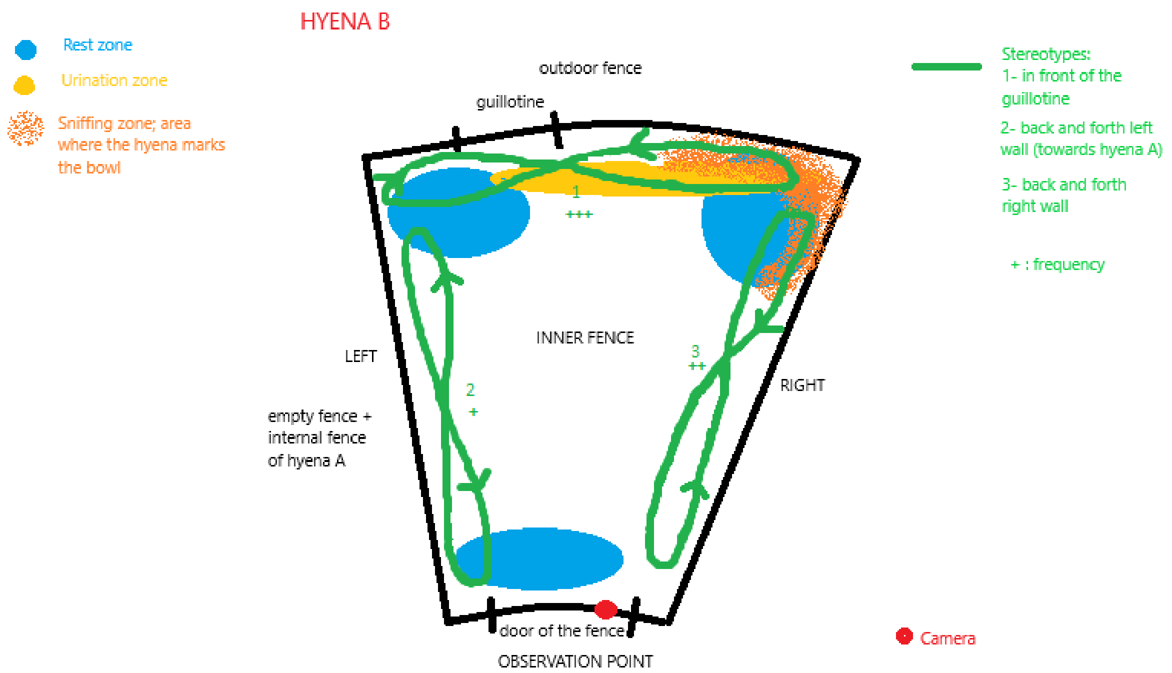


Figure 4-2: Schematic representation of the walking zones covered by the observed hyenas in their indoor fences. In light green, on the left, it is represented the walking path of Hyena A; in dark green, on the right, it is represented the walking path of Hyena B.



a)



b)

Figure 4-3: Schematic representation of the zones of activity covered by the observed hyenas in their indoor fences. a) Hyena A activity zone map. In light green, it is represented the walking path; in blue, it is represented the rest zone; in brown, it is represented the defecation area; in orange, it is represented the sniffing zone. b) Hyena B activity zone map. In dark green, it is represented the walking path; in blue, it is represented the rest zone; in brown, it is represented the defecation area; in orange, it is represented the sniffing zone.

Outdoors (Figure 4-4), they spent most of the time resting and sleeping (according to their nocturnal lifestyle), and to do that, they used always the same areas, without difference between morning and afternoon. The identified beds were three: only one was directly visible from the observation points 2 and 3; the other two were unseen because of the vegetation, in case of the bed used by Hyena B, or because it was in the area not visible from all the observation points, in case of the bed used by Hyena A (Figure 4-5). Videos collected by the camera collocated near this area of the outdoor enclosure confirmed the use of these two beds. Beds consisted of an area without grass, near rocks or trees, hidden by vegetation: similar to the so called “den” (Figure 4-6).

When they were awake, both the hyenas did maintenance activity and sometimes manifested attentive behaviour to the surrounding environment. Locomotion was performed in different areas of the exhibit; each hyena walked through a specific path (Hyena A walked mainly along the perimeter of the enclosure, while Hyena B crossed more frequently also the central areas) and usually marked the shrubs and the ground (especially where they slept) with the anal glands or by rubbing neck and hips; Hyena A did it more frequently than Hyena B (Figure 4-7).

Outdoor, they didn’t vocalize or perform stereotypes. The only behaviour similar to a stereotype was performed once by Hyena B (0.1 ± 0.30 events in 30 minutes): he went back and forth along with the “lions window”; then he urinated and defecated in the same area and at the same time.

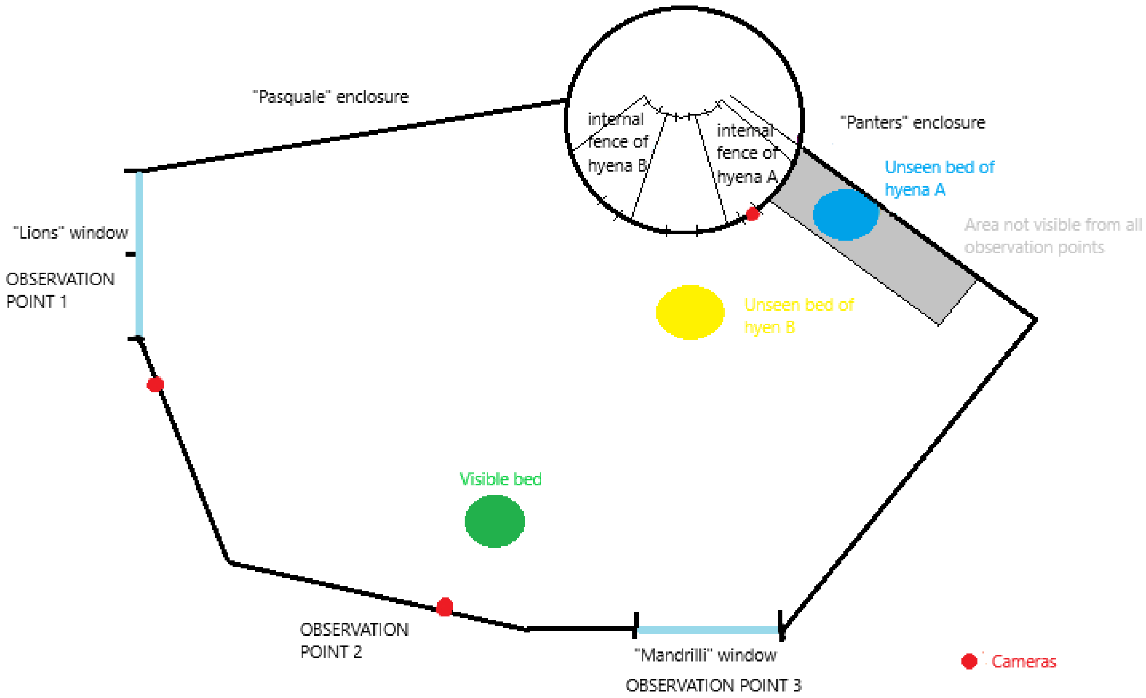


Figure 4-4: Schematic representation of the outdoor enclosure of Striped hyenas' exhibit. The three observation points are: 1. the "lions window", 2. an area of the fence between "lions window" and "mandrilli window", 3. the "mandrilli window". Beds are represented as green, yellow and light blue circles; the light grey area is that which is not visible from all the observation points.

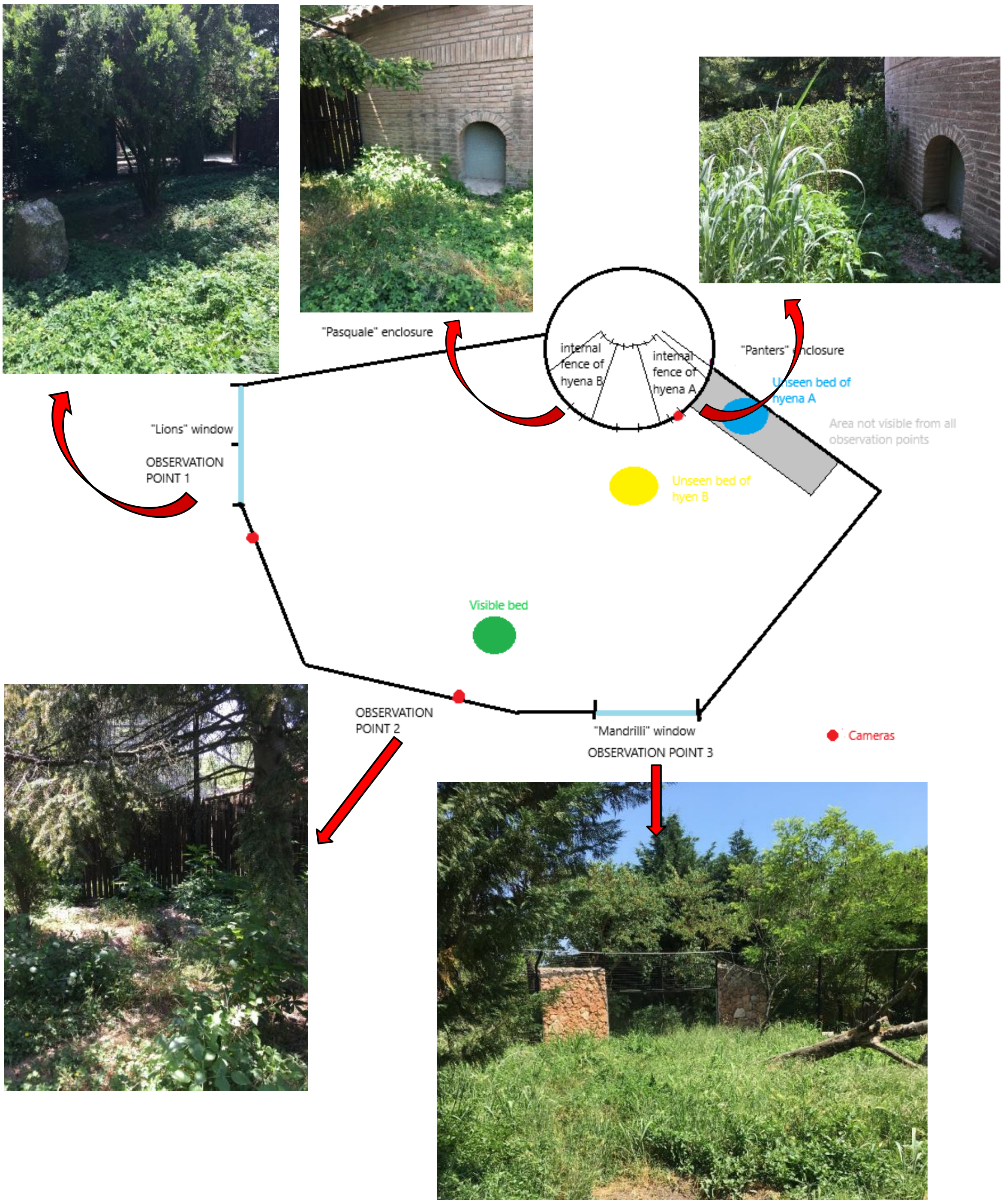


Figure 4-5: Schematic representation of the outdoor enclosure of Striped hyenas' exhibit and pictures of the observation points (view from inside) and of the guillotines through which the hyenas access to the outside.

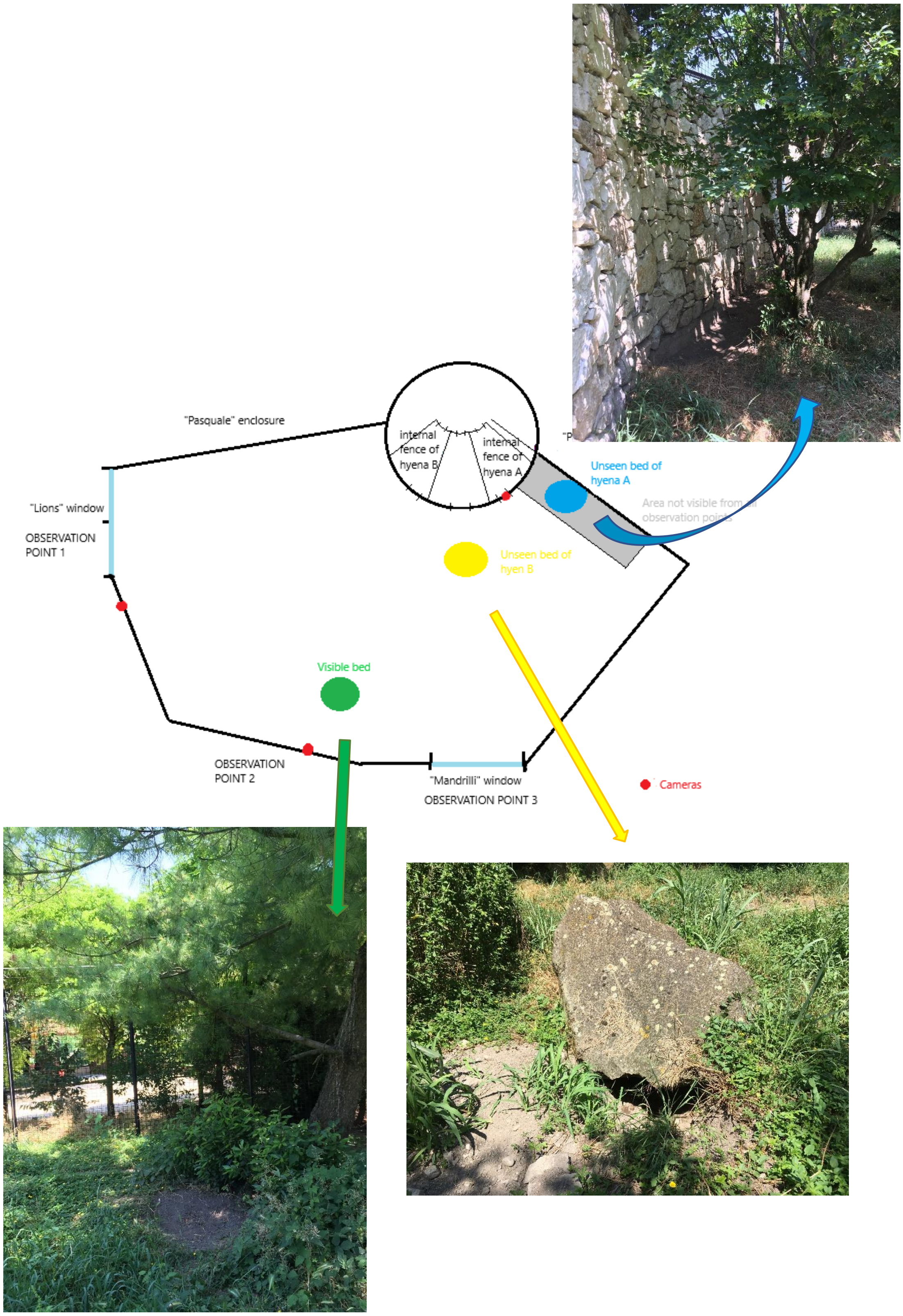


Figure 4-6: Schematic representation of the outdoor enclosure of Striped hyenas' exhibit and pictures of the three dens.

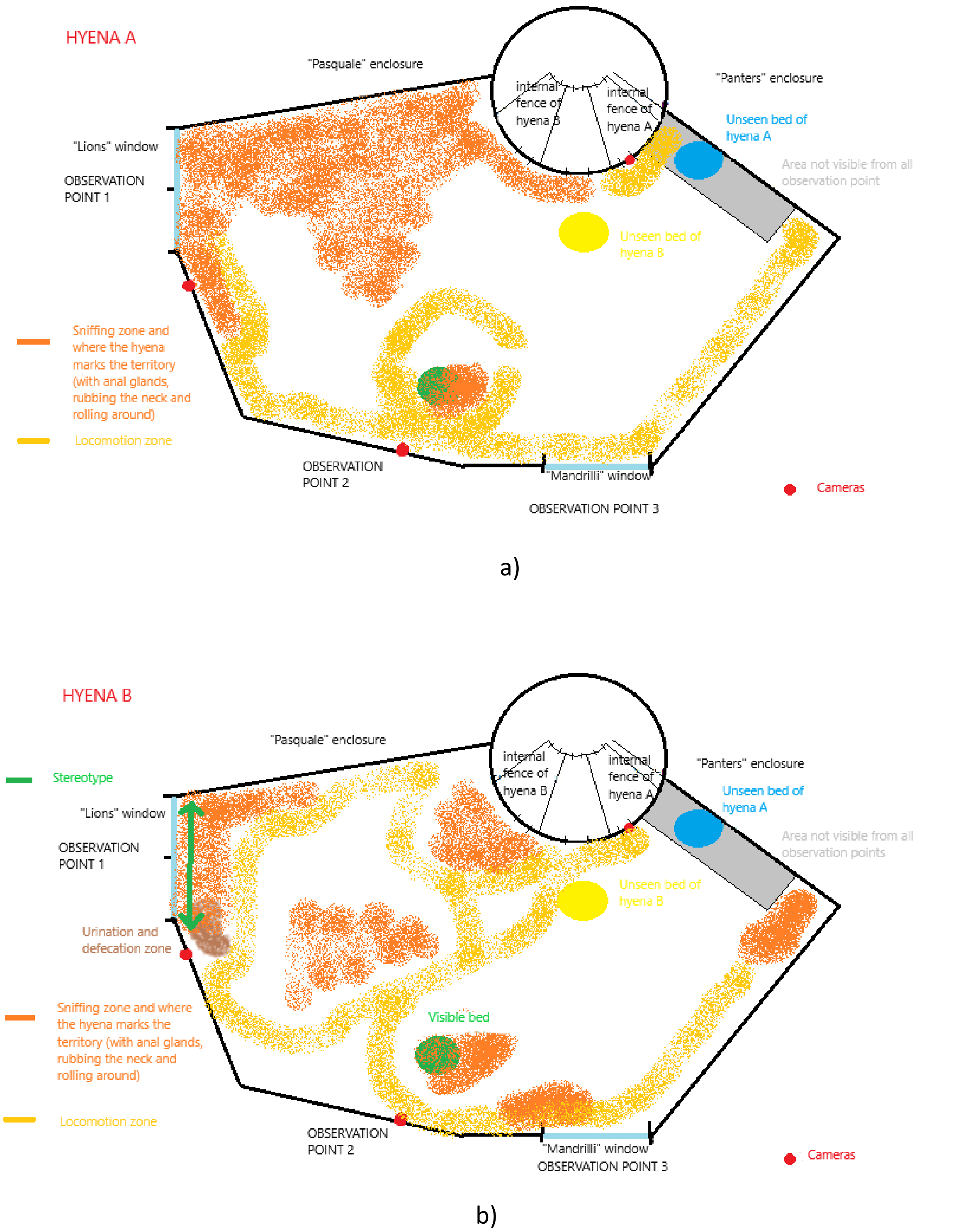


Figure 4-7: Schematic representation of the zones of activity covered by the observed hyenas in the outdoor enclosure. a) Hyena A activity zone map. In orange, it is represented the sniffing zone and marking zone; in yellow, it is represented the locomotion zone. b) Hyena B activity zone map. In dark green, it is represented the walking path where he performs a stereotype; in orange, it is represented the sniffing zone and marking zone; in yellow, it is represented the locomotion zone.

The quantitative analysis of data about the social behaviours, that were observed only inside the indoor fence, had no significant effect of the thesis.
Nevertheless, direct observations highlighted that, during the morning and the evening, before the opening and after the closure of the park, when the hyenas stay indoors in the respective pens, they were always very agitated. They walked a lot, showed attentive behaviours towards the surrounding environment and often repeated their stereotypes.
It must be noticed that the observation times coincided with the moment before the exit of either animal (in the morning) and with the feeding time (in the evening); moreover, at the time of the evening observation, the leopards had already returned to the indoor fences (according to the routine of the park) and this may have contributed to an increased anxiety of the two animals.
Both Hyena A and B marked their water bowls with the anal glands everted several times; this happened mostly in the evening.
Hyena A repeatedly looked in the direction of Hyena B, with raised back fur and swollen tail. Her fur was slightly raised even while performing the stereotypes. Instead, the Hyena B looked in her direction but without ruffling the hair; he seemed to respond to that attitude by marking the bowl of water or (more rarely) with vocalizations.

4.1.1. Resocialization attempts

Regarding to the attempts of resocialization, the first was done a month after putting the hormonal subcutaneous implant (July 4th, 2018). That was the time at which it was expected that the implant started to have an effect, so the hyenas were put in contiguous pens in the indoor area, separated only by a wire fence.
Unfortunately, this first attempt of resocialization did not give positive results because they showed the same hostile behaviour. To record observations, the table for social behaviours was used and they were assessed every 30 minutes, but it had to be stopped soon due to a problem with the fence.
On September 20th, 2018, the second attempt to put the hyenas in contiguous pens was done. Observations lasted one hour and were held in the morning. For the first thirty minutes, they showed the same abhorrent behaviour, but then the female started to look

for positive contact (maybe playful) with the male: she assumed a “sphinx position” with the tail kept up and lied down by the net, near the male. The male maintained his aggressive attitude for the whole time, except a few moments in which he lied down in the corner furthest from the female to calm down. At the end of the session the two animals were in a “neutral” attitude towards each other but still separated by the wire fence.
No other attempts have been done. It was considered not safe to try to put the hyenas in the same enclosure: the indoor fence was too small while the outdoor enclosure was good to allow them to escape or hide, but it would have been difficult to separate them in case they started fighting.

4.2. Hormonal analysis from biological samples

To the best of our knowledge, no reference values for steroid hormones in Striped hyena have been published and, therefore, a comparison with similar data can not be performed.

Individual plasma concentrations of HC, DHEA, E2 and TST are reported in Table 4-1.

HORMONE	UNIT	HYENA	
		A	B
HC	ng/ml	52.0	81.0
DHEA	ng/ml	0.74	0.43
E2	pg/ml	54.80	55.03
TST	ng/ml	2.41	0.94

Table 4-1: Hormonal concentrations (raw data) in blood samples collected on day 0 (day of the implant) before the implant was insert.

The amount of feces analyzed was 202 ± 1.97 mg, respectively 201.6 ± 2.20 mg and 202.4 ± 1.67 mg for Hyena A and B. The results of the descriptive statistics of the hormone concentrations in stool samples are reported in Figure 4-8.

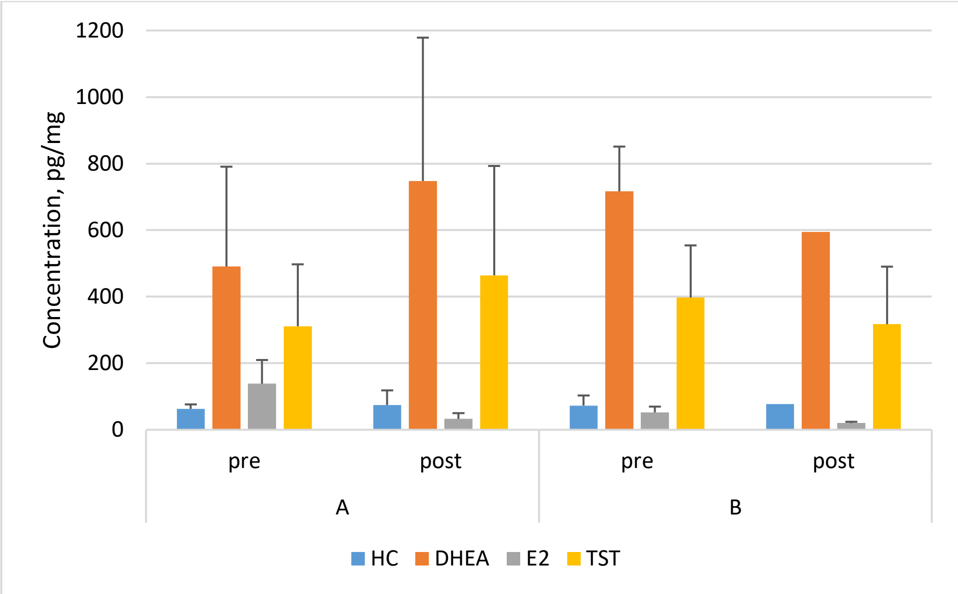


Figure 4-8: Hormonal values (mean and standard deviation) from stool samples taken from the two hyenas (A and B) in the pre- and post- implant intervals.

No significant differences were found for HC, DHEA and TST concentrations in stool samples, according to the main effects studied, nor to their interaction. Conversely, fecal E2 concentrations were significantly different between animals (P<0.01), and were affected by the sampling interval (pre- and post-implant; P<0.001) and the interaction Animal × Sampling interval (P<0.05; Figure 4-9).

E2 is a steroid hormone both present in female's and, although to a lesser extent, male's organism. In Table 4-1, is shown that plasma levels of E2 are high and almost equal between female and male. This feature is reported in male of different species, such as in the stallion, whose reproductive endocrinology is characterized by elevated synthesis of estrogens in comparison to other mammals and seasonal sex steroid production in plasma (Lemazurier et al., 2002). E2's synthesis is stimulated by the release of the gonadotrophins from the pituitary gland under the control of Gonadotropin Releasing Hormone (GnRH); this system is extremely homogeneous in all mammals (Pisu and Romagnoli, 2012).

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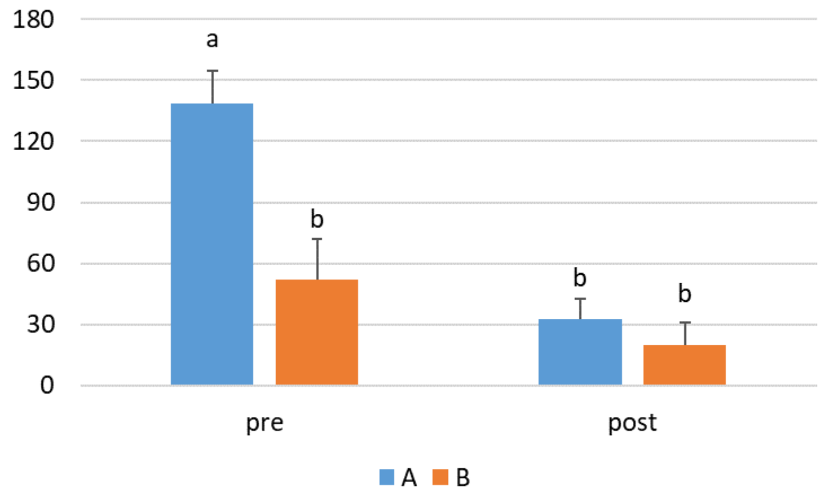


Figure 4-9: Effect of the interaction between hyenas (A and B) and sampling timed (pre and post implantation) on stool E2 concentration (least square mean and standard error; pg/mg).

GnRH agonists suppress the reproductive endocrine system, preventing production of pituitary and gonadal hormones. They can be used to prevent temporarily unwanted pairings and to suppress any behaviours related to sexual motivation. As an agonist of the GnRH, Deslorelin acetate initially stimulates the reproductive system, which can result in oestrum and ovulation in females or temporary enhancement of TST and spermatogenesis in males. After that phase, no oestrous cycle will occur.

Contraceptive methods are widely used to manage wildlife population by controlling fertility and are included in many conservation plans; their use in zoos is controlled by the AZA Reproductive Management Center (RMC) and the EAZA Group for Zoo Animal Contraception (EGZAC) (Asa and Moresco, 2019).

The use of Deslorelin acetate as reversible contraception in Striped hyenas is considered safe by relying on similar studies concerning exotic wild carnivores, such as African painted dog (Bertschinger et al., 2001), but dosages and duration of efficacy are not well established. The initial stimulation of the reproductive system lasts 3 weeks on average, in both females and males. Due to this lack of precise information about the use of this principle to ameliorate behaviour in this species, the veterinarian of the park decided to use only one 4.7 mg implant for each hyena, following a principle of caution in formulating the dosage. As after the insertion of the implants, the fecal concentrations of E2 were decreased in both animals, this suggested that the hormonal implants were effective.

The biological samples were not collected during the first days after the implantation, therefore, the temporary stimulation of the reproductive system could not be proved. Moreover, no oestrum was seen during the three weeks after the implantation, but this may be due to female Striped hyena's oestrum is difficult to identified, since oestrus signs are mild and last for one day only (Wagner, 2006).

In males, TST is related to aggression and TST release is likely to decrease following Deslorelin administration. In this case, Hyena B did not show any significant decrease in the TST levels after the implantation and neither an improvement in his behaviour was seen.

From the results it is notable that levels of TST are higher in the female than in the male. It is known that in female Spotted hyena the levels of Androstenedione are higher in females than in males. Androstenedione is a pro-hormone produced by the ovaries that can be converted either into Estrogens or TST within the target tissues. The placenta of the Spotted hyena is very efficient in converting Androstenedione into TST and puppies of both sexes are exposed to high levels of TST in the uterus: this leads to a masculinisation of the female genitals. Its ethological meaning is that the social structure of this animal is matriarchal, so the dominant female needs to be more aggressive and is bigger in size (Muller and Wrangham, 2002).

Striped hyenas are mainly solitary animals, so females do not need those characteristics: they have not masculinized genitals (Wagner, 2006), but no information about levels of androgens are reported in literature.

Another consideration is that, as the reproductive cycles of the two animals were not monitored, it is not certain that the implants were inserted at the most appropriate time.

This is one of the first studies that provides information about the concentration of steroid hormones in the Striped hyena and it could be used as comparison parameter for future studies regarding this species.

4.3. Qualitative Behavioural Assessment

Data of QBA were summarized in two principal components (PC1 and PC2) explaining in total 63.2% of the variability with 48.5% and 14.7% of it explained by the first component and the second component, respectively. Loadings of each term on PC1 or PC2 are reported in Table 4-2 and their graphical distribution is represented in Figure 4-10. The first PC summarized positive mood descriptors (*relaxed, friendly, sociable, calm, positively occupied, enjoying, content* and *happy*) with positive loadings and negative descriptors (*tense, frustrated, uneasy, nervous* and *distressed*) with negative loadings. High scores indicate high positive and low scores indicate high negative mood. The second PC summarized activity descriptors (*active, inquisitive* and *boisterous*) with positive loadings and inactivity descriptors (*uncomfortable, irritable, indifferent* and *bored*) with negative loadings. High scores indicate higher activity levels while low scores indicate higher depression, indifference and boredom.

	PC1	PC2
Active	0.095	**0.452**
Relaxed	**0.719**	0.002
Uncomfortable	**0.758**	0.031
Calm	**0.731**	0.019
Content	**0.732**	0.086
Tense	**0.716**	0.001
Enjoying	**0.701**	0.086
Indifferent	0.006	**0.322**
Frustrated	**0.713**	0.046
Friendly	**0.340**	0.002
Bored	0.235	**0.432**
Positively occupied	**0.631**	0.140
Inquisitive	0.172	**0.466**
Irritable	**0.724**	0.010
Nervous	**0.796**	0.015
Boisterous	0.038	**0.582**
Uneasy	**0.351**	0.001
Sociable	**0.252**	0.000
Happy	**0.691**	0.079
Distressed	**0.299**	0.172

Table 4-2: Qualitative Behavioural assessment fixed term list and loadings of each term on the first and second principal component PC1 and PC2. Bolded values ≥ 0.25 means loading on PC1 or PC2

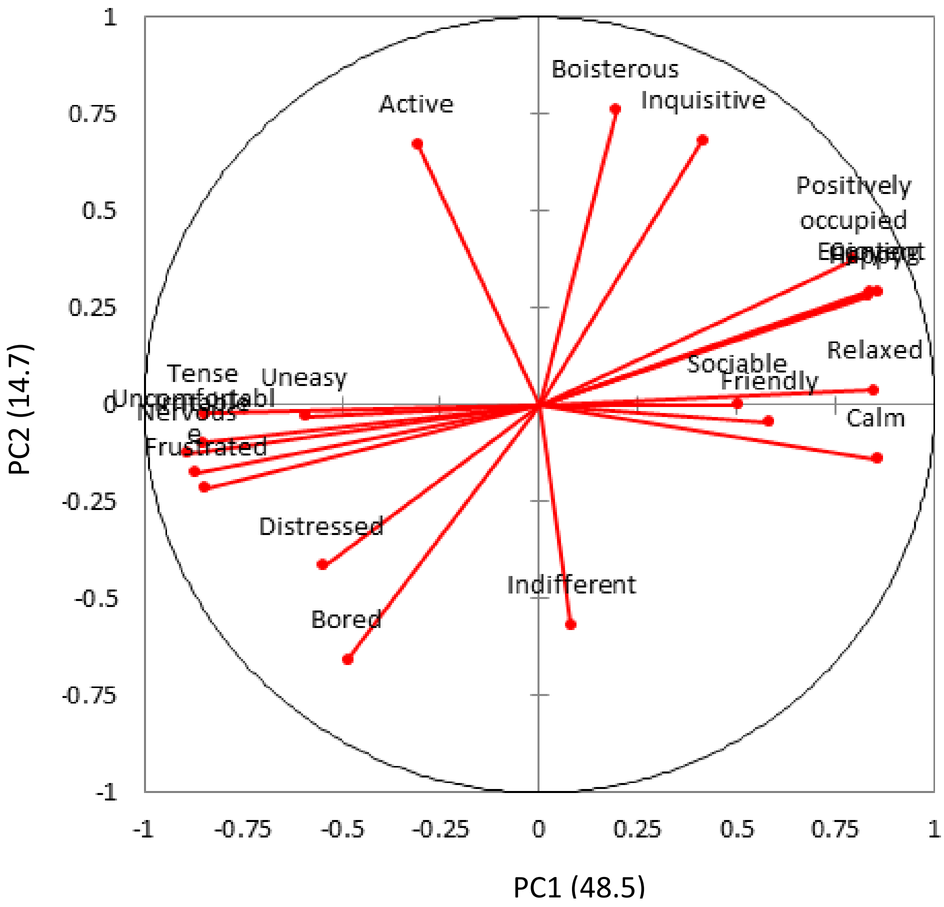
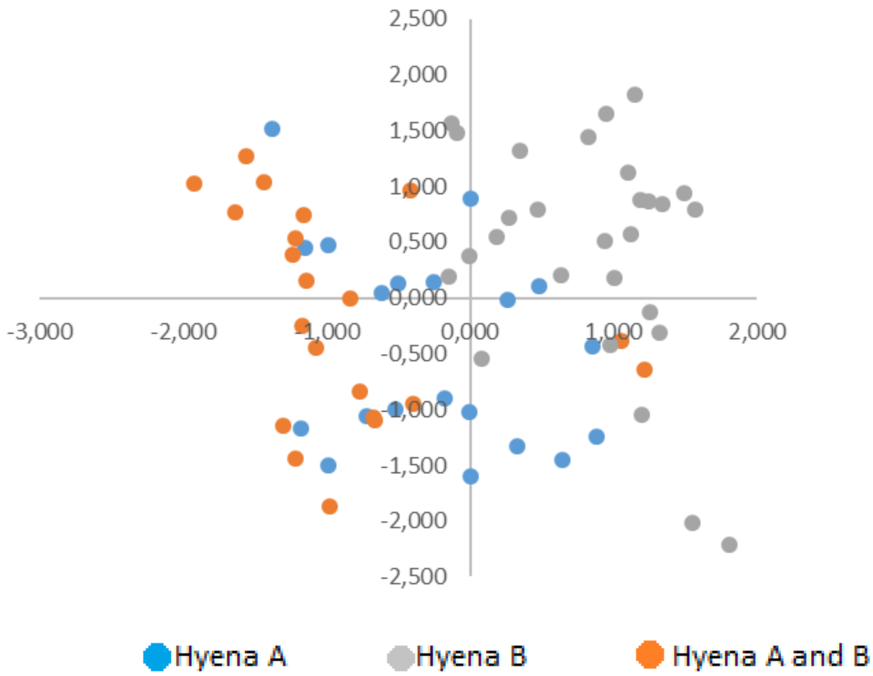


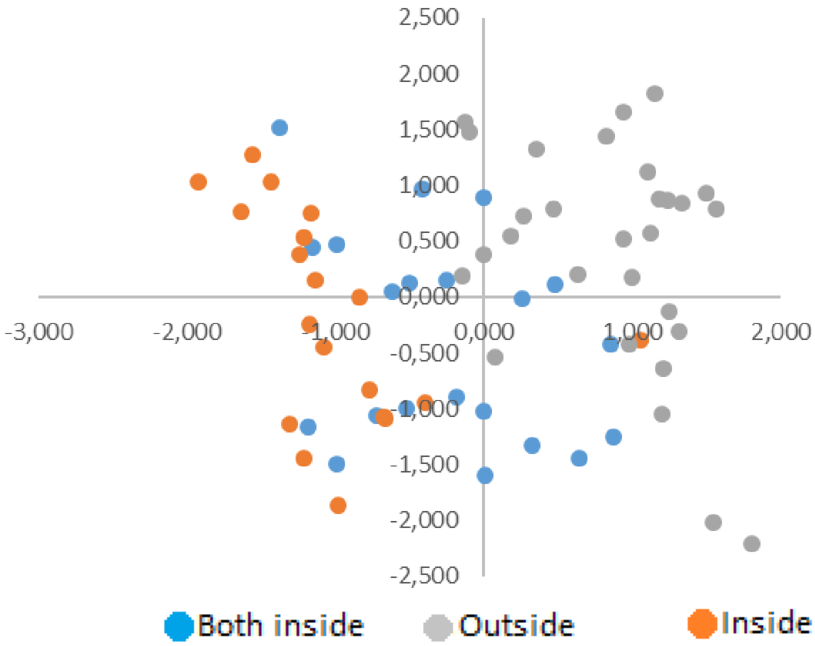
Figure 4-10: Graphical representation of the QBA terms. PC1 (horizontal axis) summarized positive mood descriptors (relaxed, friendly, sociable, calm, positively occupied, enjoying, content and happy) with positive loadings and negative descriptors (tense, frustrated, uneasy, nervous and distressed) with negative loadings. High scores indicate high positive and low scores indicate high negative mood. PC2 (vertical axis) summarized activity descriptors (active, inquisitive and boisterous) with positive loadings and inactivity descriptors (uncomfortable, irritable, indifferent and bored) with negative loadings. High scores indicate higher activity levels while low scores indicate higher depression, indifference and boredom.

Results of the analysis of variance showed a significant effect of the thesis on both PC1 ($P < 0.001$) and PC2 ($P < 0.01$). Results revealed that a more positive mood (PC1) was recorded when hyenas were outdoors with similar estimates for Hyena A and B, intermediate values when both hyenas were indoors and Hyena B indoor whereas the lowest estimate was for Hyena A indoors. This result would suggest the need to ensure a better welfare by allowing both animals the opportunity of daily access to the outdoor enclosure. Hyena B had the highest PC2 estimate when outdoors indicating a greater level of activity whereas the lowest estimates were attributed to Hyena A indoors and when both hyenas were indoors with the other combinations having intermediate values. It seemed therefore that both hyenas showed discomfort and stress when they were kept in the indoor enclosure either individually or together with Hyena B being more active and in a more positive mental state compared to Hyena A.

The graphical representation of the PC1 and PC2 according to Hyena and to location are reported in (Figure 4-11a and b). The latter graph allows to point out that there is an effect of the location on hyenas' emotional state and in particular when they are outdoors, they manifested high positive mood while indoors they had a majority of negative attitudes.



a)



b)

Figure 4-11: a) distribution of PC1 and PC2 according to the Hyena(s), hyena A (light blue), B (grey) and together (orange); b) distribution of PC1 and PC2 according to the location both indoors (orange) outdoor (grey), and indoors (light blue).

The results of QBA in this study in terms of variability explained could support its application as a method to assess emotional expression in hyenas, although it is not yet applied routinely to zoo animals and it is not possible to statistical inference. A pilot study investigated Human-Animal Relationship (HAR) in the zoo-housed giraffe and suggested QBA being a valid method to investigate it and its implications on animal welfare within the zoo environment (Patel et al., 2019). In an analogous way, QBA has been successfully applied to dogs suggesting its applicability in captivity contexts as those common in zoos. Dogs in shelters live in confinement and have necessity in terms of welfare that are common to all animals in the same living situation, such as provision of environmental enrichments to reduce boredom (Walker et al., 2010). The fixed list of QBA descriptors applied to video-based assessments of dogs living in a shelters (Arena et al., 2019) was consulted along with fixed lists (FL) for other animal species used in Welfare Quality and subsequent EU projects. However, it was not set starting from free choice profiling (FCP). The use of FL may have more problems in achieving observer consensus than using FCP because individual observers can have different ethical values and understanding of the meaning of the descriptive terms they score (Fleming et al., 2016). However, several studies using FL in farm animals have shown that observers can also reach consensus and some findings found little evidence that observer ratings were influenced by the use of FCP or FL methods (Clarke et al., 2016).

One of the challenges in the interpretation of these results was to understand if the environment in which the hyena was recorded could have influenced the observer's score in a more positive or negative sense because the observers were not blind. This major shortcoming could be discussed as suggested by Fleming et al. (2015) who highlighted that taking the environment into account and evaluating the animal's situation allows observers to make a more discerning and powerful judgment of an animal's behavioural style, but the observers' judgment of that context also makes qualitative assessments vulnerable to undesirable bias. There is the necessity to manage the emotion perception to limit potentially distortive effects on the integrity of scientific assessments; that could be done through appropriate instruction, training and experimental design.

However, several studies have proven that even observers with little experience with the animal species in question can valuably contribute to qualitative assessments, reaching consensus with other observers (even with more experienced ones) in scoring the animals with the only difference between animal-experienced and inexperienced observers related to the fact that experienced observers are less likely to use the extremes of their raw visual analogue scales. The reason why QBA does not necessarily rely on the observer having experience with animals is because they both (expert and inexpert observers) perceive the differences in the animal's expression and statistical processes, identifying the common dimensions of the score, are independent of subjective interpretation (Clarke et al., 2016). The QBA method is often not enough as the only evaluation carried out. Recent research has shown statistically significant correlations between QBA scores and physiological indicators relevant to welfare, but to help interpret what the difference indicates about welfare, the judgment of experts is needed (Fleming et al., 2016). Another study on the correlation between a qualitative method (QBA) and a quantitative method (clinical/health evaluation) on veal calves, highlighted that QBA was sensitive to factors that did not affect clinical measures. Thus, the two methods appear to provide complementary information and may be suitable to be used in an integrative holistic animal welfare assessment approach (Brscic et al., 2009).

4.4. Literature research and collection of experiences

Results from the review of scientific literature reveal that, regarding the situation in wildlife, hyenas are important and influential components of most African and some Asian ecosystems. Unfortunately, human interests often conflict with those of hyenas to a greater extent than with many other groups of animals. Although there are status survey and conservation action plans that deal with the four living species of the family Hyaenidae, the most important challenge is to overcome cultural and public attitudes, improving the relationship between humans and hyenas. This can be done through the communication of research findings on hyenas, not only in scientific journals, but also in popular articles and books, and concerted education campaigns.

The data collected during the compilation of the action plans suggest that, of the four Hyaenidae species, Striped hyena is the one in most need of conservation attention, but it is also the less studied. That's why it is extremely difficult to assess the status and distribution of these animals and to monitor population trends: they are nocturnal, live at low densities and aren't easily distinguished from the other Hyaenidae. Moreover, Striped hyena lack of positive interest because it evokes many superstitious fears due to the reputation and documented cases of injuries to adults sleeping outside, snatching and killing of children and grave robbery. Most cultures consider the Striped hyena to be a predator of livestock. Besides, it is widely exploited as an aphrodisiac and utilized for traditional healing. In most African countries Striped hyena can be hunt and this led to illegal commerce of trophies, such as skulls and skins.

Historically, all four hyena's species have been commonly kept in captivity, but often not kept well. Zoos obtained them as an afterthought to their collection, using them to fill empty cages until something "better" came along. Later, in subsequent planning processes, many zoos allocated larger and better facilities to taxa considered more charismatic by the public and staff. As a result of this wrong approach to hyena's husbandry, they have been sporadically propagated and too often been relegated to inferior exhibits. Now they are not common in most of the world's captive collections (Mills and Hofer 1998).

Often, when hyenas are kept in captivity, they showed behavioural problems, that become evident when a new animal is to be introduced.

Out of a selected list of 50 European and non-European zoos where the Striped hyenas are currently present, only Augsburg zoo, Utica zoo, zoo de la Barben, Minsk zoo, Twycross zoo, Kiev zoo, Bratislava zoo, and CERZA zoo provided a feedback. They reported that the fighting between genders is almost a normal behaviour and many of them have similar socialization problems with this species, solved in all cases by keeping the animals separate and this can be easily attributed to the mainly solitary nature of this species. Everyone agreed that keeping the animals separate is stressful for them, suggesting that the access to the outside enclosure should be guaranteed daily to both animals and not every two days.

Moreover, between the two genders, aggressiveness, biting and injuries are very common. Since these problems stated when they became sexually mature (2 – 3 years old), that should have become a concern. Biting and aggression could be part of their reproductive behaviour and it’s reported that the female is often more aggressive. It could be possible to join a female and a male when she is or not in oestrum adopting different approaces: anyway, female´s oestrum is difficult to detect because she does only some vocalizations, increases the interest in the male, and everything lasts only for short time (one day). The male does not show any changes in behaviour. A further hypothesis aroused through the collection of experiences was related to the fact that hyenas in this case (arrived to the Valcorba park as to the park as 5 months old cubs) might have been taken from parents too early and hand reared and have thus lost continuity of social learning and which have led to behavioural problems. In addition, it is not confirmed that using Deslorelin as a contracept approach is a good way to achieve the goal.

Many of the feedbacks suggested to contact the coordinator of the regional breeding program led by the EAZA for Striped hyenas, but, unfortunately, it was not possible to get in touch with him.

Also, the Italian wildlife park from which the two specimens came was contacted. They confirmed that the animals were hand reared and they didn’t encounter behavioural problems in their parents, but they confirmed that the pair were kept constantly separate and reunited only for reproductive reasons.

The feedbacks are summarized in a Word Cloud, reported in Figure 4-12 in a visual format to highlight important textual data points: the more frequent a specific word appears in a source of textual data, the bigger and bolder it appears in the word cloud.

As shown, the most relevant words were: *animals* (16); *female* (14); *time* (11); *enclosure* (9); *male* (9); *old/years* (8); *striped/hyenas* (8); *outside/outdoor* (7); *species* (6); *breeding* (5); *problem* (5); *inside* (4); *zoo* (4); *separation/separate* (4); *together* (4); *injuries* (4); *aggressive/aggressivity* (3); although no robust scientific evidence could be given to these results (Ramsden and Bate, 2009).

Figure 4-12: Visualization format of Word Cloud processing.

5. CONCLUSIONS

This case study aimed at resocializing two siblings Striped hyenas (Hyaena hyaena) that live in a wildlife park in northeastern Italy, in order to improve their welfare status by allowing a daily access to the outdoor enclosure for both of them. However, this resocialization attempt failed, and results of this study contributed to gain knowledge on the different hypothesis and reasons why the resocialization did not work. The case study approach was used since it was not possible to carry out any experimental trial and many notions were lacking before setting the study. Moreover, research demands had to be adapted to the zoo context and management that prioritize different needs of owners, staff, visitors, and animals hosted. More detailed information about the biology and captive management of Striped hyenas were gained during the study, also through the sharing of experiences from other zoos and experts' advices.

In general, the quantitative analysis revealed that the animals expressed more positive behavioural attitudes when kept in the outdoor enclosure. They were clinically healthy and did not show extreme negative attitudes which suggested that the animals had, in some way, adapted to the situation. They showed more frequent stereotyped behaviours when housed indoors, thus an improvement of animals' management (e.g. enrich the indoor housing and provide enrichment when housed indoors) could achieve a better welfare status. Results of this study proved also that a deeper knowledge of the biology and the ethogram of each species is required in order to design an adequate exhibit that allows them to express all their behavioural repertoire. Unfortunately, such information is not available for all the species housed in a zoo, because the species are less common within parks or just less studied. Indeed, the Striped hyena is not well known and many of the information on this species derive from the analogy with Spotted hyena. Over the years, that led to some errors in its management including the group housing (in this case a couple) while Striped hyenas are mainly solitary. Additionally, hyenas are allowed to access the outdoor enclosure during the day while they are nocturnal animals. The park itself recognized that this approach was not completely adequate for the hyenas, and they asked for support and consultation. This is the prove that zoological gardens are nowadays developing a deep consciousness of the importance of animal welfare.

Qualitative behavioural assessment turned out to be an effective method of detecting the mental state of animals and demonstrated that even unexperienced observers could correctly interpret the welfare of animals pointing out differences in response between the two hyenas. A major shortcoming in this case could arise from the fact that observers were not blind in regard to the location, although other studies have demonstrated that this was an enforcing strategy to recognise animals' mental state when observers were correctly informed.

The Deslorelin implant is a method of reversible contraception widely used in this species, but to monitor it effectiveness it would be necessary to perform a close tracking of the oestrous cycle, before and after implantation. Unfortunately, in this case, it was not possible to properly perform this monitoring. However, we assumed that the implant started working based on the changes in the levels of E2, highlighted by the results of the hormonal analyses from stools samples. Despite this, there were no ameliorative changes in the behaviour of the two animals which maintained the same hostile attitude towards each other. Therefore, it was possible to say that the use of Deslorelin as a contraceptive was not effective in the attempt to resocialize the hyenas. In case of positive outcome and resocialization of the hyenas, a permanent sterilization of the animals would have been probably chosen as alternative to the implant. This in particular due to the fact that the resocialization of the animals was not aimed at reproductive purposes because the two hyenas are siblings and inbreeding has to be avoided, thus mating is controlled by specific conservation programmes of the species. Currently, the management of the hyenas in the park is not changed, and they are kept separate in the same exhibit and have access to the outdoor enclosure every other day. Before this study, it was not known that behavioural problems were common in the captive management of this species. Thus, in order to improve their welfare status, the solution that is thought to be adopted in the near future is to rearrange one of the exhibits still present in the park and move one of the hyenas into a different exhibit. This was further supported by the spatial use of their actual exhibit.

Enforcing the role of the modern zoological garden, such study could help to increment the knowledge of this poorly studied animal species and provide information to allow to improve animal welfare through a proper management in captivity. In particular when considering that the Striped hyena is a near-threatened species and it is likely to be of interest in future conservation programs.

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ANNEX A: BEHAVIOURS

Date ____________________

Hyena's Identification ___________________

INDIVIDUAL AND SOCIAL BEHAVIOURS

		NORMAL AND AFFILIATIVE								COMPETITIVE		ABNORMAL	UNSEEN
Start time	End time	Attentive Behaviour	Maintenance	Autogrooming	Territ Expl Locom	Social	Reprod.	Inactivity	Vocaliz	Dominance	Submission	Stereotype	

Date ____________________

Hyena's Identification ___________________

INDIVIDUAL BEHAVIOURS

		NORMAL								ABNORMAL	UNSEEN
Start time	End time	Attentive behaviour	Maintenance	Autogrooming	Territoriality	Exploration	Locomotion	Inactivity	Vocalizations	Stereotype	

ANNEX B: QBA FOR VIDEOS

Video number _______

Please observe the animals in the unit, and then asssess their behavioural expression ('body language') by scoring the following terms:

Min. Max.

Active

Min. Max.

Relaxed

Min. Max.

Uncomfortable

Min. Max.

Calm

Min. Max.

Content

Min. Max.

Tense

Min. Max.

Enjoying

Min. Max.

Indifferent

Min. Max.

Frustrated

Min. Max.

Friendly

Min. Max.

Bored

Min. Max.

Positevely Occupied

Min. Max.

Inquisitive

Min. Max.

Irritable

Min. Max.

Nervous

Min. Max.

Boisterous

Min. Max.

Uneasy

Min. Max.

Sociable

Min. Max.

Happy

Min. Max.

Distressed

General comments or observations:

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