UNIVERSITY OF TRÁS-OS-MONTES E ALTO DOURO

Veterinary Medicine in Exotic and Wild Species

"Behavioral enrichment and management of two captive Snow Leopards (*Panthera uncia uncia*)"

Master Thesis in Veterinary Medicine

Diana Raquel Neves Fernandes

Supervisor: Maria de Lurdes Ribeiro Pinto, DVM, MSc, PhD

Vila Real, July of 2017

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Examination Committee

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For Ariana and Kamal

“Every creature is better alive than dead, men and moose and pine trees, and he who understands it aright will rather preserve its life than destroy it."

Henry D. Thoreau

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To my Family, not only for their massive support towards this harsh final stage, but for all the years in which they allowed, put up with and even promoted my personal eccentricities. To my uncle Hélder, for helping me in every aspect of my travels and for diving in this wild journey with me. I thank you all for backing me up in this incredible Adventure, for sparing no efforts so I could discover a world without borders, gain experience and grow personally and professionally. It would definitely never happen without you.

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Resumo

A comunidade Zoológica tem sofrido alterações marcantes, ao longo das últimas décadas, na conduta diretiva das suas instituições. Apesar de continuarem a ser centros lúdicos com fins lucrativos, objetivos de outra natureza têm, agora, maior prioridade: a conservação da fauna e flora mundiais e a Proteção do Bem-Estar Animal. Inúmeras espécies mantidas em cativeiro têm dificuldade em adaptar-se aos ambientes artificiais em que vivem e em lidar com o stress a que neles são submetidas, acabando por desenvolver distúrbios comportamentais ou aumentar a sua suscetibilidade a fatores de risco que possam exacerbar diversas patologias. A análise comportamental é uma área em desenvolvimento que se tem provado uma ferramenta essencial na avaliação do bem-estar geral dos animais cativos, ao permitir a deteção precoce de problemas comportamentais e a correta intervenção para o maneio e resolução dos mesmos.

Neste trabalho, é descrito um estudo comportamental realizado com felinos da espécie Pantera das Neves (*Panthera uncia*), um macho e uma fêmea residentes no Zoo de Santo Inácio, Portugal, nas quais foram identificados determinados padrões de comportamento anormal como comportamento estereotipado (pacing) por parte do macho, e agressividade, ansiedade e relutância em sair da recolha por parte da fêmea. Os animais foram observados de manhã, à tarde e ao final do dia, recorrendo ao método de amostragem focal contínua, após ser construído um etograma organizado em 8 categorias funcionais de comportamento. Este trabalho teve por objetivo avaliar os efeitos do enriquecimento ambiental no comportamento dos dois indivíduos e determinar se os seus problemas comportamentais poderiam ser assim corrigidos, bem como o seu reportório comportamental aumentado. Paralelamente, foi criado um protocolo de maneio comportamental que tinha como objetivo proceder á introdução gradual dos dois animais com fins reprodutivos. O estudo foi dividido em 3 etapas: Controlo (fase I); Enriquecimento Ambiental (fase II); e Enriquecimento Ambiental e Social (fase III). Os resultados demonstraram diferenças estatisticamente significativas entre as fases para as categorias Afiliativo, Atividade e Agonista no período da manhã, bem como para a categoria Afiliativo no período do fim do dia, para a fêmea. No macho, as categorias que sofreram diferenças estatisticamente significativas incluem Atividade, Marcação, Estereótipo e Afiliativo no período da manhã; Afiliativo nas tardes; e Exploratório e Afiliativo no final do dia.

Palavras-chave: Enriquecimento Ambiental, Enriquecimento Social, *Panthera uncia uncia*, Bem-Estar Animal, Medicina Veterinária, Maneio reprodutivo

Abstract

Parks and Zoological gardens have undergone outstanding changes over the decades. Although they continue to be recreative centers with profitable ends, major goals have won priority: the conservation of worldwide fauna and flora and the protection of animal welfare. Numerous captive species have difficulty coping with the artificial environments in which they live and dealing with the stress they are submitted to. They may, eventually, develop behavioral disorders or increase their susceptibility to risk factors that will exacerbate other pathologies. Behavior analysis is an evolving field that is proving itself as an essential tool in the assessment of the overall welfare of animals in captivity as it enables the early detection of behavioral problems and the use of effective intervention techniques concerning their management and resolution. A behavioral study was conducted in the months December through March in a male and a female of Snow Leopards (*Panthera uncia uncia*) housed at Saint Inácios's Zoo in Portugal, in which were identified certain abnormal behavior patterns. The first presented stereotypic behavior (pacing) whereas the second showed signs of aggression, anxiety and reluctance to leave the indoor enclosure. The animals were recorded during the morning, afternoon and evening, using the Continuous Focal Animal Sampling method, after the construction of an Ethogram and its organization into 8 functional behavioral categories. The aim of the study was to evaluate the effects of environmental enrichment on the behavior of the two individuals and to determine if their behavioral problems could be rectified as well as their behavioral repertoire expanded. A behavioral management protocol was developed simultaneously in order to progressively introduce the animals to each other, bearing in mind reproductive ends. The study was divided into 3 stages: Baseline (phase I); Environmental Enrichment (phase II); and Environmental and Social Enrichment (phase III). Results have shown statistically significant differences between the different phases, for the Affiliative, Active and Agonist categories during morning period, as for Affiliative category in the evening as well, regarding the female. In the male, categories which suffered statistically significant differences included Active, Marking, Stereotypy and Affiliative, during morning; Affiliative in the afternoon; and both Exploratory and Affiliative in the evening.

Keywords: Behavioral Enrichment, Social Enrichment, *Panthera uncia uncia*, Animal Welfare, Veterinary Medicine, Reproductive Management

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List of Acronyms, Abbreviations and Symbols

AZA	Association of Zoos & Aquariums
AW	Animal Welfare
BCS	Body Condition Score
CITES	Convention on International Trade in Endangered Species of Fauna and Flora
DLSR	Digital Single-lens Reflex
EEP	European Endangered Species Programmes
ESB	European Studbooks
FAWC	Farm Animal Welfare Council
GSLEPP	Global Snow Leopard and Ecosystem Protection Program
IUCN	International Union for Conservation of Nature
SL	Snow Leopard
SLSS	Snow Leopard Survival Strategy
SPSS	Statistical Package for the Social Sciences
SSP	Species Survival Plan
TAG	Taxon Advisory Group
WAZA	World Association of Zoos and Aquariums
ZIMS	Zoological Information Management System
~	Approximately

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1. Introduction

The enigmatic and rare Snow Leopard which inhabits the high and extremely harsh mountainous regions of Central and South Asia is the least known of all the big cats, and most of the knowledge about its behavior and biology comes from captive studies (19, 52, 53). This wild cat's future is uncertain as it is listed as Endangered in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species since 1988 and the current population trend is known to be decreasing (132). It is also included in the Convention on International Trade in Endangered Species of Fauna and Flora (CITES), Appendix I (01/07/1975)(29). Wild and field observations are scarce and difficult due to its elusive nature, exceptional camouflage, remote and virtually inaccessible habitat and broken population, which is why it has gained the title of "ghost of the mountains" (9, 51, 64). Behavioral and reproductive indicators of welfare problems of this species in captivity currently require remediable plans of action, in which several aspects of the animal's environment are modified and refined in attempt to reduce or eliminate stressful stimulus and aberrant behavior (60). The concept of environmental enrichment was first introduced in the 20s, but only recently it yield an exponential evolution. A global awareness of its importance and benefits in animal area allowed its raise to the tittle of a "management rule" to apply systematically in all zoological institutions. It is a principle that aims to enhance the animal's quality of life, ensuring their physical, psychological and even social well-being (91). In the present study, a series of enrichment items were used in a non-discriminative way in order to alter the animal's routine and environment as a means to serve the following objectives:

- Decrease levels of exhibited abnormal behavior such as stereotypy and agonist demeanor (including aggressive and fearful behaviors) as well as to elicit a new range of behaviors that would increase species-specific natural behavioral repertoire.
- Analysis of overall behavioral changes after enrichment provision.
- Creation of a specific enrichment plan for this species at Saint Inácio's Zoo which could be extended to the other felids in the institution.
- Introduction of two animals of the same species with compliance for conservative and reproductive goals, as well as the analysis of its implications for Animal Welfare.

2. Literature Review

2.1. Zoos' History, Evolution and Current missions

Zoological institutions have served many different purposes and undergone a remarkable evolution throughout the years until the present days. The first models of modern zoos emerged during 18th century, a period known as "Age of Enlightenment" and characterized by educational and scientific endeavors such as animal behavior and anatomy research, which brought a new perspective and goal to the outdated "Menageries" (99). In the 1970's, public opinion began to change and society become more sensitive to the ethical concepts of environmental and animal welfare, leading to the emergence of movements which drove zoos to develop new intentional statements. Such intentions would emphasize the conservation of endangered species, animal welfare and more naturalistic exhibits' construction, turning the entertainment of visitors into a secondary objective (21,118). In this day and age, modern zoos have a far more important role and are beyond the old tight and empty iron barred cages with concrete floors (Figure 1). Despite the fact that they are still profitable and recreational centers, they are now driven by a new set of missions and ethical principles: education and public awareness, scientific research, and reproduction /conservation of endangered species along with the preservation of their habitats (29,98). In order to overcome a still widespread infamous reputation which infers that zoos are prisons, holding captive animals for the amusement of humans in conditions which are fully inadequate to ensure their quality of life (104), it is imperative that these missions establish an ethical commitment to one another, so they can ensure the well- being of the animals intended to protect.

Figure 1. Snow leopard in cage at Bronx Zoo 1906 (WCS Photo). Adapted from Snow Leopard Trust in 18th January 2017.

The enhanced role of human actions brings new escalating conservation challenges and emerging diseases, which pressure impaired long-term survival of threatened free-ranging and captive wildlife species, while having hazardous effects on ecosystems and public health. (42,65,68). Current extinction rates are outstandingly high (about 1000 times previously valued ones) and likely to be underestimated once biodiversity statistics are affected by a gap of information on species taxonomy, distribution and status (105). The Zoological community has a role to play in the future of biodiversity conservation as, apart from saving numbers of species, it also encompasses the protection of functional and integrated ecosystems (33).

Currently, there is a number of institutions which follow a professional code of ethics and are responsible for accrediting modern zoos as well as promoting program coordination and cooperation between zoological gardens and aquariums worldwide. They provide its members with services that meet the highest standards and best practices in animal care, and support scientific research, conservation, and public engagement (5). The World Association of Zoos and Aquariums (WAZA), for example, is the unifying organization of the zoological community (including more than 300 members) and the founding member of IUCN (149). All member institutions have established Taxon Advisory Groups (TAG) for all the different species of animals that are kept in zoos and aquariums. Each TAG focuses on the sustainability and conservation needs of an entire taxa and develops a Regional Collection Plan accordingly, which identifies essential conservative and research goals, develops an action plan and liberates specific recommendations for *ex situ* and *in situ* population management (149). TAG's are composed by professional specialists who are also responsible for advising, managing and supporting cooperative animal management programs like Species Survival Plans (SSP), European Endangered Species Programmes (EEP) and international Studbooks (SB) (5, 45). Most endangered species are part of cooperative international and scientifically managed breeding programs whose final goal is to sustain populations that are healthy, genetically viable, demographically stable and capable of self-reproduction, so they can serve as a "gene pool" and surplus for eventual reintroduction in their natural habitat (148,150). Despite the practical and financial difficulties inherent to breeding programmes (32), the extremely small capacity of zoos and the relatively uncommon and unsuccessful levels of reintroduction of propagated species, one must not depreciate the importance of zoo's conservative efforts and its ability to reproduce key species which have no other preservation options (33). In recent years, zoos have bred about 19% of all known mammals' species and at least 9% of the birds (32).

International Studbooks are documents where all records and data relevant to the whole captive population of a certain species integrated in a breeding program, are compiled and continuously updated

(149). They will provide vital information on pedigree and breeding history of individual animals along with several changes in captive populations, and they will assist with references on pairing choices in order to ensure the maximal genetic diversity within the population. These references will recommend which individuals should breed with whom, how often and where (10,64,149). Studbooks also recommend breeding restrictions in order to avoid future uncontrolled population expansion and, therefore, achieve stability (64, 148).

2.2. Animal Welfare and Behavioral Assessment

Despite significant advances concerning the Animal Welfare (AW) topic through recent years, the majority of the research conducted has involved farm and domesticated animals (60,86). Zoological collections still include many poorly understood species as well as individuals with different life experiences and particular temperaments (60). AW is a wide multi-disciplinary concept for which many definitions have been proposed. AW as a scientific field started with the Brambell Report on the welfare of intensively farmed animals, issued by the British government in 1965 (14) and later revised by the Farm Animal Welfare Council (FAWC) in 1979, having resulted in the decree of five formalized and rightful freedoms that would form a logical and comprehensive framework for analysis of animal welfare (Table 1) (134). FAWC claims that the welfare of an animal includes both its physical fitness and mental state and that "any animal kept by man, must at least, be protected from unnecessary suffering". Webster (1994) considers that although the "absolute attainment of all five freedoms is unrealistic", they still represent an "attempt to make the best of a complex and difficult situation". Some of these freedoms, like the freedom from fear and distress or freedom from pain, are anthropocentric constructs. Fear and pain are normal and essential in appropriate situations, where they work as natural defense mechanisms and may have adaptive and fitness value (72). Conclusively, the freedoms define "ideal states rather than standards for acceptable welfare" (134) and are best viewed as useful and practical principles that provide the basic philosophy to minimize suffering and promote a state of good welfare and assessment of any husbandry system (147). Today, AW can be scientifically assessed to determine the quality of life of individuals, and it implies the integration of the animal's biological function, as well as the subjective emotions and sensations it experiences as a result of the surrounding environment (91). Consequently, the individual's health is highlighted as a "state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (151). Broom (1986) also utters that "the welfare of an individual is its state as regards its attempts to cope with its environment".

Welfare is, therefore, a characteristic of an individual (17,18) and will vary on a dimensional continuum from very poor to very good (72,91) as a measure of the animal's perception of its external circumstances and lived experiences (88). The ability of an organism to tolerate and respond to a range of stimulation, including noxious stimuli, in order to maintain mental and body stability, is called "Coping" (60). Coping implies the concepts of homeostasis and adaptation, as well as brain activity, endocrine, immunological, physiological and behavioral complex response mechanisms (18).

Table 1. The "Five Freedoms" which constitute the primary basic principles for Animal Welfare. Adapted from the National Archives Website, assessed in 15th February 2017.

1. **Freedom from Hunger and Thirst** - by ready access to fresh water and a diet to maintain full health and vigor.
2. **Freedom from Discomfort** - by providing an appropriate environment including shelter and a comfortable resting area.
3. **Freedom from Pain, Injury or Disease** - by prevention or rapid diagnosis and treatment.
4. **Freedom to Express Normal Behavior** - by providing sufficient space, proper facilities and company of the animal's own kind.
5. **Freedom from Fear and Distress** - by ensuring conditions and treatment which avoid mental suffering.

Scientific consideration of subjective emotional states in AW has been disregarded since these are difficult to identify and quantify (61,72). However, active promotion of positive feelings, such as pleasure and contentment, plays a primary role in assuring good welfare status (12,18) provided that this is determined from an overall balance of experiences. Addressing only the negative emotions and states will not necessarily give rise to positive ones but will merely serve to achieve a neutral situation (88,91). Animals have a wide range of needs (Figure 2) which must be always met appropriately according to each species and based on scientific principles, so as to minimize negative welfare states while promoting positive ones (91). Failure or difficulty to cope with the environment occurs together with the presence of negative emotional states (e.g. suffering) and subjective experiences, and it represents a state of poor welfare, from a holistic view of well-being. (17,144). Animals seek to control interactions with their environment and avoid unpleasant stimuli. When unable to do so, and simultaneously denied resources they are very strongly motivated to obtain, they will achieve a distressful state of frustration and anxiety. (39,91). Stress is currently defined by its consequences and described

as detrimental as a reaction to a challenging stimuli that will activate the organism's Hypothalamus-Pituitary-Adrenal (HPA) axis and produce a response with adverse effects that disrupts homeostasis (18), resulting in overall reduced fitness (60). It can lead to a variety of short and long term responses characterized by a range of physiological abnormalities (i.e. alterations in hormonal profiles, metabolic changes and cardiovascular malfunction) (18) as well as psychological disorders (maladaptive demeanor such as increased occurrence of pacing, aggression, self-mutilation or fear behaviors) (39). It is therefore essential to provide animals with productive environments that reward them with fresh challenges, opportunities and choices over time which allow them to express innate behavior and control interactions with their surroundings. (18,91).

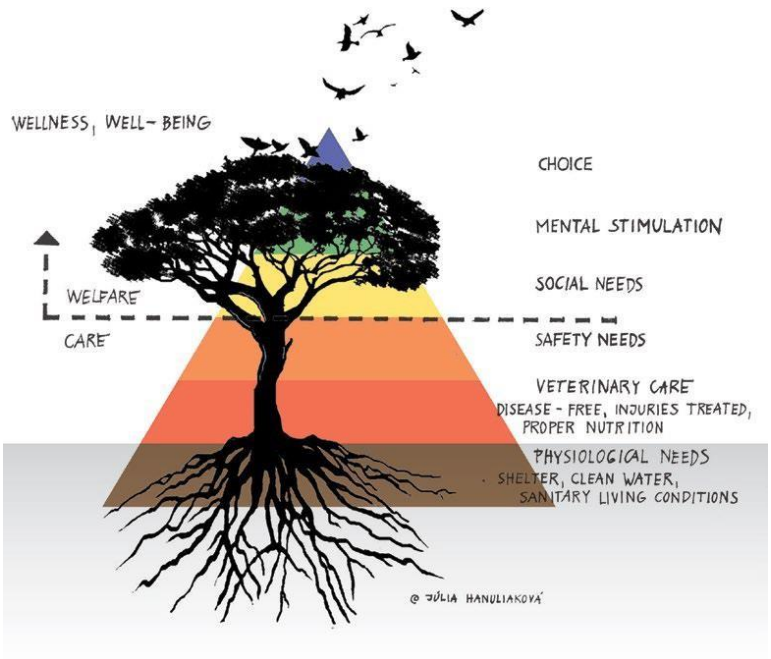

Figure 2. "Maslow's Hierarchy of Needs" pyramid expresses the aspiration of World Zoo and Aquarium Animal Welfare Strategy in directing attention towards the highest categories of Maslow's pyramid of wellness and well-being, through the accomplishment of animal's wide range of needs. Adapted from Mellor et al (2015).

Overall assessment of Animal Welfare is by no means straightforward and should be carried out in a scientific and objective way, avoiding anthropomorphism and taking no account of ethical topics about the practices or conditions being compared in its evaluation (17, 146). Animals are now acknowledged as "sentient beings" (46), considered to have value of their own and to be able of conscious feeling and subjectively perceiving both positive and negative emotions and experiences. Despite the whole concept of AW involving moral values and judgments about our obligations to the animals under our care, it is only after scientific evidence on welfare assessment has been obtained that ethical questions and decisions are ought to be

taken (17,18). Qualitative and quantitative scientific methods for determining acceptable high standards of AW are complex and multi-disciplinary (60) and involve indirect (by taking into consideration the animal needs) and direct measurement of variables, both conducted through observation and experimentation. It is imperative that a variety of "welfare indicators" are used (17) in order to obtain a comprehensive view of the animal's biological state which is characterized by several interacting components that result in neural, endocrine, sensory, immunological and behavioral responses when faced with challenge (18). WAZA recommends that zoos and aquariums apply a simple "Five Domains" model (Figure 3) to facilitate the assessment and understanding of AW, which schematically outlines 4 functional domains (i.e. "Nutrition", "Environment", "Physical Health" and "Behavior") and one "Mental" domain. This useful model links a variety of internal/external conditions with the negative (aversive) or positive (pleasant) experiences they give rise to, and integrates its effects in order to draw the sketch of a welfare status (91). Some physiological indices can't be obtained safely in non-trained zoo animals without the use of anesthesia or sedatives (e.g. changes in heart and respiratory rates, blood parameters, hormonal profiles or neurotransmitters' levels) (18). Alternatively, other internal parameters can be collected noninvasively, for example, measuring reproductive or stress hormones and their metabolites in urine or feces (e.g. fecal estrogens or fecal glucocorticoids) (17,60). The HPA axis' frequent and high activityis the most often used physiologic measure (60), very useful in assessing stress responses and impaired immune system associated with environmental disturbance. It is usually monitored noninvasively through analysis of fecal cortisol/corticosterone (16,144). Although efforts should be made to include the importance of both positive (pleasure, contempt) and negative feelings or sensations (suffering, fear, pain) in the assessment of AW, these imply complex brain constructs and functioning mechanisms which are not easy to evaluate in a fully objective way (18). Dawkins (2004) argues that animal welfare should be assessed by answering two essential questions: 1) Are the animals healthy? and 2) Do they have what they want?, and that these two queries concentrate both the physical and the mental features of AW. Some indirect methods that help answering these key questions and are frequently used for AW assessment and improvement, include behavioral observation associated with tests that identify individuals' choices/preferences or study their motivational strength by "asking" the animals what they want and how much they want it. The way an animal responds to given opportunities in its environment offers valuable information about its emotional state and motivation, and helps to determine a particular hierarchy of needs (16,88).

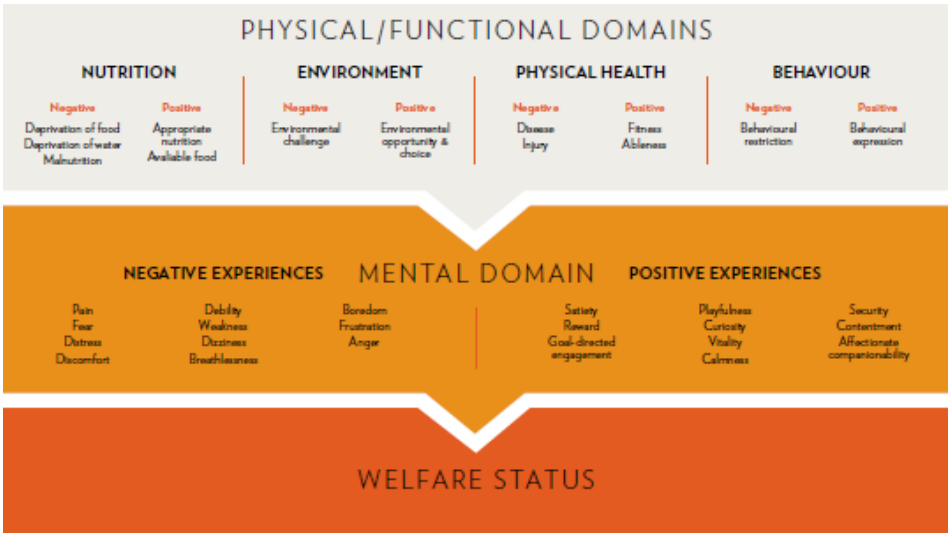

Figure 3. WAZA's "Five Domains" Model, designed to facilitate animal welfare understanding and assessment. Adapted from Mellor *et al* (2015).

Animal Welfare is considered to be a scientific branch of applied animal behavior and can be measured through behavioral assessment (111). Understanding behavioral diversity, its function and its relation with the animal's perception of their external circumstances is important as it might help to prevent what is considered an abnormal demeanor or to improve conditions that are appropriate for normal repertoires. Skinner (1938) considers that behavior is "part of the total activity of an organism" and that "is that part of the functioning organism which is engaged in acting upon or having commerce with the outside world". Behavior as a specific response, therefore represents the first line of defense to environmental challenging stimuli and may delineate a rough sketch of an animal's coping success against external stressors (91,144). It plays a major role in answering Dawkin's (2004) questions: 1) Are the animals healthy? and 2) Do they have what they want?, since it encompasses animals' own decision-making process and represents a phenotypic expression of emotions (39) while it also may be used in the clinical assessment of animal's health status (e.g. assessment of pain, nutritional requirements and hormonal conditions) (39,145). Behavior assessment through applied behavior analysis and behavioral monitoring studies is a technique that has several benefits supporting optimal animal care, making it essential to improve animal welfare and to meet conservational goals. These studies have emphasis on scientific data collection through direct and objective observation of measurable behavior as well as the circumstances under which they occur, and concern the functional relationships between environment and expressions of behavior (35,145). Systematic observations and record keeping have numerous advantages as a management tool in zoos and other related facilities: they represent a non-

invasive and, in the majority of cases, a non-intrusive technique (39) that allows documentation of normal behavior patterns and identification of any changes on regular activity, establishing a database of background information on individuals on a consistent basis (145).

Modern Zoos and Aquariums have a responsibility towards the animals under their protection, through their whole life stages. They should work in an organized way in order to achieve high standards of AW, comply with animals' wide range of needs and minimize the incidence of negative states while promoting positive ones (91). This involves providing: 1) appropriate, safe and naturalistic environments; 2) proper diet; 3) adequate veterinary care; 4) appropriate social contact and 5) environmental enrichment (45,91).

2.2.1. **Stereotypic Behavior**

As stated before, behavioral responses may help the animal coping with its external environment, however, if failure or difficulty to do so persists, they may develop into behavioral pathologies whose demeanor diverges in pattern, frequency and context from that which is exhibited by healthy conspecifics in their natural habitat or assumed optimal living conditions (18,35). These class of disorders may include a diversity of aberrant, stereotypic and self-injurious behaviors (18) which may cause harm to the animal. Stereotypies are repeated, relatively invariant sequences of behavior which serve no apparent functional goal (17,35,84). They seem to be quite common in captive animals and are often displayed by farm and zoo individuals that appear to be under stressful and difficult conditions, such as those implying conflict, fear, frustration, severe lack of stimulation or control of the environment, and even the imminent arrival of food (17,31,83). Examples of captive-induced stereotypies include, among others, compulsive oral or locomotive behaviors such as bar-biting; tongue-playing; neck-twisting; tail-chewing; head-swinging; eye-rolling; jumping; self-biting; excessive grooming; and pacing (83,111). Stereotypy is a symptom (78) of undoubtedly importance in welfare assessment and is extensively suggested to be an indicator of welfare problems (17,84) once it is typically associated with sub-optimal and inadequate environments (83). Moreover, it will also interfere with the driving goals of captive facilities (including reintroduction projects and educational value) as well as attract more criticism by the media and society about the role of Zoos in the preservation of animal welfare (31). However, the relationship between stereotypic behavior and welfare is still not straightforward. Thus, caution must be taken in order to consider the complex mechanisms involved in stereotypy emergence as well as the contexts in

which it develops, when using it to track suffering and poor welfare conditions. Stereotypic behavior is thought to develop from normal behavioral repertoire (83), as a remnant of displacement or defensive activities that are initially conducted in an attempt to maintain control of a challenging situation (35). Some stereotypies are difficult to demarcate from natural behaviors (78)(Figure 4). Yet, with repetition, the behavioral output may become independent of the original stimuli that elicited its performance in the first place (83) and reach a development stage where it gets fixed, centrally controlled and will be performed in a varied set of situations, where it will be gradually harder to interrupt or extinguish (84). Stereotypies are heterogeneous in their properties when related to context and functional pattern of behavior from which they arise, eliciting stimuli, motivation, development over time, physical characteristics and impact in welfare (83,84). They will also differ in repetitiveness, frequency and intensity and will depend on the species and the individual that is performing them (83).

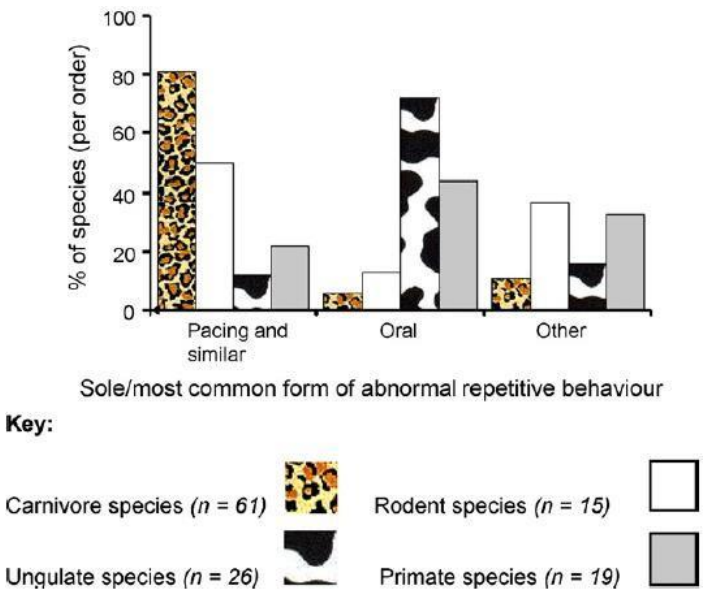

Figure 4. Taxonomic distribution of different forms of stereotypies. Carnivore species favor locomotory repetitive behavior (pacing) comparatively to other species of mammals. Adapted from Mason *et al* (2007).

Although literature agrees in the ground broad definition of stereotypy, it is not always clear about its classification and how it can be quantitatively used to assess welfare. Some authors suggest that any level of stereotypy exhibition may indicate poor welfare while others defend that only increasing levels do so (84). Wiepkema (1983) considers that if stereotypies surpass more than 5 % of a population welfare then reaches unacceptable levels, Broom (1991) for more than 10% of an animal's time. It is important that, in order to avoid confusion, any behavioral pattern classified as a stereotypy is extensively defined in a clear, objective and

quantitative way (83). Stereotypies are not clear to be maladaptive themselves except when they represent a severe energy cost to the animal and imply self-mutilation or other detrimental effects (e.g. serious weight loss, physical injuries, painful conditions) (83). It is important then to reflect that within stereotypy inducing environments, animals with absent or low-stereotyping levels should never be overlooked or credited to be faring well since stereotypic individuals may actually be the least compromised ones when regarding their welfare. Psychotropic drug therapy using anti-depressants (55), selective serotonin reuptake inhibitors (62,107) or opioid antagonists, (78), has been applied to tackle down stereotypy. However it should be borne in mind that simply preventing a stereotypy might, in some cases, be counter-productive as it would only address the physical expression of a behavior that may have beneficial effects, without understanding its underlying motivation. Stereotypy must, at all times, be taken seriously as a "warning sign of potential suffering" but never used as a single indicator for welfare assessment. (84).

2.3. Veterinarian's Role in Conservation Medicine and Animal Welfare

In the past, the primary role of wildlife veterinarians was the intervention and management of free-ranging populations experiencing a health crisis (40). Epidemic wildlife disease was mainly addressed due to its zoonotic menace (e.g. rabies, brucellosis and tuberculosis) and its harmful outcome in the health of domestic animals or game species considered economically relevant. (40,41). In the meantime, veterinarians from zoological institutions focused on the *ex-situ* individual, providing healthcare and ensuring compliance of welfare criteria for captive collections while their main part in wildlife conservation projects was considered to be the chemical immobilization of animals in order to enable research (41,68). Presently, differences in the role of wildlife and zoo veterinarians are fading as a result of the expansion of the role of the latest in conservation efforts. Such tendency was inevitable due to marked changes in Zoo's missionary priorities and an increase in the movement of animals between facilities or for intended release in the wild. (42,65).

Veterinarians are irrevocable generalists, which makes them proficient in the holistic approach of disease dynamics. They have not only a broad education in comparative medicine (not a single-species focus) but also in many specialties such as surgery, clinical medicine, anesthesiology, epidemiology, nutrition, pathology, toxicology, theriogenology and behavior. This makes them excellent at understanding both mental and physical needs of an animal, and how well adapted it is to its environment (41,65). Wildlife vets are also highly trained in

recognizing, diagnosing and understanding disease impact on public health as well as on individuals, populations and whole ecosystems; and, in choosing the most advisable preventive and therapeutic options on a case by case basis (40,41). Their ubiquitous knowledge, skills and expertise therefore turns them into valuable key players in planning, implementing and effectively assisting both *in-situ* and *ex-situ* conservation projects (42). As a result, deep collaboration between veterinarians and professionals of other scientific fields such as applied biomedics, epidemiology, ecology, biology and evolutionary genetics, is becoming positively accepted as a new way of integrating health sciences into conservation (41,42).

When it comes to decide whether to prioritize the interests or rights of animals individually or to focus on the global viability and health of populations and species in specific situations zoological veterinarians are those in the best position to offer a balanced view concerning what is the best for the animals, either from an individual or population perspective. They are expected to actively promote and safeguard animal welfare on the grounds of scientifically justified practices which encompasses a wide range of activities, summarized in Table 2 (94). The veterinary clinician works under an ethical code of professional conduct which implies a commitment with integrated principles of animal welfare and the individual responsibility of ensuring a rational enforcement of the "Five Freedoms". All animals must be treated with respect, dignity and compassion as well as with thoughtful consideration for their species-typical biology and behavior (2). Accredited zoological institutions ought to have veterinary clinicians working closely with a variety of other institution's organizational staff (such as animal care providers; endocrinology, behavioral or nutrional field specialists; ecologists; regulatory board of directors; and even educational, marketing or facility design departments) and who actively participate in management decisions (41,42). Clear communication among the different interest groups existing within a zoo allows for the creation of holistic medical programs whose lead foundation is preventive medicine and through which animals can be regarded as one population/community, while still having their individual needs attended (3). Keeper staff should be trained for clinical health assessment, so they could be better qualified at understanding, identifying and promptly reporting any signs of injury or abnormal behavior that could indicate early disease stages (42). All data collected and medical records should be condensed in databases like Medical Animal Record Keeping System (MedARKS) or Zoological Information Management System (ZIMS), for cooperative consultancy of disease occurrence and share of information between institutions at a global level. A standardized, computerized medical record-keeping system is a useful tool for management of health care and husbandry practices. By documenting and analyzing medical

information that is easily assessed (e.g. clinical notes, treatments, anesthesia, parasitology) it can help future disease assessment or comparison with other populations (93). Complete reports of necropsies on collection animals should be kept as well, once they can provide information about species anatomy that has great value for surgical procedures. One of the most exalted contributions of the zoological veterinarian nowadays is its aid in successful captive breeding programs. Apart from securing animal general health, the veterinarian is able to access reproductive health and behavior, monitor breeding cycles, establish management protocols, help in implementing artificial reproductive techniques and effectively control selective reproduction, with resource to permanent or reversible contraceptive methods (65,40,41,42).

Table 2. Applied Veterinary Medicine and Research in supporting both *ex-situ* and *in-situ* conservation programs

- Health assessment, surveillance and log-term monitoring for feral and domestic animal populations within park borders
- Identification of critical health factors with impact on wildlife population dynamics
- Development and enforcement of new health care technologies and methodologies
- Provision of preventive, diagnostic and therapeutic health care for wildlife species
- Improvement of safety and efficiency of methods of animal handling and securing the addressing of other ethical and welfare concerns
- Active participation and inspection of zoological management decisions such as husbandry, nutrition, animal shipment and pest control
- Scientific biological data collection, analysis and management
- Active participation in captive breeding programs
- In situ and ex-situ reproductive and health management of threatened species
- Disease risk analysis and creation of health screening and quarantine protocols for wildlife translocation projects
- Management of emerging disease and health crisis intervention
- Research on zoonotic, anthropozoonotic and interspecies transmission of disease
- Interdisciplinary collaboration in conservative efforts
- Guidelines and policy development at local, national and international levels
- Training of field personnel to expand their skills in addressing wildlife health issues

2.4. Behavioral Enrichment

Captivity can affect drastically animal behavior (Morris, 1964). By confining animals to a cage or an enclosure we reduce the complexity of their environment, severely narrowing the natural control they should detain over it and restricting the range of behaviors they are able to exhibit (87). Where animals have very limited choices, we are the ones planning almost all aspects of

their life (e.g. feeding schedules, what to eat, where to sleep, who to live or to reproduce with). Effects of sensory deprivation and physical variety in the environment may result in aggression, boredom, anxiety, frustration and, ultimately, both physical and physiological illness (18).Thus, captive establishments have an ethical and legal obligation to provide for the holistic welfare of all animals under their protection.

The History of enrichment starts in the 1920's with Robert Mearns Yerkes (23), a psychobiologist best known for his work in intelligence testing of both humans and primates and his writings about the importance of enrichment for gorillas and chimpanzees in captivity (8). The Swiss zoologist Heini Hediger (1908-1992), known as the "father of zoo biology", was a visionary in the proxemics in animal behavior. In the 1950's, and for the next several decades, he wrote about human responsibility in providing constructive environments for wild animals in zoos and described the importance of studying animal's territorial surroundings, having a particular influence on the construction and planning of naturalistic enclosures (23,81). His revolutionary concepts are well expressed in his book in which he refers that "Anyone who sets out to build homes for animals should be quite clear that the cube is the most unbiological and therefore most inappropriate of all spatial forms" (57).

The American psychologist and behaviorist B.F. Skinner had strong implications on enrichment as well. He used operant conditioning to strengthen behavior, created the principles of reinforcement and introduced the process of shaping, techniques that are still used today in many animal training for husbandry and medical procedures, and represent a gold standard for dealing with behavioral problems in a variety of settings. (8) He also reported sterile environments causing animals to engage in repetitive behaviors (23). Hal Markowitz also made significant contributions to the expansion of social consideration about enrichment since the 1970s, being a pioneer in marine mammal research and extending the work of former authors, concerning operant conditioning,. in order to apply this science to improve animal's life (23).

There have been tremendous improvements regarding animal care and captive settings throughout recent years. Replication of wild conditions and stimulation of wild biological repertoires in captive animals have been a long term conservational goal for many zoological institutions (58). This way, behavioral enrichment programmes have become an accepted practice whose most generic priority is addressing and reducing stereotypy (84,122). In fact, Swaisgood and Shepherdson (2005) associate enrichment with a reduction of 53% in the time spent in stereotypy performance (Figure 5).

Behavioral enrichment, also known as environmental enrichment, is currently a principle of animal husbandry that has been scientifically proved to be beneficial (29,91). It should be fully

incorporated in the daily routine of animals as a tool to maximize their quality of life (135) and discourage undesirable behaviors that emerge as "artifacts of captivity" (e.g. stereotypies) (29,101). Enrichment is officially defined as "a dynamic process for enhancing animal environments within the context of the animals' behavioral biology and natural history. Environmental changes are made with the goal of increasing the animals' behavioral choices and drawing out their species-appropriate behaviors, thus enhancing animal welfare" (136). The aims of behavioral enrichment can be achieved by creating productive environments which encourage each animal to express the natural mental activities and behavioral repertoire of the species, and by adding stimulus that offer complexity and novelty to its routine as well as opportunities that enable it to restore the sense of control it should have over its environment (138). Behavioral programmes have a role in fighting against inactivity and obesity, in reducing / eliminating stereotyped and aberrant behavior (e.g. aggression, sexual frustration), and in decreasing levels of stress which can, alone, indulge reproduction (101,122,137). Preservation of core biological behaviors is also essential to the survival of the individuals targeted for release and reintroduction in the wild and, therefore, for the success of conservation programmes (101). However, enrichment will not only be beneficial for the animals but for the general public as well. Through promotion of natural species-specific behaviors, enrichment will create valuable and more accurate educational opportunities for the visitors to learn about animal's natural history, biology and conservation. Consequently, it will enhance guest's experiences and their perception of the Zoos' missions (29,138).

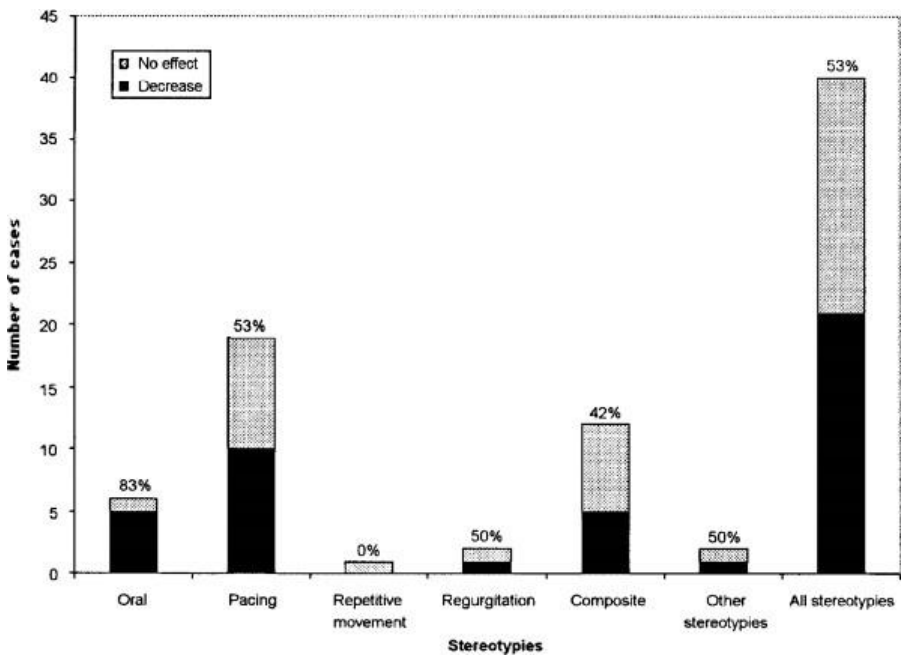

Figure 5. The effects of enrichment on stereotypy performance. Adapted from Swaisgood and Shepherdson (2005).

2.4.1. Types and Contextualization

Enrichment in its most varied forms elicits investigatory, foraging or marking behavior, social interaction, and creative play. It also provides shade and privacy through hiding places or escape routes, leading to a more efficient use of space (101,139). However, the results of every approach will depend on intrinsic factors such as species, age, sex and individual personality (146). Simple ideas can have a remarkable effect on animals; ideas that are often cost effective, require no construction or repair and are readily to install, maintain, modify or replace. Frequently, several material lying around zoo ground (e.g. pine cones, log trees, fruits, browse, feathers, varied subtracts, cardboard boxes) would serve for a non-permanent addition of novel objects and may be easily applied as a source of enrichment (138). Five, not mutually exclusive, enrichment categories are generally recognized which will cover the basic needs of an animal and increase a positive utilization of its environment: 1) Sensory; 2) Structural / Physical; 3) Social 4) Cognitive and 5) Food/Nutrition (136). The animal's environment can thus be manipulated and modified in countless ways through a holistic view of the animal's biology, being the limit our own creativity.

2.4.1.1. Sensory

Sensory enrichment refers to the five perceptual senses of sight, smell, hearing, taste and touch (29). Olfactory stimulation is of the main importance in behavioral enrichment as many species use olfactory signalment to communicate with intra and inter specifics as well as to maintain their territories. They are also driven by their sense of smell to locate prey, reproductive mates or food (63,122). Olfactory enrichment may include addition of scented material and both packaged and natural odors. Essential oils and aromatic compounds (e.g. lavender, chamomile) are documented to reduce anxiety and encourage positive effect in some animals, including dogs and pigs (47,54,86). Specific smells of a species natural habitat or material from natural prey (e.g. feces, urine, fur, feathers, shed skin) can be employed successfully (28). The same material when coming from natural predators is, however, suggested to have a detrimental effect on their respective preys (146). Captive felids in particular are shown to grossly benefit with the introduction of spices such as cinnamon, nutmeg and ginger; excitatory herbs like catnip or lemongrass (146); and synthetic pheromones as Feliway (127). Daily removal of natural odors from enclosures through husbandry routine practices should be avoided as it leads to clearance of important olfactory cues in the animal's environment (146).

The value of auditory stimulation to the well-being of animals is still controversial in the literature. It can be vaguely divided into sounds specific of a specie's natural habitat and sounds that are not found in the wild (46). Natural auditory cues may, yet, not be considered biologically relevant or meaningful to the animals concerned. In fact, some studies reveal them to be counterproductive and to increase agitation and anxiety (102,96). Auditory signals provided in the institutional setting and which indicate some welfare advantages include specific styles of music and radio broadcasts (101). Country and Classic music (Mozart's Sonata K. 448 in particular) are featured to have some potential as an enrichment tool with some welfare benefits in a set of different species. It is important to bear in mind, however, that the addition of extra noise to environments which can be pretty loud themselves may also have a negative impact in their dwellers, including hearing impairment and communication disconcert between animals (101,146). In some cases, the most significant aspect regarding acoustic stimulation so far, may be the overall reduction of the ambient noise (146).

Visual means of enriching the captive environment of animals may include addition of mirrors, moving toys, televisions or other computer-assisted equipment as well as simply allowing the sight of activities outside the enclosure or of a prey (29,146).

The tactile category includes a varied set of toys, subtracts and other artificial/natural *manipulanda*, which can be provided in different shapes and textures, either permanently or through a rotation scheme in order to maintain interest, and in close association with the other classes of sensorial enrichment (101,139)

2.4.1.2. Foraging

"Foraging", as a concept that comprises searching, retrieving, acquiring and processing food (139), is a time consuming activity that constitutes a major portion of the daily time budget of animals in the wild (29,100). Captivity, on the other hand, provides animals with a more limited selection of food types, usually processed diets that are dispensed in highly predicted locations, at fixed feeding times, and in an easily consumed form which does not require natural foraging tactics (100,101). Absence of appropriate diets and inhibited expression of natural feeding behaviors is associated with reduced sensory input (10) and may contribute to obesity (101), poor oral health (152) and to the development of certain forms of stereotypies and aberrant behaviors (101,120), which may also be directed towards cohabitants or enclosure's furnishing with consequent harmful effects (101,121). Feeding animals through more versatile and natural ways is one of the most widely used enrichment techniques (30,152). It simulates natural food

acquisition hurdles and promotes biological foraging strategies (49), improves physical condition and nutritional balance (152), and reduces occurrence of abnormal behavior (120). The enriching source of this category comes from provision of varied and novel food items accompanied by an improving in the method for food presentation (11,29,77). A wider selection of food items with different size, textures, consistency, taste and nutritional value, also takes into account the stimulation of the senses included in the sensory category of behavioral enrichment (139). Examples include bones, frozen fruits, blood or fish, whole carcasses, edible furniture, chopped vegetables and miscellaneous scented browse (139,152). Novel techniques for food delivery may comprise hiding, scattering or burring dietary items in unpredictable places in the enclosure or under foraging substrates (101,130) as well as puzzles and other feeder devices such as artificial mechanical preys (152). Randomizing feeding schedules, locations and frequency of meals will alter the way an animal searches for food, increasing its efforts to extract, catch, process or ingest food with expansion of overall foraging time (101).

2.4.1.3. Structural

A revolution in zoo enclosure design has led to a proliferation of more naturalistic exhibits which replicate as closely as possible the wild habitat of the species concerned (57,149). These exhibits are also functionally evaluated with the priority of creating stimulating and appropriate captive environments according to the specific behaviors and biological needs of each species (138). The basic components of an animal's physical inanimate environment is its structure (i.e. size, shape and design) and the substrate within it (i.e. "base on which an animal lives") (139). Structural enrichment highlights the utmost importance of the quality of the space available in overcoming space restrictions. It involves, among others, altering the size or complexity of an animal's enclosure; providing territorial subdivisions with specific functions and significance (e.g. areas for locomotion and sanitation, sleeping quarters and marking places); making simple rearrangements of furniture; and increasing both horizontal and vertical space by adding multiple accessories (27,57). Physical enrichment, temporary or not, may comprise perching or climbing structures, swings, ropes, hammocks, logs and branches; elevated platforms which would serve as a viewpoint or resting spots; edible furniture; infrastructures which would provide hiding and shelter from weather conditions; water features such as pools, lakes and waterfalls; and devices that control temperature or humidity (29,136). Substrates elements usually used include a variety of artificial and natural flooring or bedding, which will serve as

foraging material as well (e.g. shredded paper, woodchip, leaves, hay, grass, straw, sand, mud and gravel) (139).

2.4.1.4. Social

Social enrichment involves all forms of social interactions provided direct or indirectly by conspecifics, humans and other species of animals, through physical contact, verbal communication or even olfactory signalment (136). Many studies instigate the importance of housing appropriate social groupings in the welfare of captive animals (109, 121), even for species considered as primarily "solitary" in nature, as it is the case of some big cats (43,80,130). The term "mixed-species exhibits" implies inter-specific associations of animals that would naturally occur in the wild (29). Implementation of regular training sessions has, among other enormous advantages, the benefit of improving the human-animal relationship, regarding veterinarians, zookeepers and even visitors (89).

2.4.1.5. Cognitive

Mental stimulation may be carried out through provision of training sessions, puzzle feeders and other cognitive devices that require animals to solve a problem (29,136). Training, based on both classical and operant conditioning principles is a revolutionary way of intellectually challenging the everyday routine of captive animals while managing them to comply with basic husbandry tasks or medical procedures without being forced to do so (139).

2.4.2. Environmental Enrichment Program Planning

For an enrichment program to succeed, it is very important that it is methodically planned in accordance with its objectives and desired outcomes, otherwise it may be more harmful than beneficial (29). The foundation and logistics of a successful, goal-orientated and self-sustained program can be outlined through AZA reviewed guidelines and protocols so as to represent a master plan from goal setting to re-adjustment, addressing safety issues, providing and keeping up-to-date resources (138). All animal care staff members and all the professional sectors in the

zoo must be involved in the development of an animal enrichment program, as each one plays a critical role in its success (4,29). Disney's Animal Kingdom developed the 'S.P.I.D.E.R' framework (Figure 6), a solid model which works as a valuable tool in the development, implementation and maintenance of institutional training and enrichment programs (4). These programs provide species with appropriate challenges, opportunities and stimulation for all taxa (4,91). S.P.I.D.E.R is an acronym for the first letter of each component of the framework (119), as follows:

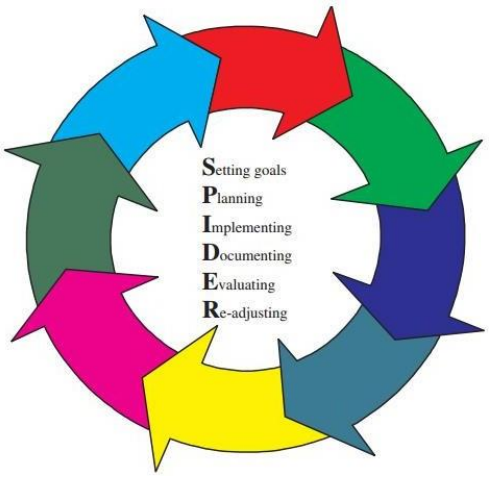

Figure 6. Disney's Animal Programs introduced the S.P.I.D.E.R. model which has been utilized by all animal care staff since 1998. Adapted from The Macaque Website, assessed in 20th June 2017.

S – Setting Goals

The question "What do we want to achieve with this enrichment?" must be answered in order to determine and prioritize the behavioral goals which will head specific enrichment strategies and schedules for each individual as well as evaluate the possibility of negative effects and how to avoid them (136,138). At this stage, general and specific behaviors which are ought to be encouraged or discouraged are clearly identified (4). Often, enrichment goals rely on anthropomorphic perceptions of what we, humans, think would be enriching to animals (44). Promotion or discouragement of behavioral repertoires requires knowledge and understanding of both the natural and individual history of the species concerned (e.g. their physiology; unique methods of communication; social structure; feeding strategies and sensory abilities; medical or behavioral problems; individual personalities; living and growth conditions) with consideration for all the components that influence a complex behavior (4,139). For example, felids appreciate resting on elevated points, from where they are able to eye their surroundings and sight potential prey. Therefore, inclusion of elevated platforms may serve a good method of environmental enrichment (79). There are numerous factors to be considered, still: exhibit

and holding area design, degree of control over animals (e.g. ability to gate or shift), past history with enrichment items, natural and captive diet, keeper schedules, budget and managers'/public acceptance of unnatural enrichment items on exhibits (44).

P – Planning

This stage specifically involves the process of active delineation of a successful goal-orientated enrichment plan. It takes into account how an individual might respond to a certain initiative; what resources are needed; the main existing constraints or limitations (i.e. material, motivation, time, manpower and financial privies) and how to manage them; where, when and how the enrichment is due to take place; safety and health concerns regarding both the animals and the people involved; and finally, the institutional philosophy, enrichment approval process and official documentation (4,138).

It is possible for enrichment devices (e.g. *manipulanda*, furniture, food, vegetation) to pose a myriad of dangerous, even fatal hazards to animals (135). One of the veterinary medical tenets is "above all, do no harm" (44). It is, therefore, crucial to carefully evaluate risks involved in providing enrichment, and create a standard checklist of potential safety hazards concerning the animals, staff and public. Items lodged in mouth, ingestion of devices, entanglement, suffocation, entrapment of body parts, tooth damage, impaction, disease-causing, toxic or irritating material and food-items, and aggression inside a social group are examples of practical enrichment casualties (56,136). Any of these cases may require anesthesia and/or surgery to be corrected and, ultimately, they may cause the death of an animal (56). Most accidents occur due to a rift in coordination/communication throughout all stages of an enrichment plan (e.g. overnight and unsupervised enrichment) and lack of maintenance of the enrichment items or exhibit's furniture over time (44). Information on physical/ medical occurrences where enrichment gone wrong should be always available for cooperation and ready reference in anticipating/preventing similar incidents in other institutions (136).

I – Implementation

Implementation implies the proactive execution of an enrichment initiative which should be scheduled in a flexible and easy to follow calendar, so as to create opportunities for updating variations in schedules and novel enrichment ideas (4).

D – Documenting

Documentation is an integrative part of any program that allows to determine the successes or failures of an enrichment initiative, to examine trends and animal's particular responses and to evaluate all the other stages of an enrichment strategy. Ongoing records may be done through daily, weekly written logs, direct observation rating scale reports, photos, video and computerized tracking programs (138).

E- Evaluation

Evaluation is a significant process that is often overlooked (4). It implies measuring the effectiveness of enrichment and it should happen on a regular basis, through individual evaluation and observation. These are to be posteriorly discussed in meetings or official conversations in which it is attempted to objectively analyze the information and data gathered, to determine trends and patterns in animal's response, to ensure that resources are being used effectively, to routinely discuss progress and to provide a basis for revision, adjustment and improvement of such strategies. (4,136,138).

R- Re-adjustment

The last component of the framework actually takes place during all the process of development of an enrichment plan (4). The goals of a plan are regularly re-adjusted and enrichment activities refined, improved or discontinued to increase effectiveness of a strategy, which may, at some point, be started over again (138).

2.5. Snow Leopard, *Panthera uncia* (Schreber 1775)

2.5.1. Taxonomy

Taxonomic classification of the Snow Leopard has been difficult and controversial to establish. Currently, the literature consensually places the SL within the genus Panthera (Table 3) and admits that the Pantherine lineage consists of 6 big cats - *Panthera leo* (lion), *Panthera onca*

(jaguar), *Panthera pardus* (leopard), *Panthera tigris* (tiger), *Panthera uncia* (snow leopard) and the closely related *Neofelis nebulosi* (clouded leopard) (38,66). However, despite the strong structural similarities in the skull with the other species contained in the genus Panthera (7), unique differences in the hyoid bone, which prevent the SL from roaring, allowed for the possibility to classify this cat alone in the individual genus Uncia (Gray, 1984), making its scientific name *Uncia uncia* (Popock, 1916) (59,148). New comprehensive studies of phylogenetic analysis defend that the Snow Leopard evolved with and it is most closely related to the tiger while the lion, leopard and jaguar are tightly grouped in another distinct clade. (38,66)

Table 3. Snow Leopard's (*Panthera Uncia uncia*) Taxonomy

Taxonomy	
Phylum	Choradata
Class	Mamalia
Order	Carnivora
Family	Felidae
Sub-family	*Pantherinae*
Genus	*Panthera*
Species	*Panthera uncia* (Schreber 1775)

Figure 7. Snow Leopard (Panthera Uncia uncia).Adapted from Roger Hall Scientific Illustrations.

2.5.1. **Population Status and Conservation**

Generating reliable estimates regarding the size of the total wild Snow Leopard population and its current distribution is difficult due to the hurdles associated with the rough conditions of most field studies carried out, the methodologies or traditional tracking techniques applied and the high degree of subjectivity implied in its interpretation (64, 125). The numbers vary according to the published literature: Jackson *et al* 2010 suggest it to lie between 4500 and 7500, IUCN indicates 4,080-6,590, whereas more recent estimates from Global Snow Leopard Ecosystem and Protection Program Plan (GSLEP) from 2013 point it out to drop in an interval from 3920 to 6390 individuals. Nevertheless, it is known that SL density is extremely low and that population is fragmented into small clusters dispersed through a vast habitat range (64,59,129). Major threats to SL existence derive mainly from anthropogenic causes and have a strong economic basis. Those include livestock depredation, which is intensely associated with negative attitudes and retaliatory killing towards the cats; chronic habitat loss and decline of natural prey due to hunting by humans or competition with domestic stock (what

subsequently exacerbates the Snow Leopard-Human conflict); and poaching along with illegal trade of the luxuriant pelages, bones or other body parts in international markets, as valuable exquisite coats or for usage in traditional Asian medicine, respectively. (124,125,129). Less outstanding but still significant menaces are climate change and expansion of human population along with large scale infrastructure such as mining, roads or fenced railway lines. The risks for the elusive SL are aggravated by the lack of awareness and education of the local inhabitants and lack of law enforcement or international cooperation between governments (64,124). Attempts to reduce the impact of these multifactorial threats comprise both *in-situ* and *ex-situ* conservational measures. The first official record of a captive SL comes from the London Zoo, in 1891. First recorded birth dates back from 1906, however, it was only in the 1950s that increasing interest and knowledge of this beautiful specimen led to establishment of the first standard management protocols (147). Currently, endangered captive SL are part of two international scientifically managed breeding programs, the SSP and EEP. It has been estimated and is now assumed that a well-managed captive population of 230 Snow Leopards is able to maintain about 90% of its originally genetic variability for nearly 200 years (129,147). In fact, although the species has been bred in captivity for more than 100 years it has maintained itself impressively healthy, with only 3% of its genetic diversity lost (10). The Snow Leopard stands out as an example of good captive management work and coordinated approach to conservation and is now one of the few non-domestic felids for which captive global population is numerically and genetically secure (10,129), with recent estimates counting for 395 individuals through 154 facilities across 4 continents (Asia, Australia, Europe, North America)(125). Most SL are allowed to breed no more than 2-3 litters through their lifespan (129). The Snow Leopard is legally protected in all the 12 countries that make up its home range and although such protection is officially in paper (64,129), it is rarely enforced due to shortage of funds and lack of awareness, priority and political commitment to uphold regulations over biodiversity conservation (64). Still, a network of conservation areas through Central Asia is being developed and at least 5 countries have established National Snow Leopard Action Plans by now (64,129). Snow Leopard conservation goes beyond the individual address of the major threats that put at risk the cat's long-term survival in the wild, requiring a broader approach that is able to engage complex environmental, social, economic and political issues as a whole (10,64). Nowadays, this task is covered by a number of organizations and platforms that work together between them and along with local communities and governments, at an international level (126).

Lately, the improvement on technology and genetic analytical techniques (i.e. radiotelemetry, GPS satellite collaring, camera trapping, fecal DNA analysis and occupancy modelling) have allowed the incoming of a great deal of new and more accurate information, as well as the progression in conservative field branches such as research, monitoring and management. (10, 129).

2.5.2. **Morphology**

Snow Leopards measure, in total, from 1.8 to 2.3 meters in length with a height of 0.6 m at the shoulders, and weigh in average 35-55 kg (64,140). Although males may be slightly larger than the females, there is no pronounced sexual dimorphism in general. (59,64). The body of this species is perfectly optimized for its harsh and frigid habitat by a series of morphologic adaptations. The thick long tail is about 75-90% of the body length (0.8-1m) and it awards the cats with an incredible agility and ability to control balance while climbing, leaping or chasing prey across the natural steep and rocky slopes. (59,64,13). Additionally, the boast tail can be wrapped around the head and body extremities when at rest, in order to help maintain body heat (64,1126,129). The skull is relatively short (165-200 mm) and has a characteristic shape with a high domed forehead, which allows for accommodation of an enlarged nasal cavity. (59,129). This is thought to warm up the cold mountain air and maximize SL breathing capacity in such high altitudes, where oxygen is short in supply (121, 126). Dental formula of the snow leopard is I 3/3 C 1/1 P 3/2 M 1/1 (70,106). As in the other Panthera spp. they have a partly ossified hyoid bone. However, the vocal folds are small and pointed as in other small cats and lack a thick pad of fibro-elastic tissue, preventing them from vibrating at low frequencies, therefore making SL unable to roar (70,125,135). The coat is long and thick, with dense woolly underfur to provide perfect insulation (59,64). It molds twice a year and gets thicker and longer in the winter, growing over 5 cm along the body and 12 cm on the ventral abdomen (126,129). The exotic pattern of spots and dark rosettes that cover the smoke- grey background color of the pelt, allow perfect camouflage in the rocky arid habitat and patchy snow. These marks coalesce along the dorsal back to form 2 elongated lines that extend from the head to the base of the tail. (59, 64,129). Like in other big cats, the pattern of the pelage of every animal is unique so each Snow Leopard can be individually identified (1429). SL also have a well-

developed chest, small rounded ears that help to minimize heat loss, and massive front paws with extra fur on their pads that optimize weight distribution and are better fitted for locomotion on the rocky terrain or silent progression through the deep snow (64,126,129). The hind limbs are powerful and longer than the forelimbs, enabling the cats to jump up to 6 meters high and leap significant linear distances (59, 129).

2.5.3. **Habitat and Diet**

Habitat of the Snow Leopard is characterized by its extremely harsh typography and severe climatic conditions. The rugged terrain is typical of alpine and sub-alpine ecological zones: arid, sparsely vegetated in some regions and broken by cliffs, ridges, ravines, steep slopes and rocky outcrops (59, 64). Elevations of mountain rangelands can attain more than 5,500 meters but the SL may be found at lower ones, especially in the winter, when they seasonally migrate by following its prey to regions of 600-1500 meters (59,126). Although they avoid crossing large unbroken spaces, SL may, however, be associated with relatively flat and open coniferous forest. The rangy territory extends itself for 1.2-1.6 million km^2 through 12 Asiatic countries (Afghanistan, Bhutan, China, India, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia, Tajikistan, and Uzbekistan) (Table 4) but home range will vary with prey availability and presence of humans or other competitors (i.e. common leopard and Asiatic wild dog) (64,129). In general, home ranges size is smaller (12-39 km^2) in productive areas whilst it may go up to 500 km^2 where prey density is low (64,129).

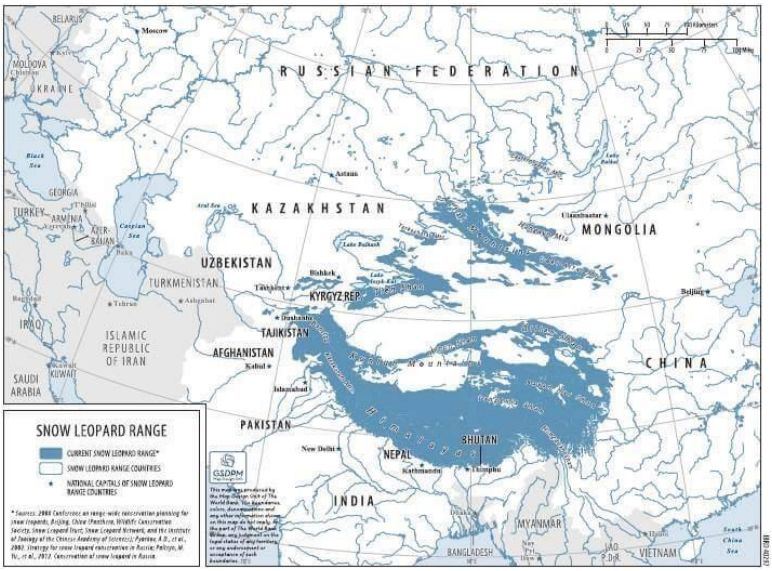

Figure 8. The Snow Leopard home range. Adapted from Global Snow Leopard and Ecosystem Protection Program (2013).

The Snow Leopard is an apex opportunistic predator that preys on a number of species: mountain wild goats and sheep but also domestic livestock; deer; boars; small mammals like marmots, hares and rodents; and game birds (51,59,64,). It is capable of hunting any prey up to 3 times its own weight. The main quarry consists of the Bharal (*Pseudois nayaur)* and the Ibex (*Capra ibex),* weighting between 55-76 kg and making about 45-66% of the cat's diet. Domestic stock is estimated to compose 15-30% and supplementary prey items, 12-25% (64). Several authors have reported SL consuming large quantities of plant matter as grass, twigs and other vegetation (129).

2.5.4. **General Biology and Behavior**

There is no reliable information of SL longevity in the wild (64). Snow Leopards are considered animals of easy temperament, shy demeanor and the least aggressive of the large cats. In general, there is low incidence of aggression associated with these animals and no attacks to humans have been reported so far (51,126). Even when disturbed or confronted, they are more likely to flee away than to defend themselves or their territory. In captivity, they are known to be unusually tame and gentle (129). In the wild, Snow Leopards are typically solitary, however not unsociable (59,64): mating pairs will travel together and copulate during estrus, females will rear/stay with the young until they reach independence and sibling can remain together for some time after dispersing (64,

125,129). Telemetry studies reveal large spatially overlapping territories of both males and females, however, usage of a particular area is temporarily separated (64,148). Males are reported to maintain the highest spatial separation within an overlapping home range, with an average of 5.4 km, while females seem to be more tolerant between them, keeping a distance of about 2.6 km (148). Range-wide population estimates vary in reliability due to the difficulties inherent to the tracking of these animals but densities calculations in some areas fluctuate between <0.1 to 10 or more individuals per 100 km^2 (125,129). Regarding captivity, former literature suggests pair bonding and living as a possible social organization (51,53) and there is no evidence of any well-established dominance pattern (50).

Wild Snow Leopards have a crepuscular activity pattern, meaning they are more active during dawn and dusk/night. The cats are quite active as they patrol and travel around their home range, leaving signs in sites which are marked frequently by more than one individual (64,129). These core areas work as a pool of communicative olfactory signalment (64). They will usually remain in a place for one week or more before shifting to another area (126) and can cover great distances in a short period of time: records in Nepal show movements of 7-12 km daily (64) but average distances approach 1.9 km and 2.2 km, regarding males and females, respectively (148). In Mongolia, there is record of a female covering 28 km in a single day (64). Winding walking routes may consist of mountain ridgelines, valley floors, stream-beds, course of deep gorges and cliff edges (129,148). Snow Leopards look for sheltered and elevated places for bedding and resting quarters like rocky caverns or secluded clefts on cliffs (148). In captivity, SL are also known to be more active during early in the morning and at late evening but spend a high proportion of its time resting and sleeping with only brief activity phases occurring during the rest of the day (51,53,59). Knowledge of hunting methods is scarce and most information comes from records of eyewitness accounts. It is suggested that the cats rely on a surprise effect by resorting to a stalking and ambush hunting style, using the cover of the broken terrain to hide their approach and get close enough to launch an attack from above on their prey (59,125). They will pursue the quarry with incredible agility across the cliffs, steep slopes and down the rocky outcrops and kill it with a bite to the nape or throat (129). Kill of a very large ungulate usually happens every 10-15 days (125,126). Snow Leopards are considered a very slow eater (51): if not disturbed they may remain in the area where theyhide the kill, keeping themselves occupied feeding and resting until all meat is consumed, for as long as one week (64,148). As a solitary top predator, Snow Leopards are widely assumed to

maintain a dispersed social system by patrolling and marking their home ranges with a set of scent communicative signalment that allows them to identify and gather information about other snow leopards present in the area, so as their gender and reproductive status (70,129). It is also suggested that the scent marking system helps to locate other individuals and to maintain spatial and temporal distance if avoidance is preferred, playing a vital role in the efficiency of management of overlapping territories and their resources (64,70, 129). Olfactory signs containing pheromones are left by spraying urine or anal gland content into a prominent vertical surface, scraping the soil with the hind feet, what is usually accompanied by normal urination or defecation, and rubbing the cheeks (where facial sebaceous glands are located) against the furnishings of the rocky habitat. (53, 70). Clawing on tree trunks can work both as an olfactory and visual signalment, due to scent derivative from interdigital glands in the paws and the visual marks left by the protractile claws when scratching. The scents are then received by displaying the Flehmen response and drawn into to the vomeronasal organ, what allows assessment and interpretation of this pheromones' rich messages (70). Snow Leopards have a wide repertoire of facial expressions, meaningful movements of the tail and characteristic whole body postural displays that are the foundation of visual signalment (59,70) so useful in sending clear messages about individuals intentions when encounters are inevitable. Vocalization is the ultimate and least known method for felid intraspecific communication. Snow Leopards cannot purr or roar (70,129) but they yowl, growl, meow, snarl, hiss and spit as in other Felidae (59,129). Their vocal repertoire also includes a very characteristic and friendly soft puffing sound known as Prusten (53,58), a long drawn out caterwaul used by the female when in heat (128,129) and a typical copulatory cry made by the male during mating (9,53).

2.5.5. **Reproductive Biology and Behavior**

Felids are one of the most threatened group of mammals (73) and 29 of the 38 species listed in the IUCN Redlist of Threatened Species hold declining wild population's trends (132). *Ex-situ* breeding programs goal is to sustain genetically viable and healthy captive populations (147,148), however, it is acquainted that many of the non-domestic felids do not reproduce well in captivity (19,20). Knowledge and understanding of reproductive physiology and hormonal cycling of individual wild felid species, along with aspects of ovulatory patterns, anatomy, behavior and effects of seasonality, are vital for their thriving

in captive environments and overall conservation. Health and efficiency potentials of captive breeding are maximized by the scientifically supported demand of higher standards and welfare management efforts for the development of successful *ex- situ* breeding programs through enforcement of both natural and assisted reproduction techniques (20). Detailed information about reproductive parameters and biology of wild Snow Leopards is scarce and most of the knowledge regarding is extrapolated from studies developed in captive animals (82,117). For the last two decades, there has been a striking increase in the scientific knowledge about endocrine cycles of nondomestic felids, which patterns have been described and published for half of the species (20). A summary of Snow Leopard's reproductive parameters and biology is available in Table 4. The reproductive cycle of the SL is now assured to be strictly seasonal and moderately affected by photoperiod (20,51,73). Breeding befalls during the late winter and early spring months with a reported peak in February (9,129), and females will be receptive for only a few days during this period of time (129). Births usually take place in April, May or June (59,129), when the climate and prey availability are optimal for overall survival of the cubs in the wild harsh environment (129). In captivity, they may happen until early September, with a peak in May (103,148). Johnston et al (1994) demonstrated a distinct influence of seasonality on seminal and endocrine reproductive patterns of the male Snow Leopard. Testicular volume was greatest during winter and lowest during the summer months, along with optimal ejaculate quality and maximal hormonal concentration (LH, FSH and Testosterone). Although males reach sexual maturity at about the same age as females, their reproductive life is longer. Reproductive age span of males goes up to 14 years but reproductivity decreases abruptly after 12 (9,10). The reproductivity of females has its peak during 8 years old, slowly decreases until the age of 11 and has a drastically decline after that (9). Estrus, as exhibited by copulation, is set in a variable but limited time frame and may last from 2 to 8 days (129,148). This period is characterized by endocrine oestradiol peaks (19,20,117), consistently correlated with a remarkable precopulatory increase in affiliative and close contact behaviors like sniffing the anogenital area of the female by the male, social allogrooming, head and body rubbing, following and play (53,117). There is a rise in the frequency of scent- marking (9,64), levels of activity and vocalization (Prusten, mainly). Females may present lack of appetite, restleness and rolling on their back, and were observed to produce a continuous yowling sound that is thought to serve to attract males (9,116). They present themselves to the male by walking in front of him with their tail raised (9) or by lying on the ground in lordosis with the tail turned aside (53). Copulations in the SL can exceed the 20 times a day (82,129), may last 15 to 45 seconds (129) and are similar to the other big cats: although

ventral/ventral mating has also been reported (53), the male usually mounts in ventral-dorsal position while gripping the fur on the female's neck (9,53). A successful copula is characterized by the male´s very characteristic piercing cry at the end of the thrusting act, when full penis intromission is achieved (50,53). Female may growl and may be aggressive towards the male when he dismounts, by fighting swear at him, pawing or even chasing (9). Cats have been traditionally characterized as induced ovulators, requiring frequent matings to stimulate sufficient LH surges for final ovulation, but recent studies show that some species may also experience spontaneous ovulation. This ovulatory pattern is, however, nonexistent or, at least, very rare in the Snow Leopard (20,117). Estimates of inter-estrus period were variable among studies but a general interval of 15-39 days was reported (50,126). Schmidt et al (1993) identified similar periods' length of oestradiol and sexual behavioral peaks accounting 3.6 and 3.03 weeks, respectively, for females housed alone, and a larger interval of 6 and 8 weeks, for females housed as a breeding pair and actively mating but with no production of offspring. Gestation period varies between 90 and 110 days (9,148). Like all Felids, the Snow Leopard has an endotheliochorial type, zonal placentation (20,73). In the wild, female gives birth in a nest in a rocky sheltered den, lined with the soft fur from the underparts of her venter (59,129). Reported parturitions in captivity last an average of 2-3 hours and, in most cases, take place during the night/early morning (52,82). The newborns are altricial: fully dependent on the mother, with eyes closed and unable to regulate their own body temperature (9,52,59). Behavioral development of the cubs is widely described in literature due to hand-rearing methods in zoos. The young will start to open their eyes around the 7-9 day (9,59,82). After 3 weeks they will start to follow the mother (82,129) and within a couple of weeks will master upright walking and directional movement (52). This is also the period when the cubs start to develop social and solitary play (57,143), a behavior associated with hunting learning activity (59). Although weaning age is considered to be around 5 months, the cubs will start to eat solids at 6-8 weeks (9,59**)** when the mother is less available and shows an increasing reluctance to nurse (52). The cubs will not be fully independent until they reach one year of age. This period is very critical regarding their survival and the mean mortality rate is estimated to be around 42% (10).

Table 4. Summary of Snow Leopard's biological reproductive parameters

Reproductive Parameters		
Reproductive season		January to mid-March
Sexual maturity	Male	3-4 years
	Female	3-4 years
Estrus		2-8 days
Inter-estrus period		15- 39 days
Gestation		90-110
Inter-birth interval in the wild		Up to 2 years
Inter-birth in captivity		1 year
Mean Parturition length		2-3 hours
Mean litter size		2-3 (range 1-5)
Weight of newborns		450 ± 90 grams
Mortality in the first month		32%
Weaning age		5 months
Age at dispersal in the wild		18-22 months
Longevity in captivity		19 years (up to 21)

3. Material and Methods

3.1. Material

3.1.1. Location

The study was conducted at Saint Inácio's Zoo which is a private institution that belongs to the French group Thoiry since 2014. It opened doors in 2000 and, apart from its recreational and profitable ends, its major goal is wildlife conservation. The zoo carries out its mission by advocating environmental and social responsibility in support of worldwide fauna and flora protection, ensuring animal welfare and participating in international reproduction programs. The park has 15 hectares, houses 260 different species and participates in several European Studbooks and 40 EEP, including the one of the Snow Leopard (*Panthera uncia uncia*)(figure 9) (153).

Figure 9. Saint Inácio Zoo's Map and localization of the Snow Leopards' enclosures.

3.1.2. Subjects

The Zoo houses two adult, captive born Snow Leopards with no genetic relationship: a 2 years and a half young male, named Kamal, and a 6 and a half years old female, named Ariana (Table 5).
General information on their individual history and past experiences was collected from zoo records (ZIMS) as well as zoo personal in order to create an identification sheet for each one. In the past, they had randomly received some enrichment items but neither one had been part of a solid and individually planned enrichment program before. Ariana had been transferred at a very

young age (less than 1 year) to the Zoo of Santo Inácio, and was living there for a long period of time when the male arrived. During her time in Zoo of Santo Inácio she has dwelt in the same park and has never been bred neither housed together with another conspecific. Kamal has remained in a nearby location since his arrival. The information sheet also included morphologic particularities that would allow to physically differentiate male from female during observations (Figure 10).

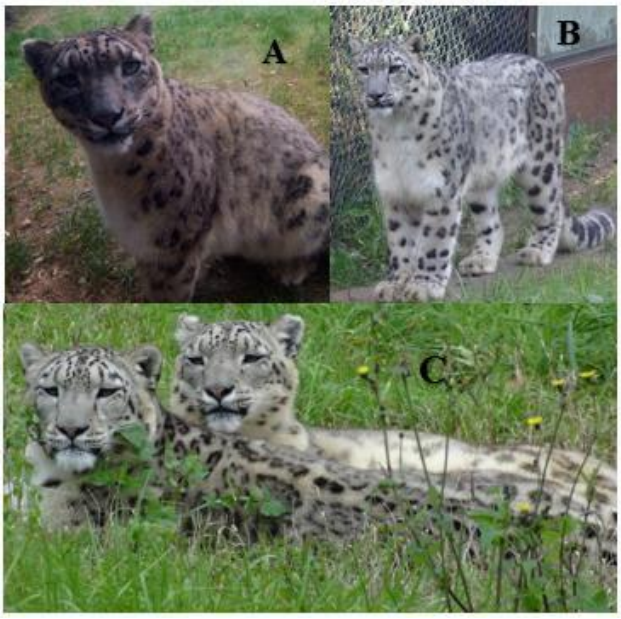

Figure 10. Subjects of the study and their morphology. A) Ariana is heavier, has a larger head bone structure, ragged years and yellowish fur. B) Kamal is more graceful and has a pale-grey coat with two big spots over the ischial tuberosity, making the shape of a butterfly. C) Kamal (in front) and Ariana (behind) laying together.

Table 5. General information about the two Snow Leopards participating in the study.

Animal	**Gender**	**Birth**	**Transferred from**	**Arrival**	**Enclosure**	**Social Status**	**Time in the zoo**	**Time with other cats**	**Behavioral problems**
Kamal	Male	July 08, 2013	Wilhelma Zoo (Germany)	March 10, 2015	A	Solitary	~ 6 months	0	Yes
Ariana	Female	May 12, 2009	Warsaw Zoological Garden (Poland)	March 11, 2010	B	Solitary	~ 5 years 6 months	0	Yes

3.1.3. **Enrichment and recording material**

Two digital single-lens reflex (DLSR) still cameras (Figure 11) were used to videotape the animals so their behavior could be posteriorly analyzed in more detail: a Nikon D3200 and a Fujifilm Finepix S2000 HD.

The material used to enrich the snow leopards' environment was chosen after other enrichment studies in the family *Felidae*. The items consisted of solid objects that the animals were able to manipulate and various odors like nutmeg, catnip and other animal's substrates (Figure 12). A list of all the material used in the study is presented in Appendix I.

Figure 11. DLSR Cameras used in recording sessions.

3.2. **Methods**

3.2.1. **Housing and Husbandry practices**

Enclosure type and management techniques were similar for Ariana and Kamal. At the beginning of the study both animals were housed singly in contiguous parks separated by a wire mesh, so they could see, hear, smell and limitedly touch each other. Kamal occupied park A while Ariana dwelled in Park B (Figure 13).

Outdoor enclosures A and B have a total area of 525m^2 and 366m^2, respectively. Both are covered and filled with natural substrate – soil and grass- and furniture, including a lake, some deciduous trees, bushes, logs and rocky hills. The parks are not fully closed on the top but are limited on all sides by a high wire fence with low- voltage electric wires that prevent the animals from pushing against the mesh or trying to climb it. Indoor housing is made of concrete walls and ads up to an area of 17 m^2 in total. Each indoor enclosure has a raised platform for the animals to lay down and rest and a small window that allows little light to enter if access doors are closed. There is no ventilation system. All the enclosures are connected by guillotine doors that are used to shift the animals from one place to another.

A head keeper and two assistants are in charge of the husbandry routine of these two animals. They are usually moved into the outside enclosures before zoo opening hours (at about 10 a.m.) and are shifted inside at closing time (7 p.m. in summer and 5p.m. during the rest of the year) when the SL are fed. However, the schedule is liable to external factors (such as the keepers' own routine) and will vary according to them, especially on Mondays when the Zoo is closed. Indoor housings are cleaned everyday with running water and Virkon. Outside enclosures and electric wires are also inspected on a daily basis and feces or any harmful objects picked up. Once a week the lake is disinfected and cleaned up. The two SL are fed 6 days per week on a varied diet of horse, chicken, cow and rabbit's meat and fast every Monday, when they occasionally receive bones. The basic diet is supplemented 3 times a week with 20g of Carni-zoo which is a supplement, particularly designed for carnivorous animals, that contains all the necessary minerals, vitamins and trace elements (e.g. taurin, carnitine and a good Ca:P ratio). The quantity of meat offered differs from male to female, and it will go from 1.2 to 1.7 kg for Kamal and from 1 to 1.4 kg for Ariana. The lower value refers to meat-free bone and the highest to meat with bone.

Routine veterinary care is given by regularly executing fecal examinations every 3-4 months and a deworming plan is readjusted according to the results. The deworming of the big cats is made with Milbemax® (Milbemycin oxime and Praziquantel) at a dosage of 1 tablet per 10 kg or with Caniquantel Plus (Praziquantel and Fenbendazole) during three consecutive days at a dosage of 1 tablet per 10 kg. No vaccination protocol is currently established.

Regarding enrichment material used during this study, before offering the enrichment, all the items were checked together with the zoo curator and the veterinary team in order to access the risks

involved and anticipate any physical or medical hazards that could outcome. Objects were always disinfected with Virkon before being placed in enclosures, and staples and packaging tapes were removed from the cardboard boxes. Feces and feathers were tested for parasites in order to prevent disease transmission: the feces were subjected to fecal direct smears and fecal flotation methods, whereas the feathers were submitted to adhesive tape examination.

Figure 12. Several items used for environmental enrichment: A) Cardboard boxes with herbivores substrates. B) Burlap with substrates and cardboard boxes with rapines' feathers. C) Catnip scented balls. D) Southern Ground Hornbill's feathers.

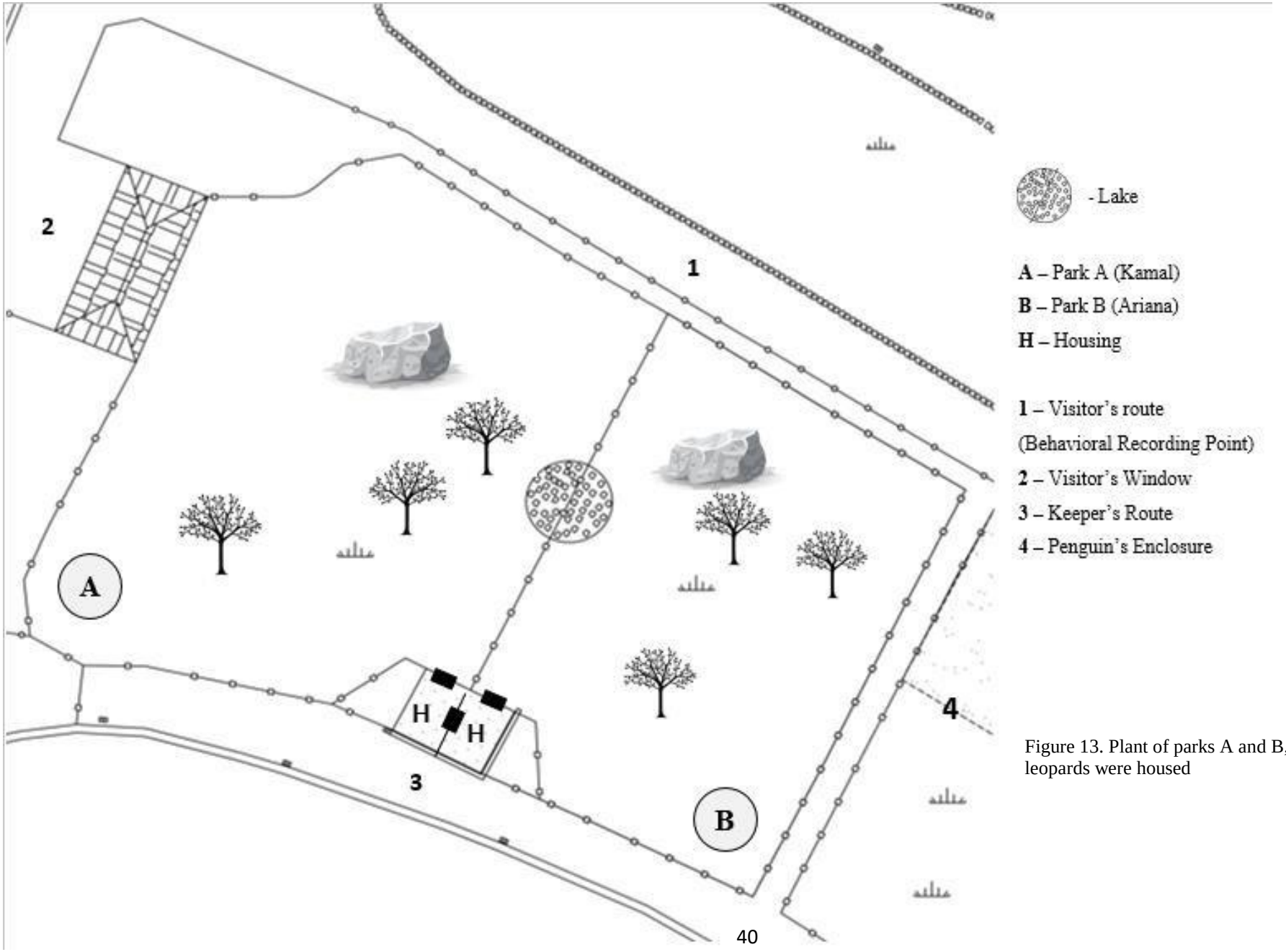

Figure 13. Plant of parks A and B, where the two snow leopards were housed

3.2.2. **Experimental Design**

3.2.2.1. **Study Definition and Behavioral Sampling Methods**

The study was conducted during the fall/winter months in Portugal, from November 2015 to March 2016. Prior to this, a preliminary *ad libitum* study was carried out for a period of 3 weeks in which brief reconnaissance observations of the animals in outdoor parks were randomly made throughout the day. The observations allowed for the construction of an ethogram and behavioral observational sheets, the identification of specific behavioral patterns (such as "Pacing") and the recognition of an activity time-budget, which outlined the behavioral observation period of interest for the main study. The *ad-libitum* phase was also essential for the observer to train her skills on behavioral recording methods and establish initial levels of accuracy, as well as for the animals to adapt and go undisturbed in her presence, reducing the bias associated.

According to the results of the preliminary study, three intervals for behavior recording were stipulated so to observations occur at proximate times each day: 1 hour in the morning, after the animals were shifted outside between 9 a.m. and 11 a.m.; 30 min in the afternoon between 2 p.m. and 3:30 p.m.; 30 min in the evening between 4 p.m. and 5p.m, before animals were shifted inside. These intervals were decided based on the fact that animals were more active during the morning and at the end of the day, but observations would always be dependent on factors related to the zoo logistics such as opening/closing time, keeper's routine and weather conditions. Moreover, enrichment devices could only be placed in the morning before the animals came out, which would correspond to their peak of activity. The 30-minute evening observation would not sometimes be completed as the animals were closed earlier due to rain/storm or the own keeper's routine.

The study was divided into 3 phases:

- **Phase I - Baseline**: Ariana and Kamal were observed one at a time in their natural environment for a total of 9 days each, during a period which went from 24 November to 22 December 2015. No enrichment devices were offered, however, the animals ended up suffering a structural type of enrichment in those days in which they were shifted from one outdoor enclosure to another, changing their usual habitat. These shifts were part of a protocol on behalf of a behavioral management program that was developed

simultaneously with the main environmental enrichment study. The goal was to progressively introduce the two SL to each other at the peak of the reproductive season in order to successively achieve mating and breeding.

- **Phase II -Enrichment**: The snow leopards were observed in a total of 17 days; on 10 of which they received all kinds of enrichment types. On the other 7 days, under baseline environmental conditions, there was no enrichment. The period went from 29 December 2015 until 22 January 2016. Ariana and Kamal were recorded in the same days but enrichment items would be given to each one on alternate days. Shifting between outdoor enclosures was still a protocol but it would happen in a day when the animals were not recorded.

- **Phase III - Enrichment and Social Enrichment**: The behavioral recording of this phase was realized between 14 March and 25 March 2016, when the pair was already together and socially stabilized; it accounted for a total of 9 days, 5 of which they received enrichment.

Sampling in the morning began as soon as the housing guillotine was open and each observation was recorded from a public viewing area (Figure 13).The enrichment program specified for this study in particular was carefully planned following the "S.P.I.D.E.R framework" steps, and all the sessions and respective items were scheduled in a calendar. In order to prevent habituation, the enrichment type was different in subsequent enrichment days. All the enrichment devices offered, except for the structural ones like big rafts serving as platforms, were removed from the enclosures at the end of the day. Enrichment sessions were assessed through evaluation sheets (Appendix VI) which were created based on a protocol that was generously provided by the Phoenix Zoo's Behavioral Enrichment and International Animal Welfare Coordinator, Hilda Tresz. Interactions with enrichments items were evaluated through direct and indirect observations, the later one with recurrence to an indirect observation rating scale (IORS). During all the phases of the study, both animals were recorded the same number of days for behavioral assessment, both singly and as a pair (social enrichment), using the Continuous Focal Animal Sampling.

3.2.2.2. Construction of the Ethogram

A streamlined ethogram was created, based on a standardized ethogram for the family Felidae (Stanton et al, 2005) that combined studies of domestic and exotic cats (9 of which referred to Panthera uncia uncia) and assembled behaviors that were very likely to apply to most felid species. In addition, a browse was also carried out through the Ethogram list of large felids available on the Ethosearch website (128). The ethogram was then adjusted for the SL participating in this study, based on the information gathered during the *ad-libitum* observations, and the behaviors chosen to feature these two animals were grouped into 8 behavioral categories (Table 6) according to their functional purposes which are summarized in Table 7. The final ethogram (Appendix I) includes a list of modifiers (Appendix III) that could be used to clarify the circumstances of each behavior, a list of vocalizations (Appendx II) and a table with descriptions and contextualization of ears and tail's positions (Appendix II), which were considered important to recognize and understand in order to efficiently interpret the cats' communicative demeanor. Some of the behaviors listed were not initially displayed, emerging as new behaviors at the different stages of the study. Others such as Stereotypy and Patrol were defined in a very accurate way, specifically for the subjects under study (Ariana and Kamal). Although not considered a behavioral category, the time when an animal was out of sight and its behavior was not visible for the observer was recorded as well.

Table 6. Description of the behavioral categories studied. Adapted from Stanton et al (2015)

Categories	Definition
Active	Motion behaviors where the cat is alert and in locomotion, moving around or from one location to another.
Affiliative	Positive behaviors that promote and strengthen social cohesion. The cat seeks social contact with another animal or human and interacts with them in a peaceful and friendly manner.
Agonistic	Socially negative and hostile behaviors where the cat shows intent to cause injury or engage in harmful physical interaction with another animal or human. It includes aggressive (both offensive and defensive) attitudes towards another subject and also anxious and fearful behaviors, which suggest the cat's inability to cope with a specific situation/stimulus.
Exploratory	Investigative and reactional behaviors that represent the cat's general interest and curiosity in its environment when facing a new situation or a specific stimulus/object (eg. enrichment item).
Inactive	Behaviors characterized by an absence of action. The cat is calm/relaxed and stationary or performing minimal movements with its head, ears or limbs.
Maintenance	Behaviors that allow to cope with the basic physiological requirements of the cat (i.e. feeding, elimination) and its external hygiene (grooming).
Marking	Behaviors whose functional objective is communication. It includes olfactory signalment and tactile marking that usually leaves visual marks (i.e. claw marks) in the furniture of the environment.
Stereotypic	Abnormal, repetitive and standardized behaviors with no apparent goal or function which occurs in captive felids (e.g. Pacing).

3.2.1. **Social Introduction**

The SL were introduced to each other on Tuesday morning, 26th January 2016, close to the peak of the reproductive season, when their mutual interest was noticeable. Both animals hadn't eaten for 24 hours since Monday is the predetermined fasting day. The session lasted for 20 minutes after which the animals were separated and isolated again in their respective enclosures. A second session occurred later that day for another 20 minutes.

On the introduction day, enrichment was provided in both enclosures as an additional distraction. It consisted of tree logs spilt with nutmeg scent, which was the one they empirically seemed to like the most in previous phases, through the evaluation of the enrichment record sheets. The animals went out into their most familiar habitat (park B for Ariana and park A for Kamal), following their normal routine for 35 minutes. At that point, the guillotine door connecting both outdoor enclosures was opened. Caretakers, the veterinary team and additional zoo staff were present during the introduction session, in possession of walkie-talkies and ready to intervene in case aggression escalated. Water hoses with pressure nozzles were available and a blowgun was prepared with a combination of sedative drugs (Medetomidine and Ketamine). The reversal agent Atipamazole was also prepared. Housing access was not allowed so as to prevent hiding or aggression episodes in a close-spaced area with limited access and out-of-sight to the keepers.

After the first introduction session, the entire initial team kept supervising the following introductions for 3 days. Then, these were monitored by the observer alone who could make use of a walkie-talkie and water hoses in case of emergency, for a period of 2 weeks. During the first weeks the duration of the introduction sessions was gradually increased up to 1 hour first , and later to longer periods of time depending on the reaction of the animals to one another. From the 3rd week on, the central guillotine would stay open all day long and the animals could choose either remaining in their familiar surroundings or exploring the outskirts which the potential mate was occupying. This time, the observer was still present though not to access the introduction itself, but to evaluate the stability of the two SL as a social group and to determine the appearance of reproductive demeanors.

Table 6. Behavioral categories used in the study and the ethogram's base behaviors grouped according to their functional objective.

Inactive	Active	Stereotypic	Maintenance	Marking
Crouch	Climb	Pacing	Defecate	Clawing
Drowsing	Hunt	Self- mutilation	Drink	Head rubbing (object)
Lying	Stalk	Excessive grooming	Eat	Scraping
Sitting	Chase	Self-chewing	Groom	Urine spray
Sleeping	Jump	Self-biting	Urinate	
Standing	Locomotion			
Yawn	Running			
	Trotting			
	Walking			
	Moving			
	Lay down			
	Stand up			
	Patrol			
	Solitary Play			
	Stretching			

Affiliative	Reproductive	Agonistic	Exploratory
Allogroom	Anogenital groom	Attack	Approach
Approach with tail up	Anogenital sniff and Flehmen	Arch back	Bite
Anogenital sniff	Copulation	Bare Teeth	Carry
Follow	Copulatory cry	Bite	Chew
Head butt	Excessive affiliative behaviors	Chase	Drag
Head rub	Flirt	Crouch	Explore
Huddling	Lordosis	Defense	Flehmen
Social Play	Mount	(Submission)	Flinch
Chase	Nape bite	Fight	Forage
Stalk	Pelvic trust	Flee	Investigate
Roll	Roll	Hide	Lick
Wrestle	Urine spray (increase)	Piloerection	Paw
Social Sniff		Retreat	Manipulate (object)
Vocalization		Strike at	Paw
Prusten (chuffle)		Threaten	Rear
		(Dominance)	
		Vocalization	
		Growl	
		Hiss	
		Spit	
		Snarl	

During the period which followed the SL introduction and until their routine was normalized, an observational journal was kept, where all the social interactions, both agonist and affiliative, were recorded in detail. Although these would not account for the main study, they were crucial for

interpreting, evaluating and balancing the behavioral management protocol's effects, as well as for the characterization of the reproductive profile of these two animals in particular.

The housing guillotines were closed as soon as the animals came outside, during all the weeks mentioned. The two outdoor enclosures were now connected through a guillotine door that was opened as soon as the animals were shifted outside, and the SL could freely room through an enlarged area, which was then 891 m^2 in total. At the end of the day the animals would recall to their respective initial housing and stay separated until the next day.

3.3. Data Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (IBM SPSS Statistics 24). To determine the effect of the "phase" and "period of the day" on the dependent variables a two-way multivariate analysis of variance (MANOVA) was conducted followed by univariate analyses of variances (ANOVA) and Tuckey's Post hoc test, when appropriate. The significance level was set at $p \leq 0,05$.

4. Results

Data concerning the time each animal spent in each behavioral category during the different periods of the day (mornings, afternoons and evenings) throughout the different phases under study (Baseline-phase I; Environmental Enrichment-phase II; Environmental and Social Enrichment-phase III) is expressed in percentage. Detailed results regarding enrichment evaluation as well as social introduction (social enrichment) results are reported further on in this section.

4.1. Ariana

The activities which suffered greater changes throughout the different periods of the day correspond to behaviors included in the Active, Inactive, Stereotypy and Maintenance categories (Figures 14 to 22). In general, Ariana is more active in the evenings. However, regardless the period or the phase of the study considered, Ariana spent more than 50% of her daily budget inactive, this means, performing behaviors included in the Inactive behavioral category. These behaviors were consistently more predominant in the afternoon when comparing with the morning and evening periods. The amount of time Ariana spent out of sight (not visible) varied throughout the day, in all the phases of the study, but particularly during phase I (Figures 14, 17 and 20). Ariana exhibited an estrus during phase I, which lasted for about 6 days and was characterized by a slight increase in affiliative behaviors such as rolling, anogenital grooming (Appendix V) and vocalizations (Appendix II).

During the **morning** period (Figures 14 to 16), Ariana presented statistically highly significant differences regarding the Affiliative category: there was a clear and progressive exhibition of the behaviors included, going from null in an initial observation in phase I to 4% of the total amount of time observed in phase III ($p<0.001$). The Active category presented a statistically significant increase throughout the study which is clearly well-established between phases I and III, being the amount of time occupied by this category in phase III four times higher than in the initial phase ($p=0.001$). Agonist behaviors were eliminated from phase I to phase III in a statistically significant way ($p=0.05$), springing from an initial value of about 1% to almost 0% in both phases, II and III. Although not having a statistically significant value, the period of time Ariana spent out of sight suffered a tendency to diminish gradually throughout the 3 phases, ending with a minimal

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representation of the total amount of time observed in the mornings. The time spent in the remaining behavioral activities slightly varied during the different phases of the study, however, without statistical meaning.

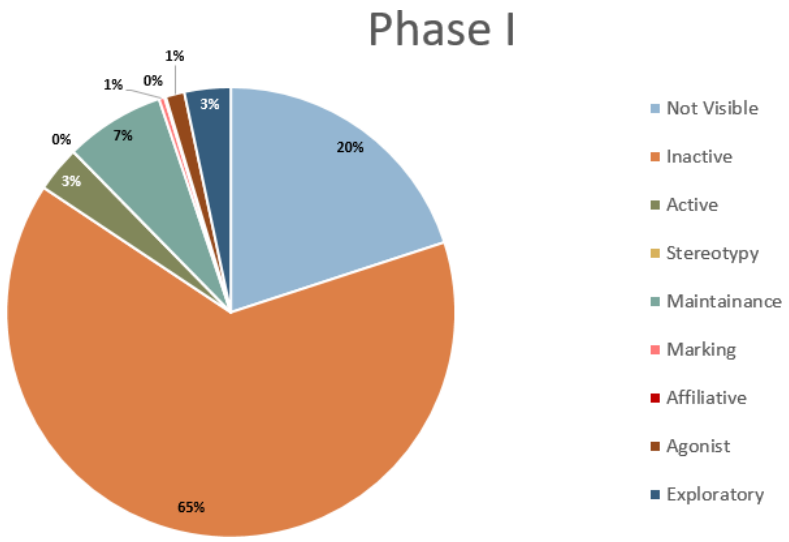

Figure 14. Ariana's behavior, distributed according to the different behavioral categories during the morning period of Phase I (Baseline).

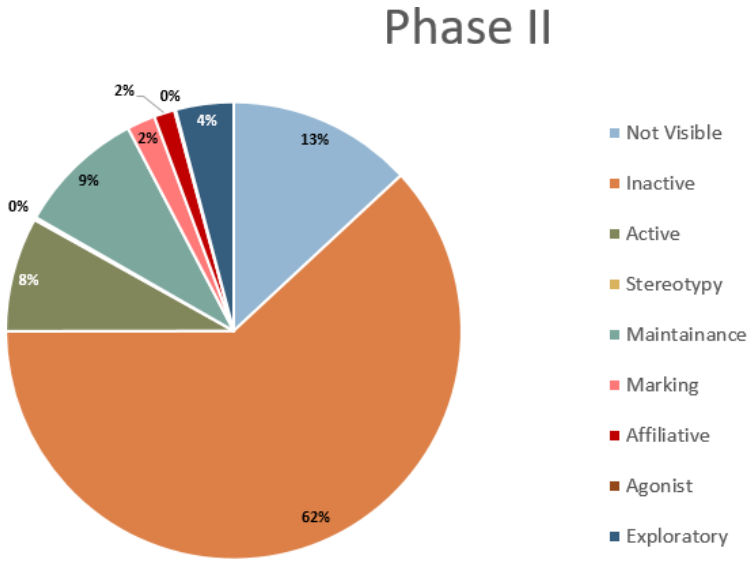

Figure 15. Ariana's behavior, distributed according to the different behavioral categories during the morning period of Phase II (Environmental Enrichment).

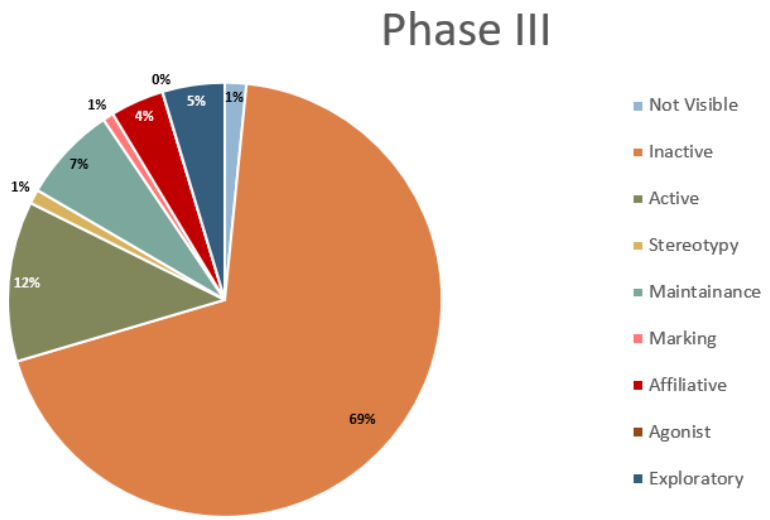

Figure 16. Ariana's behavior, distributed according to the different behavioral categories during the morning period of Phase III (Environmental and Social Enrichment).

Regarding the **afternoon** period (Figures 17 to 19), no significant statistical differences were observed between the different phases of the study. However, it is noticeable that the behaviors included in Stereotypic category were hardly visible in phase III; it is also noteworthy that Active demeanor behaviors, which remain constant in the first two phases of the study diminish in phase III; also, Affiliative behaviors increased up to 3% of the total time observed in phase III.

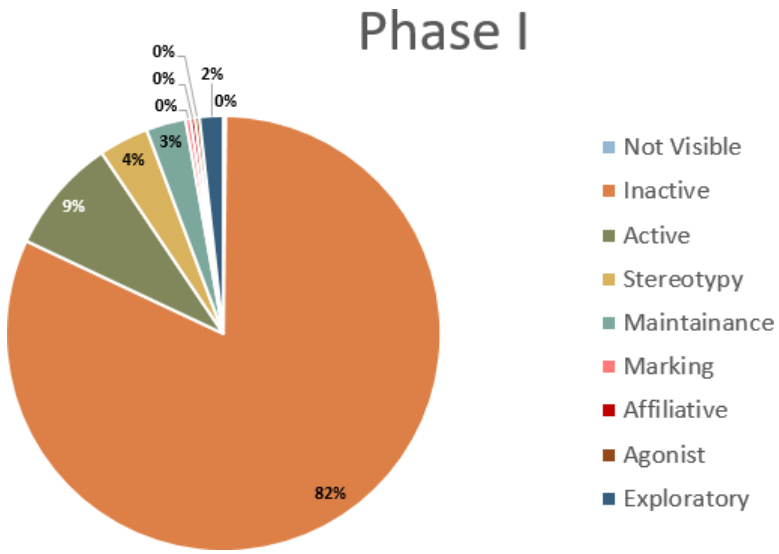

Figure 17. Ariana's behavior, distributed according to the different behavioral categories during the afternoon period of Phase I (Baseline).

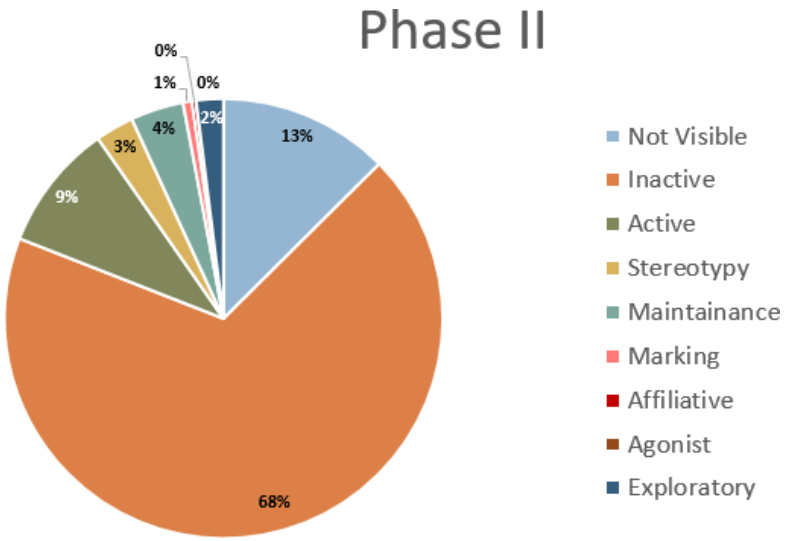

Figure 18. Ariana's behavior, distributed according to the different behavioral categories during the aftenoon period of Phase II (Environmental Enrichment).

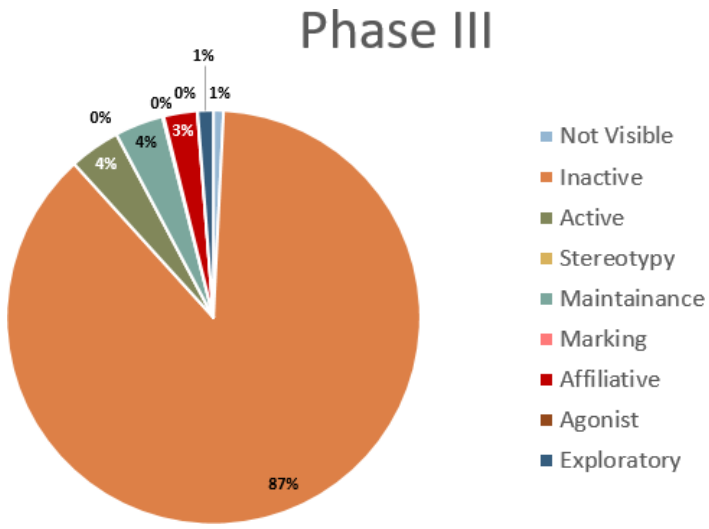

Figure 19. Ariana's behavior, distributed according to the different behavioral categories during the afternoon period of Phase III (Environmental and Social Enrichment).

As for the **evening** period (Figures 20 to 22), when the animals were about to be closed in their interior housing, statistically significant differences for the Affiliative category were determined ($p=0.013$). It was possible to observe a marked increase in the performance of such behaviors, from phase I up to a prevalence of 6% of the total evening time observed in phase III. None of theother

categories differed significantly. However, a decrease in the behaviors from the Stereotypic category was observable between phases I and II, and phase III.

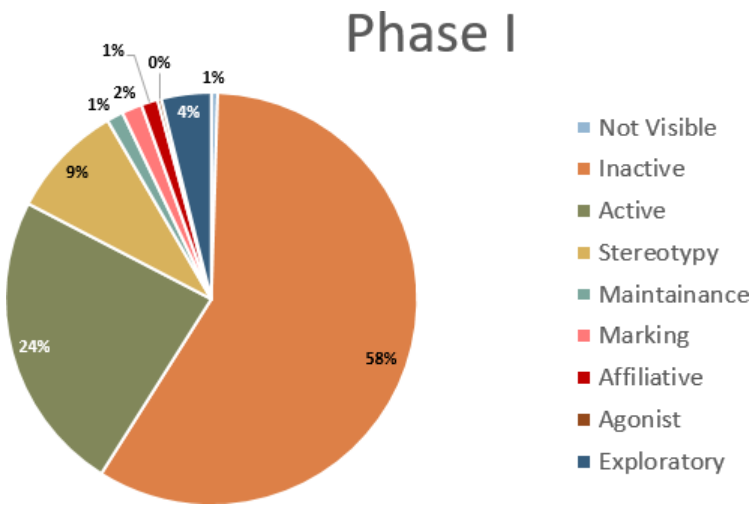

Figure 20. Ariana's behavior, distributed according to the different behavioral categories during the evening period of Phase I (Baseline).

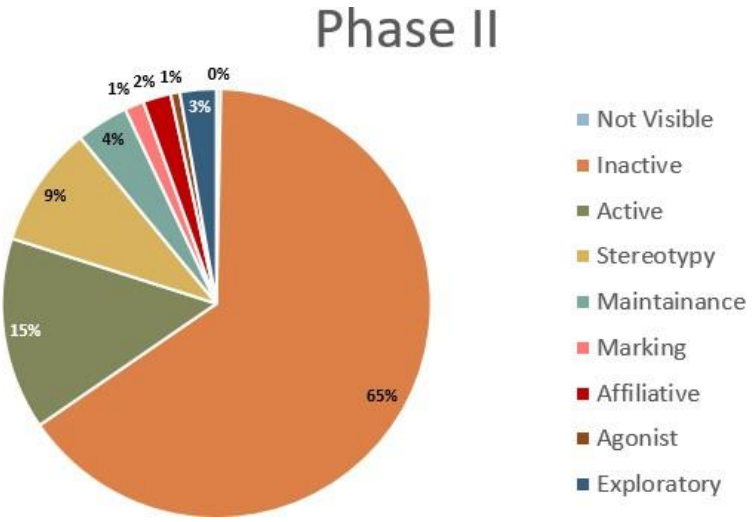

Figure 21. Ariana's behavior, distributed according to the different behavioral categories during the evening period of Phase II (Environmental Enrichment).

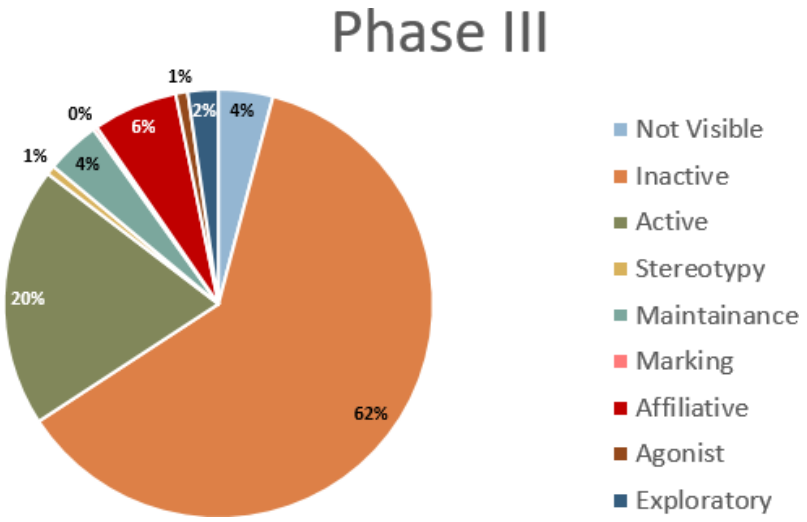

Figure 22. Ariana's behavior, distributed according to the different behavioral categories during the evening period of Phase III (Environmental and Social Enrichment).

4.2. Kamal

The behaviors that suffered greater changes throughout the different periods of the day are included in the Active, Inactive, Stereotypy and Maintenance categories. Generally, Kamal is more active during the morning period, and behaviors from the Exploratory, Stereotypy and Active categories were performed at their daily height in all phases of the study (Figures 23 to 31). Stereotypy behavior showed a tendency to diminish gradually as the day went by, reaching a minimal value at the end of the day, regardless the phase under study. Behaviors included in the Active repertoire were at their lowest values in the afternoon period in all the phases of the study, whereas Inactivity behaviors were higher during the afternoons (more than 70%) in comparison with the mornings (less than 50%).

During the **morning** period (Figures 23 to 26), behaviors from the Active category had its maximum expression in phase I, occurring a statistically significant reduction between phases I and II ($p=0.01$). There was a slight increase again in phase III, without statistical significance, though. A clear increase in the Marking behavioral category is well-established between phases I and II ($p=0.025$). Also, for phases I and II an obvious decrease of the stereotypic behaviors, from 32% to 15% was observed ($p=0.034$). Although this value increased in phase III, it had no statistical

significance and it still remained inferior to that of phase I. Concerning the Affiliative category, Kamal exhibited a noticeable increase of these behaviors from the initial phase of the study to the last one ($p<0.01$). The remaining behavioral categories did not differ significantly along the study although it is observable an apparent punctual increment of Maintenance behaviors between phases I and II.

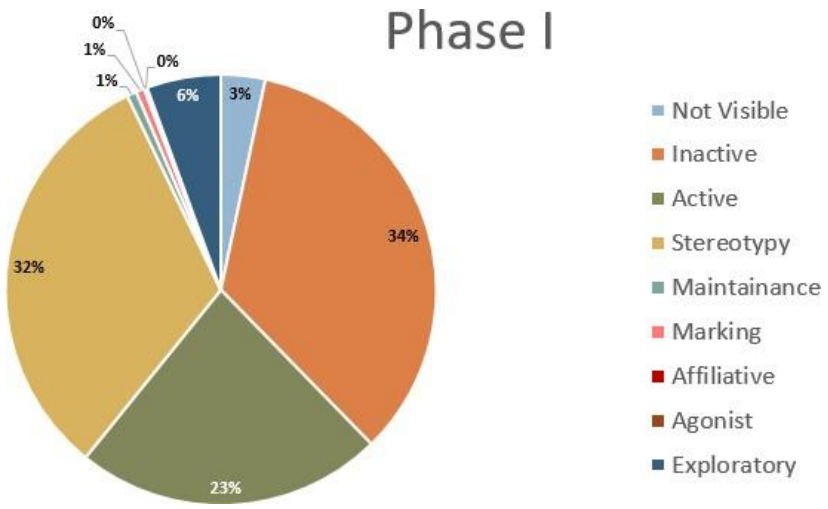

Figure 23. Kamal's behavior, distributed according to the different behavioral categories during the morning period of Phase I (Baseline).

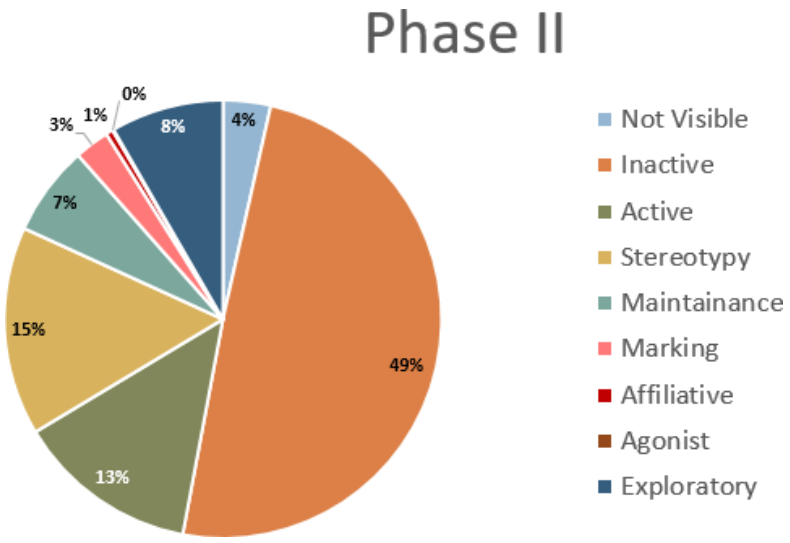

Figure 24. Kamal's behavior, distributed according to the different behavioral categories during the morning period of Phase II (Environmental Enrichment).

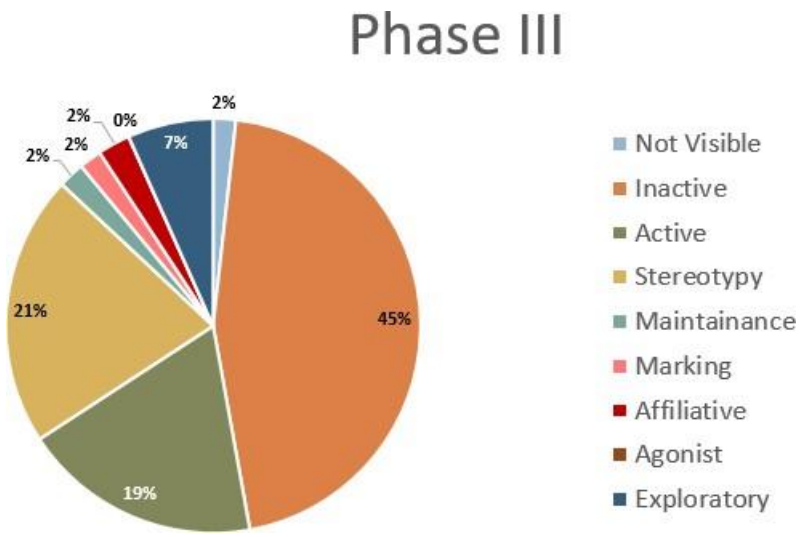

Figure 25. Kamal's behavior, distributed according to the different behavioral categories during the morning period of Phase III (Environmental and Social Enrichment).

As far as the **afternoons** are concerned (Figures 26 to 28), a significant increase in the Affiliative behaviors was observed between phases I and III (p=0.027), whereas the other behavioral categories did not differ in a statistically significant way. Notwithstanding, it is noticeable a tendency to the gradual diminishing of Kamal's stereotypic behaviors during this period of the day throughout the different phases under study, attaining a null representation in phase III.

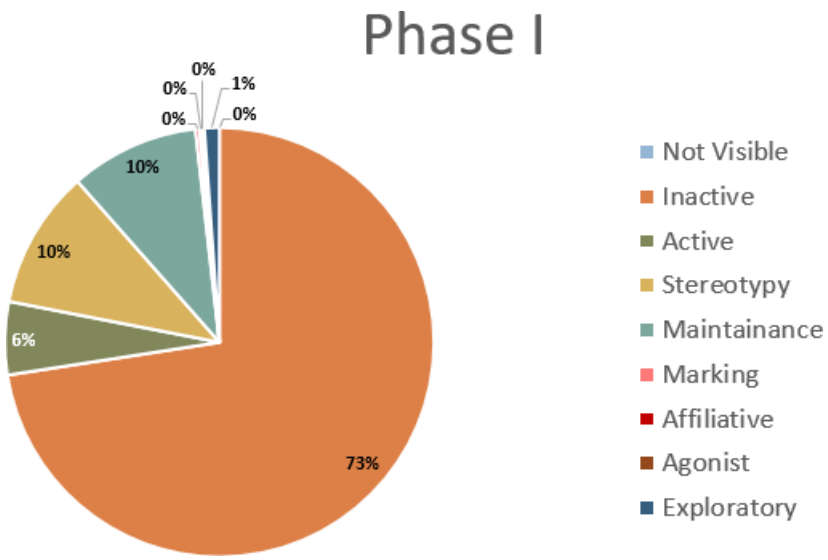

Figure 26. Kamal's behavior, distributed according to the different behavioral categories during the afternoon period of Phase I (Baseline).

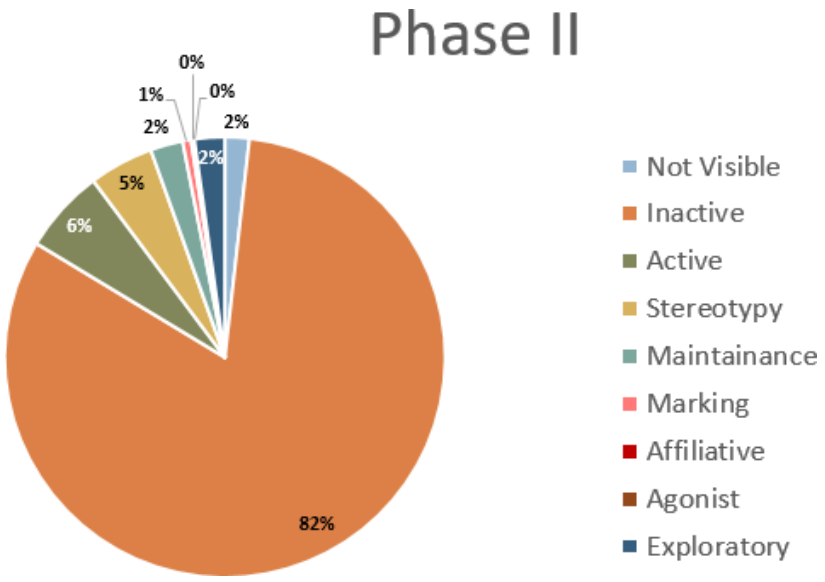

Figure 27. Kamal's behavior, distributed according to the different behavioral categories during the afternoon period of Phase II (Environmental Enrichment).

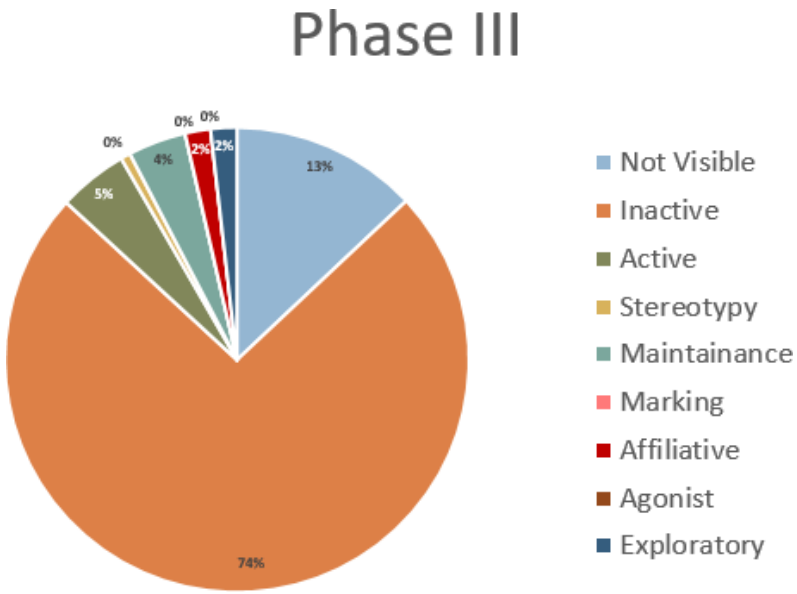

Figure 28. Kamal's behavior, distributed according to the different behavioral categories during the afternoon period of Phase III (Environmental and Social Enrichment).

In the **evening** period (Figures 29 to 31), it was possible to find statistically significant differences for the Exploratory ($p=0.005$) and Affiliative ($p<0.001$) categories, having both a far more accentuated representation in phase III (respectively 5% and 6% of the total amount of observation time during this period). Despite devoid of statistical significance, a tendency towards a gradual decrease of the Stereotypy category of behaviors throughout the 3 phases of study was apparent. The remaining behavioral categories did not show any statistically significant variations during this period of the day.

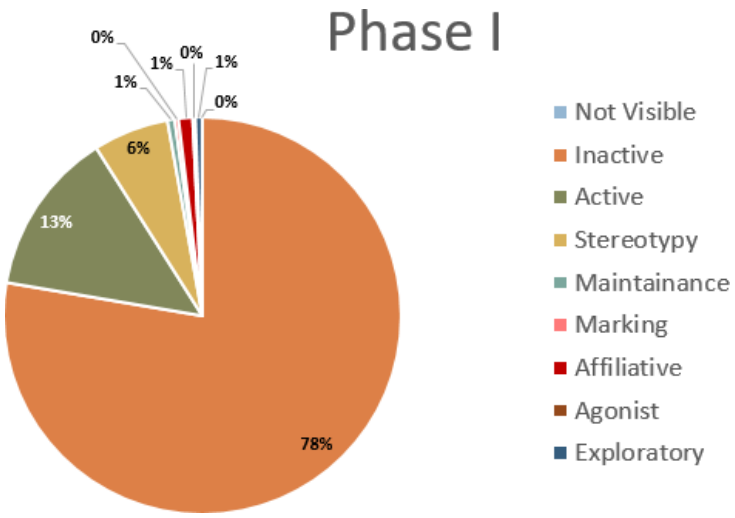

Figure 29. Kamal's behavior, distributed according to the different behavioral categories during the evening period of Phase I (Baseline).

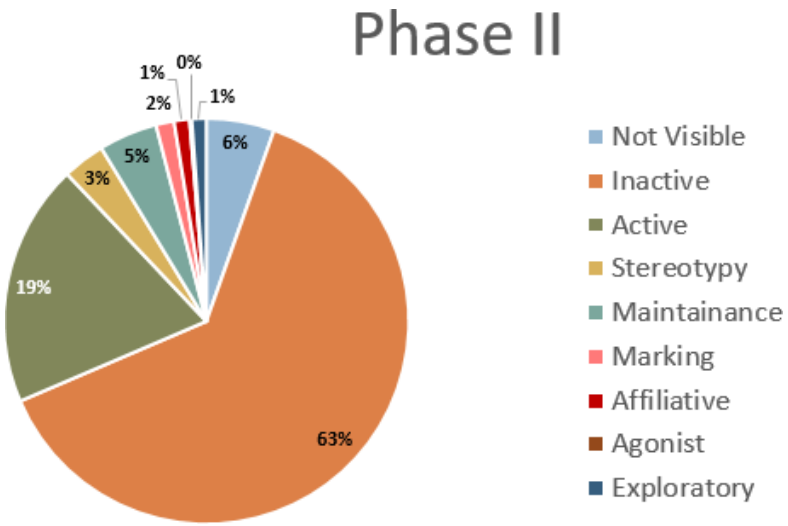

Figure 30. Kamal's behavior, distributed according to the different behavioral categories during the evening period of Phase II (Environmental Enrichment).

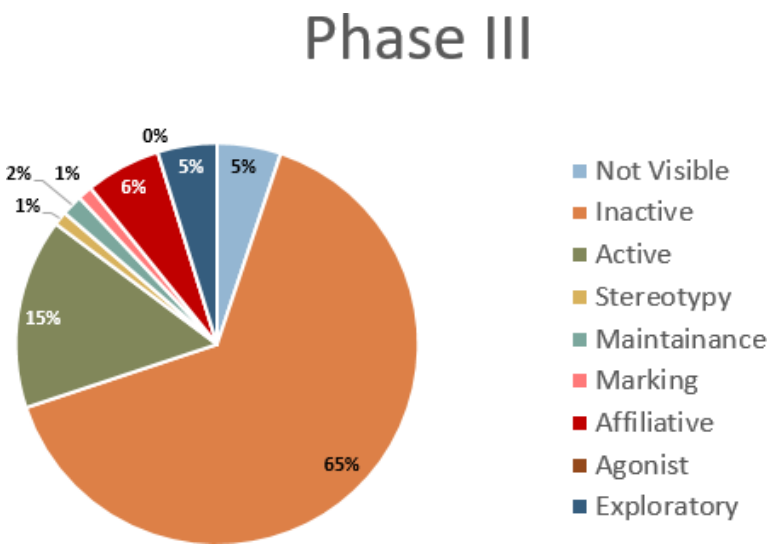

Figure 31. Kamal's behavior, distributed according to the different behavioral categories during the evening period of Phase III (Environmental and Social Enrichment).

4.3. Enrichment Evaluation

In order to assess the success of the enrichment provided, "Evaluation Sheets" (Appendix VI) were thoroughly filled after all enrichment sessions. Results, which portray a direct appreciation of the overall time the subject was actively interacting with the items concerned (e.g. touching, grabbing, pawing, smelling, and fully paying attention to) through all the periods integrated in daily recordings, are presented in table 7. All items would suffer an additional indirect evaluation along the day by filling in an IORS (Figure 32). Despite the length of the interaction, records would also evaluate the number of interactions, the success in achieving pre-determined goals and the number/quality of (new) natural species-specific behaviors elicited.

Figure 32. The same enrichment item in the beginning vs ending of the day. The image on the right is suggestive of an IORS of 2 as the item is reaped apart, and contains marks of teeth while the feathers are wet.

According to table 7, Kamal is shown to be more reactive to enrichment and to spend a larger period of time using it. Less successful enrichment items included Bones and Amur Tiger's feces, the first of which elicited no observed reaction at all and accounted for an indirect rating value of 0 for indirect observation. Although Ariana was less effusive than Kamal, she used all the items provided, with the exception of the plastic barrel filled with newspaper balls (0 in IORS). Less preferred items were determined to be Amur Tiger's feces and a cardboard box filled with newspaper balls. Interaction with bones accounted for only 55 seconds on direct observation, however, the items would be in different places at different times of the day, a strong indication of its usage (IORS 2). An enrichment which proved to be very efficient for both subjects was the provision of avian feathers which resulted in a high level of interest and interaction, and elicited a series of new species-appropriate behaviors (Figure 33).

Figure 33. Ariana investigating Southern Ground Hornbill's feathers.

The behaviors mostly stimulated where inserted in the category of exploratory, marking and active demeanor. Scented material and tactile *manipulanda* stimulated active investigation and smelling, Flehmen reaction and manipulation of items (with both paws and mouth) as well as foraging and marking behavior. Structural enrichment mostly stimulated the Active behavioral category with the exhibition of climbing, jumping and balancing on the furniture added. As enrichment was introduced, several base behaviors suffered a tendency to intensify themselves. Novel behaviors which were not observed before (neither on *ad libitum* observations neither on phase I) were now being exhibited, added to the initial ethogram and sorted out through the different behavioral categories (Figure 34). As an example, activities like grabbing, dragging, transporting/carrying on mouth, tearing apart, ripping and pulling off an object were included (Figure 34). It was also observed a cognitive stimulation as a consequence of the need for problem resolution created by the institution of certain enrichment devices. Social introduction played a very important role in the ethogram evolution as it led to exhibition of new social behaviors such as allogromming, anogenital sniff , head rubbing , following and social play (Figure 35).

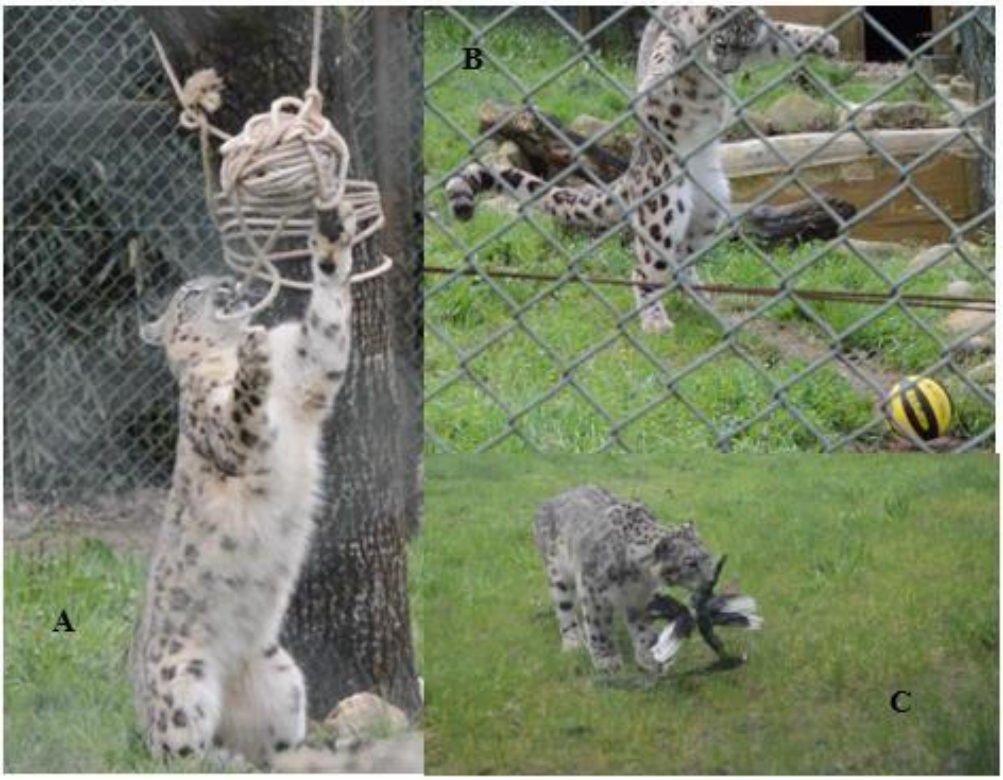

Figure 34. Example of novel and intensified species-specific behavior: A) and C) Foraging Behavior; B) Play

Table 7. Total daily period of time the subjects were observed actively using enrichment items offered.

	Enrichment items	Time (min:sec)	
		Ariana	Kamal
Phase II	Nutmeg Scented Pine Branches	4:18	13:03
	Avian Feathers	13:41	15:51
	Amur Tiger's Feces	0:32	0:6
	Structural	7:50	32:44
	Catnip Scented Balls	2:21	5:20
	Bones	0:55	0 :00
	Zebra's Feces in a Box	5:08	7:16
	Catnip Scented Pine Branches	10:55	15:04
	Nutmeg Scented Balls	4:52	3:11
Phase III	Hanging branches and rope ball	6:38	7:50
	Feathers and Burlap	2:01	3:39
	Peppermint Scented balls and Brushes	5:33	4:11
	Plastic Barrel filled with newspaper balls	0:00	0:29
	Cardboard box filled with newspaper balls	0:37	11:32

Figure 35. Novel Social behaviors exhibited during phase III: A) Ariana and Kamal using enrichment together. B) Social play.

4.4. Social Introduction.

The first contact between Ariana and Kamal was quite peaceful. Kamal was the one taking the initiative, moving without hesitation towards Ariana, who was crouching on the rocks of her enclosure, watching him closely. They smelled and felt each other prudently without showing signs of aggression (Figure 36). After this first encounter, Kamal tried further interactions whereas Ariana avoided them, leading to an obvious stressful situation that kept on going for a week: Kamal would invasively force interactions, chasing Ariana around. She would run away and, when not able to do so, she would adopt a defensive posture of aggression. On the second day of introduction, there was the first big fight, with all the evidence of hazardous physical contact including biting and loud vocalizations. During the first week Kamal would patrol Ariana's park in a relaxed way, exploring and marking it, and he would even sleep in there. As a result of his presence, Ariana would pace on the edges of the park with mouth open and gasping. After the first week, Ariana's made her first approach, to which Kamal responded effusively moving abruptly in her direction, which resulted in another aggressive encounter. Ariana's behavior changed from what was considered a stressful coping response to active aggressive posture of attack every time Kamal approached. Ariana's aggression had a tendency to increase over the weeks, leading to several aggressive encounters, some of which turned into small fights (Figure 37). In most of them Kamal adopted a submissive posture, rolling on the ground in front of Ariana with its abdomen exposed. After almost 4 weeks, Ariana's behavior changed drastically: she was performing social affiliative behaviors (e.g. head rubbing), through the fence that separated both parks following and stalking Kamal in a playful way and initiating approaches to which Kamal would respond nervously by running and trotting around. She was observed performing anogenital grooming and rolling. Every time it was Kamal the one coming closer, she would still show signs of aggression, however, these would be less exuberant. Estrus was considered to start on 25th February 2016 where lordosis presentation first took place. Ariana would allow physical contact and roll on the ground while Kamal would perform anogenital sniff and follow her everywhere, trying to mount her. Copulas started the next day. Most of them were performed with correct positioning of both animals but without penile introduction. Successful copula was considered to occur on 29th and

this specific date was the one validated as a starting point for the gestation calendar and calculation of the expected birth day. Estrus lasted for 7 days. After 2nd March no more copulas were observed. Ariana didn't go through another estrus cycle so, after 30 days, gestation was considered to be a possibility. After estrus, the subjects would still stay together and interact in a positive social way. They would patrol, lay, play and sleep together as well as groom each other. After almost a fortnight the animals were considered to be socially stabilized and acting according to their normal repertoires, so observations for phase III could start. The Snow Leopards would occasionally growl and hiss at each other but without harmful effects or any other sign of aggression. They would be observed engaged in affectionate behaviors shortly after a quarrel (Figure 38).

Figure 36. First physical contact between Ariana and Kamal.

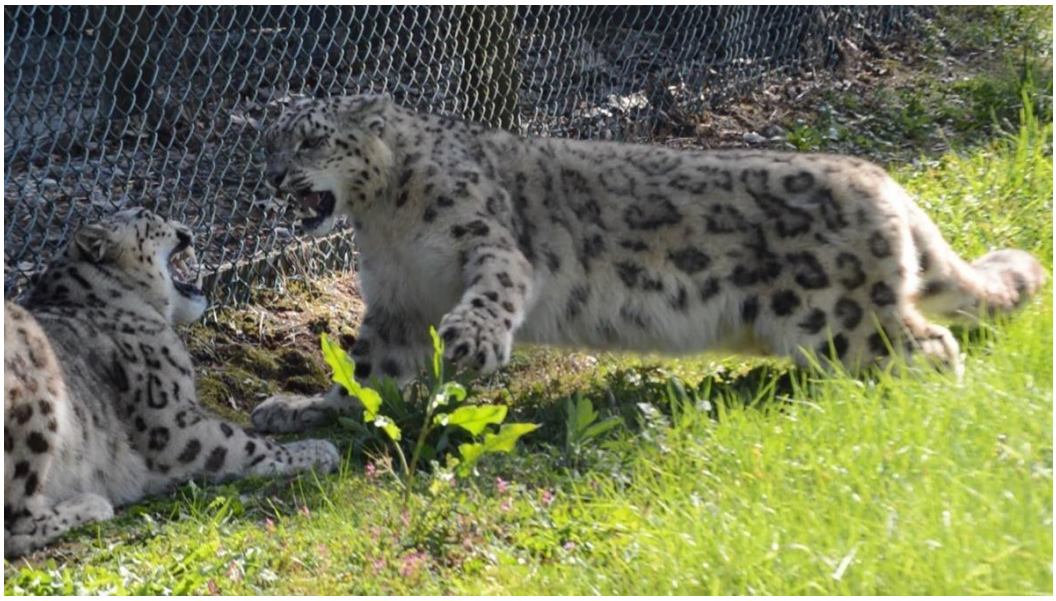

Figure 37. Ariana launching herself against Kamal, with bare teeth, extended fore limbs and protruded claws

Figure 38. The two Snow Leopards performing allogrooming.

5. Discussion

5.1. Experimental Design

In this day and age, facing a conservation crisis known as "sixth extinction" (41,18,98), many of the 38 living feline species are classified as endangered or threatened (38,66). Keeping wild animals in captivity is one of the most litigious ethical debates in our society presently and it goes on questioning the moral acceptance of captivity and deeply dividing opinions over the ethics-conservation conflict and moral obligations owed to captive animals (94).Behavioral and animal welfare programs have currently become a "must-do" in Zoo animal management. In fact, to be AZA-accredited, zoological institutions in the U.S. need to develop, implement and manage professional enrichment plans and widely apply them to the different taxa (6).

This work describes a study on environmental enrichment and behavioral modification conducted in two zoo-housed members of an endangered species of the family Felidae, the Snow Leopard, whose wild population continues to decrease, mostly due to anthropogenic causes (64). The study aimed to assess animal welfare by characterizing changes in pre-determined behaviors facing environmental enrichment provision, and evaluating the efficiency of such items in eliciting species natural behaviors as well as in increasing behavioral diversity. It also aimed to conduct the social introduction of the two Snow Leopards (which are inserted in the species' EEP), bearing in mind their reproduction. This project was defiant because it embraced a variety of fields from Veterinary and Conservation Medicine to Animal Reproduction, Behavior, Animal Welfare and Ethics, and other aspects of Zoo management. Another challenge was the fact that although there is a reasonable amount of scientific information available on this particular species, mostly from captive studies, empirical and practical information about its behavior and welfare in zoo-settings, (e.g. effects of captivity or social housing) is scarce. Generally, scientific studies on captive animals are actually conducted on few taxa (88) and most of the information gathered used to guide zoo animal welfare comes from the agricultural industry (88,60). Behavioral monitoring, as a scientific collection of behavioral data in order to determinate normal expressions of behavior , detect behavioral deviations or evaluate breeding, health-related issues and animal programs (6,131,139,142), is a demonstrated technique which supports improvement of animal welfare and

the achievement of conservational goals in zoological institutions (69). In order to access the effects of one variable (such as introduction of an enrichment device) on animal behavior, exogenous factors must be held constant (34). However, in Zoological settings in particular, the researcher is usually unable to entirely control factors such as zoo regimens (e.g. opening and closing hours), husbandry activities (e.g. feeding times, animals coming out or going in, cleaning of enclosures), keepers' schedule and rotations, animal disturbance caused by visitors, keepers and staff working nearby, biological events (e.g. estrus cycles, growth, death of an animal), and weather conditions (e.g. storms, rain, heat) (34,111). During the present study heavy rain was the biggest enemy for collecting data as it would not only prevent animals from leaving their housing but also damage enrichment items (e.g. scents, cardboard boxes) and observational equipment (i.e. video cameras), obligating the researcher to cancel some recording sessions. Unpredictable external instances as the ones mentioned above must be taken into account when interpreting the results (34). In order to ensure unbiased estimates of behavior it is important to appropriately outline an experimental design and choose an adequate sampling method before starting an observational study (142,145). In order to prepare an experimental design, *ad libitum* observations were conducted. *Ad libitum* is a casual sampling method that allows the observer to gather information and field notes about a specific individual/group of animals' significant behaviors by recording them whenever they occur; it has a great importance in the first stages of a quantified study and it helps in formulating questions, planning systematic sampling methods and deciding what behaviors are of interest to the main study and when to observe them (1,111). These preliminary observations also allowed to condense a general ethogram for the individuals being observed. An ethogram is a list of the behaviors that a specific species exhibits in its natural environment and it is considered the groundwork of behavioral research (142). It may be more detailed and compile the full behavioral repertoire of a species or include only primary behaviors that compose the animal's daily activity budget depending on the goals of the study (142) .The behaviors listed should always be mutually exclusive, and they have to be clearly defined (75) and operationally described in an extremely objective way so that an observer is able to efficiently classify and assertively recognize them. At any moment should it be forgotten that each individual is unique and, therefore, it must be considered that members of a same species are likely to behave differently between them and not to respond similarly to the same stimuli (142). This way, it was possible to identify individual variations for Ariana and Kamal regarding

normal behavioral patterns as well as deviations from what is considered as standard behavior. For example, before the study was initiated and during phase I, Kamal was reasonably active and exploratory about his surrounding environment but he also exhibited a form of stereotypic behavior, quite common in felids, known as “Pacing”. Ariana showed signs of fear/stress that were expressed by agonist behaviors towards keepers or Kamal (when he was passing nearby), by not coming out of the interior housing in the morning, especially when the keepers were close (sometimes she would stay inside for a full morning observation), and also by some exhibition of Pacing.

Both animals were observed with Animal Continuous focal sampling during all the phases of the study. This method was chosen due to the fact that it allows to focus on an individual and continuously record all the occurrences of specified behavioral categories during a set period of time (1,34). This way the probabilities of missing behaviors are minor, especially in the case of those which occur less frequently, and the biases that could outcome will decrease. Continuous focal sampling allows to retain more information such as accurate frequency of behavioral events (short duration behaviors), duration of behavioral states (long duration behaviors), rates, latency and sequence and transition times between different behaviors (34,111). It will also report social interactions once it samples both the actions initiated by the subject and those directed to it by others (1).

In synthesis, environmental/behavioral enrichment strategies aim to improve animal welfare by encouraging species-typical behaviors, enhancing behavioral diversity, increasing the ability of animals to cope with changes in the environment, reducing or eliminating abnormal patterns of behavior and reinforcing positive utilization of the environment (138,146). In general, the results obtained in the present study show that provision of environmental enrichment elicited significant alterations on some of the behaviors which were intended to be modified. It also led to an increase in behavioral diversity as the subjects executed novel behaviors when interacting with the items offered. Animals in Saint Inácio’s Zoo would occasionally receive some enrichment items (e.g. cardboard boxes and balls) in a random non-scheduled way. However, it is important to bear in mind that isolated enrichment do not constitute an Enrichment Program (138). Despite several studies exploring the efficacy of particular types of environmental stimulation on behavior of animals (11,122,129), results are likely to depend upon a variety of other factors such as sex, age, species, husbandry features, past experiences and individual temperament (138). In order to avoid

boredom or habituation (29), to benefit different changes in behavior (122) and to determine the most effective way an enrichment should be presented (91) different categories of items were offered in interspersed days. Behavioral observations and close evaluation of the enrichment provided to the two Snow Leopards allowed the construction of a list of enrichment items suitable for the species considered, which can be adapted for other wild carnivores living in the zoo such as the Siberian Tiger (*Panthera tigris altaica*), Asiatic Lion (*Panthera leo persica*) or even the African Wild Dog (*Lycaon pictus*). No kind of foraging enrichment, in respect to food or bones, was offered during phase III (when the animals were together in the outdoor parks) so to avoid any competition that could led to a fight. Other devices were offered which could still originate aggressive encounters, however, no signs of agonistic behaviors were observed. The two Snow Leopards were instead recorded performing affiliative behaviors near the enrichment items or using and playing with them together.

5.2. Behavioral Categories' Analysis

5.2.1. Active and Inactive

Observing the subject's activity/inactivity throughout different times of the day led to the confirmation of what is already known from literature available: that Snow Leopards are more active during early morning and late evening while spending a high proportion of the rest of the day resting and sleeping (51,53,58). A significant and consistent increase in Ariana's activity during the mornings throughout the phases of the study may indicate an influence of the enrichment provided in this period of the day since Ariana was typically more active in the evenings. Kamal was visibly more active than the female during mornings, however, there was a significant decrease in his activity levels from baseline to enrichment phase, during this period. Inconclusive variations in active/inactive categories for both subjects during the study may suggest a need for a closer evaluation of the quality of the behaviors performed during their period of highest activity, instead of only evaluating their activity levels.

5.2.2. **Stereotypic Behavior**

Wild carnivores are particularly prone to stereotypies, the majority being of locomotory nature. Pacing as a "repetitive locomotion in a fixed pattern, such as back and forth along the same route" (128) is the most familiar for the public in general (31,83). It is hypothesized that this locomotory stereotypy arises from the persistence and/or thwarting of unfulfilled highly motivated behaviors (31) such as the need for patrolling a territory especially in crepuscular wide-ranging species (58,103), the urge to escape from an aversive stimuli (31) and the attempt to find a conspecific or mate (71). The most widely cited motivational explanation for pacing, however, implicates the inability to express their natural appetitive foraging behavior (71,31) as the search for or hunting the prey can consume a significant amount of time and energy in wild carnivores (26). Performance of pacing during the evening, in Zoos that feed their animals in their interior exhibits at the end of the day, may be associated with the anticipation of food, for example (79). Stereotypic behavior (pacing) was established for the subjects of this study and particularly defined as a fixed pattern of repetitive locomotion in the form of walking, trotting or running, along the same route for two successive times. Animals would occasionally stop, stand and observe around. To be considered an integrated part of the pacing, the stops needed to last less than 10 seconds. Both animals paced along the edges of their enclosures. Ariana always stereotyped along the superior edge of Park A, near the Penguin's enclosure and pacing was generally much higher during evenings' observations, around closing normal hours. Kamal usually paced along visitor's path and window. Stereotypy was performed at its height during the morning when it was exhibited almost as an extension of the patrolling behavior. Its occurrence would gradually decrease during the day until the evening period, when Kamal was mostly observed following birds and gooses near his park. Pacing at this time might be explained by frustration in the inability to capture them. There is no change in Ariana's level of stereotypy with provision of enrichment, however, pacing is almost abolished during the phase where she is housed with the male. Kamal's pacing levels in the morning suffered a significant drop from phase I to phase II, to slightly increase again in phase III, what suggests a benefic effect of the enrichment items provided during the 2º phase. Freeman (1980) reports a significant drop in pacing frequencies during female's estrus period. Once Ariana was cycling during phase II, this might also have affected stereotyping of Kamal. It is also noteworthy that Ariana would sometimes interrupt Kamal's pacing, during phase III, by interfering with him at

the pacing route. Notwithstanding, stereotypic levels during morning period would always be higher than 10% in all phases. Stereotypic behavior levels during both afternoon and evenings had a gradual reduction throughout the different phases until reaching an almost null representation in phase III. The low persistence of pacing for both animals in phase III indicate that social housing may have a potential benefic effect on decreasing levels of stereotypy in the subjects. It was already stated that stereotypies are difficult to extinguish but decrease on its performance is possible with recourse to environmental enrichment strategies (130). According to several authors serious stereotypies may even reach a development stage where they are centrally controlled, and repetitive behavior is performed in a more diverse set of situations and is harder to interrupt. Therefore enrichments that do not immediately reduce stereotyping in captive animals should not be assumed failures with respect to a welfare tool (84).

5.2.3. **Agonistic and Affiliative Behavior**

Ariana's agonistic behaviors included fear and anxiety-related aggression, exhibited when the male or the keepers approached. All that was known about Ariana's history was that she was reared by her parent and that was transferred to Saint Inácio's Zoo at a very young age (less than 1 year). It is known that the mother-infant relationship plays a critical role in the process of socialization, through which the cubs learn appropriate social skills, in the development of normal adult social behavior and, consequently, in future reproductive behavior (108,87). Also, early socialization with humans (e.g. keepers and veterinarians) may affect species-specific social skills in both a positive and negative way (87). Her aggressive demeanor might be somehow explained by an inappropriate socialization but other experiences during the past years in the zoo, which might also had influence, were unknown. Agonistic behavior was more evident during the morning of phase I and was abolished in the enrichment and social enrichment phases. Representation of the agonistic category in phase III takes regard to soft warning language (e.g. hissing, pawing) with origin in play. Affiliative behaviors, which were almost inexistent in baseline phase suffered a marked increase in the mornings of phase II and in all periods of the day on phase III, when the subjects were housed together. The increase in affiliative behaviors during Phase II was probably caused by estrus, yet, it was not as high as the one noticed in phase III, which was probably related with social housing.

This may be because, although increase in affiliative behaviors is considered to indicate heat (when male is not present) (116), these are usually subtle, brief and not performed at a high level of intensity (53). Representation of the agonistic category in the male was almost null in the baseline phase and inexistent in the other two phases. Kamal's affiliative demeanor suffered a significant increase throughout the study as well, with a highest performance during the phase of social housing and enrichment.

5.2.4. **Marking, Exploratory and Maintenance**

Ariana does not present significant variations in the exploratory, maintenance and marking categories, which are considered to not be affected by the changes occurred in the environment throughout the study. Ariana also appears to mark less than the male. Marking by Kamal was generally higher during the mornings and showed a tendency to increase in all the periods of the day in phase II, with a significant variation highlighted in the morning period. These variations may be explained by males being reported to perform significantly more scent-marking than the females (53), especially during estrus (9). The significant increase in exploratory behaviors from the evening period of phase II to phase III was registered for Kamal, which suggests that both environmental and social enrichment may enhance these category of behaviors.

5.2.5. **Not Visible**

Although not considered a behavioral category *per se*, as no behaviors were visible to the observer, the time an animal was out of sight was also recorded and analyzed in this study. This was particularly important in evaluating Ariana's abnormal behavior, as she would take a long time (up to an hour in some recording sessions) to get out of her indoor closure (where she would stay out of sight) in the morning observation period during the baseline phase. When time "not visible" varies across subjects and observation days as it did with regard to Ariana, this may disturb analysis and affect overall results and their reliability (111). The referred "time" should therefore be treated

as a behavioral category in order to avoid overestimation of time spent in other behavioral classes, especially if the animal is out of sight in a situation when only few particular behaviors are likely to occur (e.g. when hiding in a den or behind vegetation) (34,111). The time Ariana spent as not visible has shown a tendency to gradually decrease during the morning period throughout the different phases of the study. In phase III she would take just about 20 seconds maximum to leave her indoor housing which is considered a positive evolution on her behavior. If this changes are due to environmental enrichment, due to entering estrus period or due to social housing, it cannot be accurately determined, however, being housed together with Kamal might had a beneficial effect in her reluctance to leave indoor housing.

5.3. Social Introduction and enrichment envisaging reproduction

The long term goal for the social introduction of Kamal and Ariana was to form a breeding unit bearing in mind their successful reproduction in captivity. The best estimate of biological fitness of an animal is its lifetime reproductive success, which may be calculated from indices such as diminished libido, delayed onset of reproductive activity, length of intervals between successive breeding, reduced litter size and number of survival offspring (17). Variables influencing reproduction in zoos include husbandry methods (76,79), physical (25,27,79) and social environment (90), which constitute factors under direct control of the zoo manager (27,90). For an animal to reproduce under captive conditions it has to respond in an appropriate way to the external factors that may influence reproductive physiology and behavior (e.g. courtship, copulation, parturition, parenting) (27,60). It is known that some degree of acute stress is needed to maintain sexual arousal and reproductive activation, however chronic stress may significantly have an inhibitory effect on reproduction (27,18). By adding novelty, providing complex environments and increasing behavioral opportunities, environmental enrichment has an important role to play in reducing detrimental stress (101) as well as in the establishment of adequate balance social interactions (28,95). Mellen (1991) determined that social environment requirements were the factors most closely correlated with successful reproduction in captive small cats. Despite the majority of big felids, including Snow Leopards, being primarily solitary in nature, they should not be considered

antisocial since they interact for mating purposes and females stay with their cubs during the rearing process, which can take up to two years (103,129). In fact, resident pairs in the wild have been reported, usually in regard to cooperatively hunts (51,36), and captive male SL were observed taking an active part in the raising of their young (51). In the past, captive animals tended to be kept alone regardless what type of social grouping was typical for the species (131). However, it is suggested that solitary animals may fare worse in captivity due to lack of interaction with conspecifics (130) and social housing may be possible, representing a remarkable means of enrichment as it increases behavioral diversity (109). Macri (2011) conducted a behavioral analysis on both solitary and socially housed SL. Although inconclusive, the study showed a tendency for socially housed SL to show more contented behavior than the solitary cats: they would pace less and express a wider diversity of behaviors, including vocalization and play (82). Several articles mention that Snow Leopards have a high degree of tolerance for each other as well as the capacity for strong long-term social bonding, and that captive heterosexual monogamous pairs may be formed (50,51,53). The same author recommend that when a pair bond appears to be established it should not be broken (6). Currently, in some captive settings, felids are already grouped in social pairs, yet, further research providing detail on appropriate social stimulation and effects on the behavior and welfare of these animals is still necessary (59).

Introduction of Ariana and Kamal took process after proper preparation, identifying goals and taking considerations since introduction of unfamiliar animals is typically characterized by significant stress that may led to fighting, physical injury and, sometimes, even death (7,108). Some social introductions may be difficult or even fail due to differences in individual unpredictable behavior or incompatibility of breeding pairs (108). It is important to acknowledge species' biology, behavior and mating system as well as animals' individual temperament in order to monitor behavioral cues (7,108). Introductions must have a veterinary team prompt on-site and should be organized and conducted in a manner that is safe for both the animals and zoo personal involved. It is suggested that introductions should gradually move from nontactile sensory contact (i.e. smell in order to perceive odors and pheromones, hearing and vision) to limited tactile contact in controlled situations (21,74). As Kamal and Ariana were housed next to each other with a mesh fence as a barrier (through which they could limitedly touch each other if willing to do so), gradual desensitization to presence, sight and limited contact was not possible. The animals were then rotated through the outdoor enclosures during phase I and II, before physical introduction,

according to a pre-defined calendar. During phase I, shifts would happen every 2-3 days so they could explore each other's territory and establish sensory contact by performing the behaviors as well as assimilating the smells and chemical signs which are so important in the olfactory signalment of feline's communication (112). In phase II shifts were more spaced in time due to husbandry issues. This management protocol was intended to allow each cat to recognize and tolerate the other at the time of introduction as well as decrease the intensity of stress, fear or anxiety-induced aggression that is associated with the introduction process (48). Also, familiarity with the potential mate's physical space would give an opportunity to locate sources of water and asylum that might allow social avoidance and decrease harassment at the time of introduction (108). Timing of introduction is very important with respect to reproductive cycles (108,51), and it only should take place when animals have stopped showing signs of aggression or anxiety when housed next to each other with limited tactile contact so to potentiate success of mating and breeding (110). Animals should first meet in a neutral environment such as indoor holding areas. However, this was not possible for the two subjects in the study because of the indoor facilities' inadequate design – small, dark, with few escape routes for the animals and weak access points for the zoo staff to intervene if physical separation was necessary. As among felids, males are referred to be the likely aggressor when introduced to a female, physical introduction is recommended to take place in female´s cage (3). In the presented case, the two SL were given a choice through the opening of the guillotine door connecting both parks. As Kamal was the one taking the initiative to leave his park and pass through the door, physical introduction ending up taking place in Ariana's enclosure. Structural and sensory enrichment was provided before the session as a means for distraction or displacement of stress-induced behaviors (108). Intensively monitored and gradually longer introduction sessions were carried almost every day for three weeks until connection between the parks was permanent. After 49 days the subjects were considered a socially affiliated pair, greeting and grooming each other, rubbing heads and bodies, entwining tails and sleeping together (74). Generally, two breeding management techniques have been developed: 1) Keeping a permanent monogamous pair or 2) Using a schedule of annual introduction of single- housed animals, at the approach of the reproductive season, for breeding processes (147). Literature suggests, however, that highly compatible pairs (those who are considered to have created a positive pair bond) may be maintained together (90) contrary to not compatible pairs that usually show a high incidence of aggression (51).Four weeks after the

introduction of the two Snow Leopards of the presented study, Ariana cycled and the pair performed successful copulas. After 60 days of the first successful copula Ariana was still not showing any signs of a new estrus cycle, what might indicate a possible pregnancy (Alan Host, personal communication, 50). However, no cubs were born and Ariana did not cycle again**.** A possible explanation for Ariana's not getting pregnant is the young age of Kamal at the time: as Kamal was less than 3 years old and males may start copulating one year prior reaching full sexual maturity (9), the sperm might not be viable yet (Alan Host, personal communication, 115).

6. Conclusions

The objectives proposed by the present study are considered to be accomplished: environmental enrichment, including social enrichment, led to positive behavioral modification by the subjects, enhanced species-specific behaviors and increased their behavioral repertoire; stereotypic and agonistic behaviors, which were intended to be abolished, were overall decreased; the subjects were successfully introduced to each other and, although the female didn't get pregnant, appropriate sexual behavior was performed. Also, the animals showed a high incidence of affiliative behaviors, suggestive of pair-bonding.

By meeting the goals of environmental enrichment, it is assumed that accomplishment of the study's objectives improved overall welfare of the animals concerned. It is also suggested, facing the lack of additional research in regarding this thematic, that social housing of Snow Leopards may be a good enrichment tool and a beneficial practice for husbandry and reproductive management routines, as a means to improve welfare.

7. Final Considerations and Future Perspectives

The final considerations expressed in the next paragraphs are the result of my deep involvement, not only in all the aspects embracing the complex project described in the present work, but also in all the exotic and wildlife animal settings (zoos, wildlife rehabilitation centers, universities and clinics) I attended through my internships:

- There is no doubt that Environmental and Social Enrichment are of extreme importance in order to promote complexity of animal's behavioral repertoire and captive animal's welfare. Moreover, it is of relevant educational value, supporting the Zoos in all their missions. Personally, and similarly to what happens in zoological institutions worldwide, Portuguese Zoos should be obligated to present an Enrichment program, properly designed and approved for the existing species under their care.
- The results of the study make me believe that housing Snow Leopards as a pair, after a well-planned and successful social introduction, has potential value as both an enrichment and reproductive management tool. What it is most noticeable is its impact in the animals' quality of life once it seems to meet their social and reproductive needs while, at the same time, it favors the conservational goals for this species. Notwithstanding, further results are necessary to scientifically support this thesis.
- Further research must be continuously encouraged and scientific work must be carried out on the grounds of evidence-based studies towards captive animals' management and welfare, as many questions remain to be answered.
- A closer cooperation is needed among Zoos worldwide as well as between Zoos and other animal institutions. Empirical and scientific information should be condensed into species-specific standard management guidelines and recommendations for good practices, available and easy to access at an international level.
- Finally, training and education must be provided to Keepers and other staff working closely with captive animals in order to improve their knowledge about the behavior and biology of the species under their care.

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9. APPENDIX

APPENDIX I

Ethogram for the species *Panthera uncia uncia* adapted from a standardized ethogram for the Felidae (Stanton et al, 2015), containing detailed definitions for all the base behaviors included.

Behavior	Definition
Allogroom	Cat licks and grooms the fur of another cat's head or/and body
Anogenital groom	Cat grooms its own genitalia.
Anogenital sniff	Cat smell the anogenital region of another's cat
Approach	Cat moves toward an interactional modifier while looking specifically at it
Attack	Cat aggressively launches itself against an interactional modifier, attempting to engage in physical combat with it. Usually the cat performs the attack with bare teeth, extended fore limbs and protruded claws.
Bare teeth	Cat exposes his canines by opening its mouth and pulling back the lips. It is usually accompanied by vocalization (hiss, growl, and snarl).
Bite	Cat opens the mouth and press down into an interactional modifier with its teeth.
Carry	Cat pick an interactional modifier off the ground with its mouth and transports it to another place.
Chase	Cat runs very quickly in pursuit of a specific interactional modifier.
Chew	Cat uses the teeth to grind an object/substrate (i.e. food) inside its mouth.
Claw	Cat drags its front claws along an object/surface. It can scratch the object, leaving visual marks on it.
Climb	Cat ascends and/or descends an object or structure
Copulation	The female cat presents and the male mounts her in a ventral/dorsal position and performs thrusting movements with his pelvic region against the anogenital region of the female. Copulation is only successful if penis intromission is achieved. This might be evidenced by the presence of a copulatory cry and female growling. Nape bite may happen and female will roll on her back and stretch after mating. There may be some aggression towards the male when he dismounts.
Crouch	Cat is alert and presses its body close to the ground with all limbs bent. The belly can be raised slightly off of the ground or touching it.
Defecate	The cat squats down and releases feces on the ground.
Drag	Cat uses its teeth or paws to pull and dislocate an interactional modifier from one place to the other without picking it up off the ground.
Drink	Cat ingests water (or any other liquid) by lapping it with its tongue.
Drowsing	Cat is lying still on the ground, with its head up and eyes closed. Cat is half-asleep and minimal movements of the head and ears can occur.
Defensive Aggression	Cat adopts a submissive posture by crouching or lying on the ground and rolling on its back, exposing the abdomen. Ears are flattened or directed backwards, teeth may be bared, eyes are narrowed and tail is moving. It is usually accompanied by vocalizations (hiss and spit).
Eat	Cat ingests food or any other edible solids (i.e. grass) buy means of cutting with the teeth, chewing and swallowing.
Explore	Cat moves around lively around a particular area while sniffing the ground and/or objects.
Fight	Two (or more) cats engage in an aggressive and potentially harmful physical contact with each other. Loud vocalizations are usually present.
Flee	Cat rapidly runs away from an interactional modifier.
Flehmen	After smelling/investigating an interactional modifier, the cat performs a grimace facial expression, which holds for several seconds while inhaling. In this posture the mouth is open, the upper lip is elevated exposing the front teeth and tongue is protruded out of the mouth.
Flinch	Cat slowly approaches an interactional modifier but abruptly stops and draw back from it. It includes retreat or flee from.

Flirt	Female cat walks around the male with her tail upright in the air, exposing her anogenital region. She can also touch the male with her body or roll in front of him.
Follow	Cat ambulates closely behind an interactional modifier in motion.
Foraging	Actively searching for or trying to acquire food (or other edible substances). Includes behaviors such as biting, biting, licking, dragging or tearing, for example.
Groom	Cat cleans and preens the fur on its body by licking, scratching, biting or chewing it. It also includes behaviors like licking the front paw and rubbing/wiping it over the head.
Head butt	Cat lightly pushes its head against an interactional modifier.
Head rub	Cat rubs its head or cheeks against an interactional modifier. While marking, this behavior can happen several times.
Hide	Cat intentionally seeks for a place away from a specific stimulus or modifier in order to keep protected or out of sight from it.
Huddle	Cat is in close body contact with an interactional modifier while lying or sitting.
Hunt	Cat actively engages in the pursuit of an interactional modifier. It includes behaviors as crouching and watch, stalking and chasing.
Investigate	Cat demonstrates interest at a specific interactional modifier by approaching, smelling and touching it.
Jump	Cat leaps vertically, from a higher point to a lower point or vice-versa, or horizontally, from one point to another.
Lick	Cat's tongue protrudes out of its mouth and passes several times over an interactional modifier.
Locomotion	Cat moves in a specific direction through the enclosure, in all four limbs. It includes walking, trotting and running.
Lordosis	Female cat presents her genitals to the male by arching her back, lowering her fore body and slightly rising her hindquarters (copulation posture). Tail is displaced to one side.
Lying	Cat's body is in contact with the ground (side, back, belly) in a horizontal position or curled in a circular formation. Cat can be alert or relaxed.
Manipulate object	Cat interacts with an object, by touching, moving, holding or picking it up.
Mount	Male cat attempts copulation by treading and straddling over the female. It can be accompanied with a neck bite and pelvic thrusting.
Movement	Cat moves around with no specific direction or makes small motion behaviors like standing up or lying down.
Nape bite	The male cat grips the female's neck with his mouth while mounting, during copulation or at the moment of ejaculation.
Other	Any behavior that does not fit in any of the descriptions of the ethogram.
Out of sight	Cat is not visible to the observer. No behaviors are recorded.
Pacing	Repetitive locomotion (walking, trotting or running) in a fixed pattern (back and forth) along the same route ("Pacing Track). The movement itself has no apparent goal. The animal stops periodically, standing and observing. This behavioral pattern must be performed two times in succession with stops <10 seconds to be qualified as stereotypic.
Patrol	Cat walks calmly but alert through its territory, periodically stopping to look around or smell something (< 6 seconds).
Paw	Cat softly touches an interactional modifier (i.e. the enclosure's window) with its forepaws. Claws are usually retracted. The movement can be rhythmically repeated several times, with one forepaw moving after the other.
Pelvic thrust	The male searches for penis intromission while mounting by making thrusting movements with his pelvis against the anogenital region of the female.
Piloerection	Cat bristles the fur on its body (neck, shoulder back and/or tail) so it stands erect.
Rear	Cat stands up and supports the body in its hind limbs while pressing its fore limbs against an interactional modifier.
Retreat	Cat passively draws back from an interactional modifier while holding visual contact with it.
Roll	While lying on the ground, the cat rotates its body from side to side, rubbing its back against the soil. The abdomen is exposed and paws are in the air.

Running	Forward motion in a rapid gait, faster than walking and trotting.
Scrape	Cat scratches the ground with its hind feet, one foot after the other, in a backwards direction. This behavior happens several times in a row and may be accompanied by urination.
Self-mutilation	Injurious behavior that is self-directed and can result in visible loss of hair and skin damage (irritation, abrasion, laceration). It includes self-biting, self-chewing and excessive grooming.
Sitting	Cat is in an upright position, with its hind limbs tucked under the body and resting on the ground while the fore limbs are vertically straight. Cat can be alert or relaxed.
Sleeping	Cat is lying still on the ground, with its head down and eyes closed. Minimal movements of the limbs or ears can occur, but the cat is not easily disturbed.
Social Play	Cat engages in interactional behaviors with another cat in a non-aggressive manner (no intention of causing harm). It includes playful activities like hunting, chasing, hitting, and wrestling.
Solitary Play	Cat moves around or interact with an object in a non-aggressive manner. It includes i.e, jumping without target, rolling, chasing attacking or hitting something.
Sniff	Cat inhales air through its nose when in close proximity with an interactional modifier (i.e. another cat, the air) and smells it.
Stalk	Slow, forward locomotion towards a specific interactional modifier. All limbs are slightly bent, head is low and eyes are focused on that specific modifier.
Strike at	The cat swipes its forepaw at an interactional modifier and tries to hit it, but no physical contact is achieved.
Standing	Cat is still in an upright position, where all the four paws are on the ground and the limbs are extended and straight, supporting the body.
Stretching	Cat straightens its forelimbs or hind limbs while curving its body inwards/outwards. It can occur when lying or standing.
Threaten	Cat is aggressive towards an interactional modifier, showing intent in causing harm, but no physical contact is made. The cat adopts a straight-forward body posture with direct eye contact, bare teeth and ears directed backwards. It is usually accompanied by vocalizations (snarl or growl) and stiff movements of the tail.
Trotting	Forward motion at a swift gait that is faster than walking but slower than running.
Urinate	The cat squats down and releases urine on the ground.
Urinate spray	The cat stands with its tail raised vertically and ejects a spray of urine or gland secretion towards a vertical surface/object. The tail may quiver while this secretions are discharged.
Vocalizations	Includes all kinds of sounds that the cat can produce and use for communication with a conspecific
Walk	Movement of the limbs at a slow gait to make forward motion.
Wrestle	Cat engages in physical contact with an interactional modifier (object/another cat), whereby struggling with and gripping it. It includes a specific behavior where the cat pulls the modifier towards itself with its fore legs while performing ranking movements with its hind legs.
Yawning	Wide opening of the mouth with a deep inhalation, followed by closing of the mouth with deep exhalation.

APPENDIX II

List and definition of the vocalizations performed by the two Snow Leopards of the study. Adapted from Freeman (1975); Landsberg et al (2013) and Stanton al (2015).

Vocalization	Definition	Context
Copulatory cry	A long, drawn –out piercing yowl of high intensity, emitted by the male cat after successful intromission during copulation	Reproductive (copulation)
Growl	A low-pitched, throaty, rumbling noise of high-intensity and long duration produced while the mouth is closed.	Agonistic (aggressive - threaten)
Hiss	A drawn-out, low-intensity hissing sound produced by rapid expulsion of air from the cat's mouth, usually during exhalation.	Agonistic (aggressive - defensive)
Prusten (chuffle)	Cat has its mouth closed and expels jets of air through the nostrils producing a low-intensity, soft, pulsed sound described as a breathy snort, usually inaudible at a distance of more than 1 meter.	Affiliative (greeting, calling)
Snarl	Cat bares teeth while emitting a sound similar to a growl, however the mouth is open and the sound is usually louder, shorter and higher in pitch.	Agonistic (aggressive - threaten)
Spit	Cat makes a sudden, short, explosive exhalation that results in a burst of noise. The spit is usually accompanied by a violent movement with the fore body (i.e. strike at)	Agonistic (aggressive - defensive)

Repertoire of the behaviors used as visual signals in social communication of the domestic cat that can be used to comprehend in detail a particular behavior. Adapted from UKCBWG (1995); Cameron-Beaumont (1997); and Rodan, (2010)

Visual Signalment	Definition	Context
Ears back	Ears are swiveled downward and held at the rear of head	Aggression (offensive); Fear (
Ears erect	Cat points its ears upward (UKCBWG, 1995).	Alert; Focused (Rodan, 2010)
Ears flat	Cat flattens its ears to its head, so that they tend to lie flush with the top of the head (UKCBWG, 1995).	Aggression (defensive) Fearful (Rodan, 2010)
Ears forward	Ears are held at the front of head (UKCBWG, 1995).	Alert; focused
Tail bent-up	Tail is bent in an upward curve (Cameron-Beaumont, 1997)	Relaxed
Tail down	Tail is held down with the end kinked out (	Aggression (offensive)
Tail parallel	Tail is parallel to the ground, sometimes slightly curved (Cameron-Beaumont, 1997).	Aggression (offensive)
Tail quiver	Part of, or the entire tail, is vibrated while raised in the up or half-up position (UKCBWG, 1995; Cameron-Beaumont, 1997).	Affiliative; Excitement;
Tail slap	Cat quickly strikes its tail on the ground (UKCBWG, 1995).	Aggression: High level of arousal
Tail swish	A violent swish of the tail; more rapid than a tail wave but smoother than a tail twitch (Cameron-Beaumont, 1997).	Aggression; Arousal
Tail twitch	A rapid flick of the tail in either a side to side or up to down motion (UKCBWG, 1995; Cameron-Beaumont, 1997).	Agitation; Annoyment;
Tail under	Tail is tucked right under the body. (Cameron- Beaumont, 1997).	Aggression (defensive)
Tail up	Tail is held in an upright position (Cameron-Beaumont, 1997).	Affiliative (friendly)
Tail Wave	A slow and gentle wave of the tail from side to side (Cameron-Beaumont, 1997).	Alert; interested

APPENDIX III

Descriptions of the modifiers which can be used with the base behaviors of the ethogram in order to specify them. Adapted from Stanton et al (2015).

Modifiers	Definition
Interactional	
Conspecific	Another individual of the same species. In this study it soleley refers to Ariana.
Human	Any human that the cat can potentially interact with. It may refer to the observer, visitors, keepers or other "staff".
Animal	Any other animal present in the cat environment (i.e. birds, Egyptian goose).
Object	Any object that the cat interacts with (i.e. branch, window, or enrichment item).
Surface	Any surfaces present in the cats parks, either horizontal or vertical (i.e. mesh or housing's wall)
Status	
Social	Cat is near a conspecific and interacts with it by performing affiliative, reproductive or agonistic behaviors.
Solitary	Cat is alone, immobile or engaged in some activities.
Dispositional	
Alert	Cat is vigilant and attentive to surroundings, with eyes open, ears forward, mouth closed or slightly open, and head up. Cat's eyes may be focused in a specific direction, or scanning the area accompanied by head and possibly ear movement (Stanton et al, 2015).
Relaxed	Cat is stationary and generally inactive, typically in a lying or sitting posture. Eyes may be closed or open, and head may be up or down and performing minimal movement (Stanton et al, 2015).

APPENDIX IV

List of materials, items and odors used for the study's environmental enrichment program.

Broom Heads

Burlap bag

Cardboard bags used to storage dry food

Cardboard boxes

Catnip spray (*Nepeta canaria*)

Feathers of Southern Ground Hornbill (*Bucorvus leadbeateri*) and a variety of Raptors and Psittacines

Fresh bones of the Portuguese cattle breed Barrosã (*Bos taurus*)

Hardground rubber basketballs acquired in Decathlon

Logs of pinaster (*Pinus pinaster*)

Natural rope

Newspaper

Nutmeg (*Myristica fragrans),* suspension (grounded nutmeg mixed in 1L of water)

Plastic barrel with various 10 cm diameter holes on it

Rafts and boughs for structural enrichment and increase of vertical space

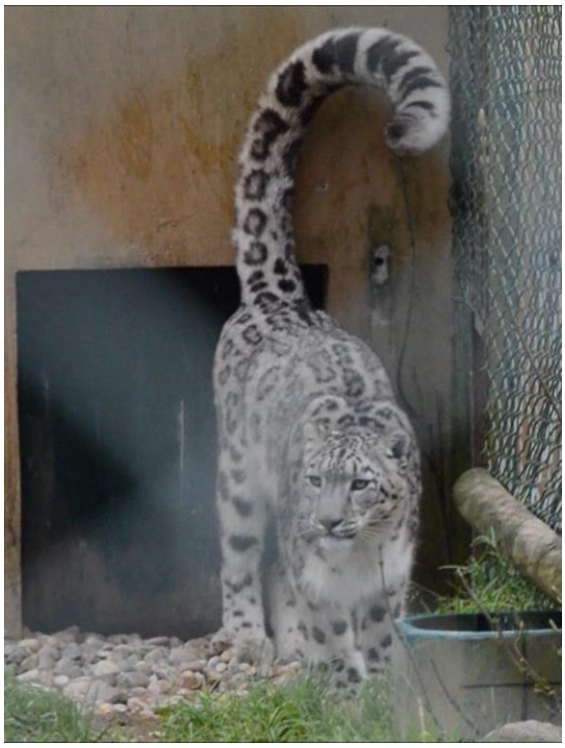
Siberian Tiger's (*Panthera tigris altaica*) feces

Sisal yarn

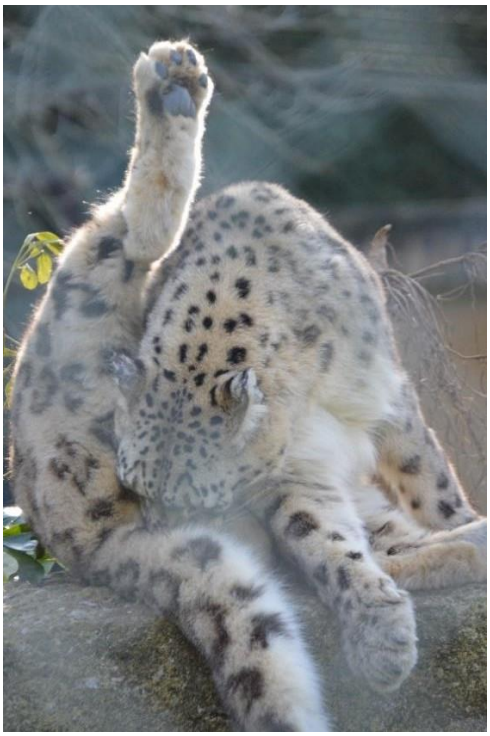
Grévy's Zebra's (*Equus grevyi)* feces

APPENDIX V

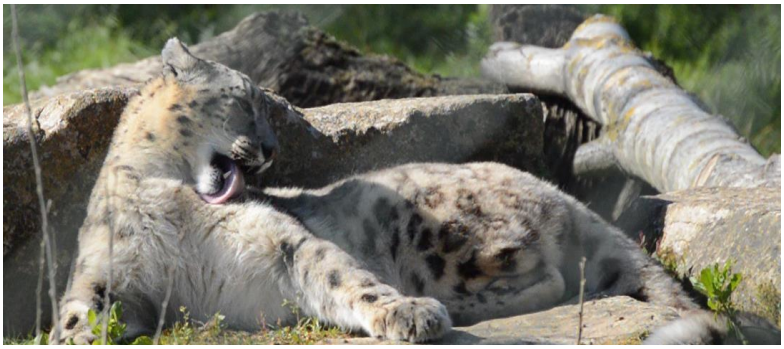
Representative Images of some of the subjects' behaviors.



Marking behavior – Spraying.



Anogenital grooming.



Grooming.

Balancing out – The long tail helps to maintain equilibrium.

Rolling.

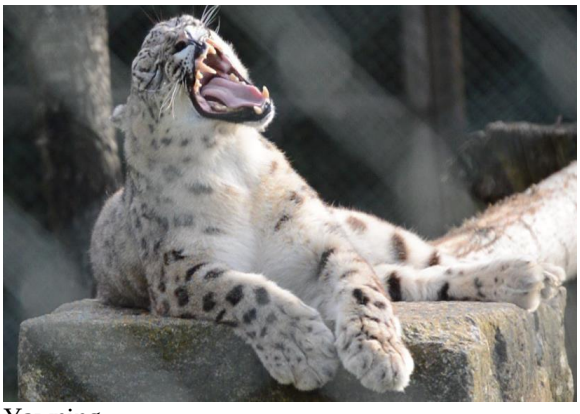
Clawing..

.Solitary play

Copula.

Social play.

Head rubbing.



Yawning

APPENDIX VI

Behavioral Observations Sheets

Enrichment Evaluation Sheet

Date: Last Evaluation: Weather:

Species: Animal: Exhibit:

Name of enrichment	

Type				
Social			**Manipulative**	
Training			**Foraging**	
Structural			**Sensory**	

Location	
On-exhibit	
Off-exhibit	
Both	
Areas	

Material		
Artificial		
Naturalistic		
Components and Material		

Goal	
Which behaviors do we want to encourage?	
Did the animal exhibit these behaviors before?	

Safety	**Are there any safety issues associated with this item?**		
Yes		**Which?**	
No			

Total material/supply cost invested	
Time spent by keepers in this enrichment	

Did item elicit species-specific behaviors?				**Did item elicit new behaviors?**			
Yes		**No**		**Yes**		**No**	
Whic0h?				**Which?**			

Was the animal seen using the item?								
No		**Yes**						
		Average length of engagement (minutes)		**Morning**	**Afternoon**	**Evening**	**Total**
		Number of visits to the item (frequency)						
Was there any indirect measure of the animal using the item?								
No		**Yes**						
		Specify:						

Indirect Observation Rating Scale:	
0	No evidence of interaction, item seems untouched.
1	Minimal evidence of interaction. Item is slightly moved from place between two observations and/or contains slight marks of teeth or claws.
2	Significant evidence of interaction. Item is moved from place between a series of observations and/or has strong evidence of being manipulated (destroyed, teared apart, heavy marks from teeth and claws).

Was the Goal achieved?					
Yes		**No**		**Explain**	
Overall recommendation for this form of enrichment:					
Continue		**Don't Continue**			
Suggestions					
Modifications for improving item's effectiveness					
Modifications for item's applicableness to other species					

Focal Animal Data Sampling 60 min

Date: Begin-End: Enclosure:

Species: Animal: Keeper:

Husbandry: Feeding:

Environment Enrichment: Y/N Kind: Weather:

Time (m)	Local	Human	NV	Inactive	Active	Stereo	Maintain	Mark	Repro	Affiliative	Agonist	Exploratory	Behavior	Modifiers
1:00														
2:00														
3:00														
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Focal Animal Data Sampling 60 min

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Focal Animal Data Sampling 60 min

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57:00														
58:00														
59:00														
60:00														

Notes	Comments
Latency in coming out	

Focal Animal Data Sampling 60 min

Distance Exam:

Geral Exam (BCS, wounds, claudication, discharges, mass, etc):

Abnormal Behavior

Date: Begin - End: Enclosure:

Species: Animal: Keeper:

Husbandry: Feeding:

Environment Enrichment: Y/N Kind: Weather:

Time (m)	Local	Human	NV	Inactive	Active	Stereo	Maintain	Mark	Repro	Affiliative	Agonist	Exploratory	Behavior	Modifiers
1:00														
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Notes	Comments