UNIVERSIDADE DE SÃO PAULO
FFCLRP – DEPARTAMENTO DE PSICOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM PSICOBIOLOGIA

Influência de enriquecimentos ambientais e tamanho do recinto no comportamento de felinos silvestres em cativeiro.

Juliana Damasceno

Tese apresentada à Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, USP como parte das exigências para obtenção do título de Doutor em Ciências, Área: Psicobiologia.

RIBEIRÃO PRETO – SP

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“VERSÃO CORRIGIDA”

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Orientador: Prof. Dr. Mateus José Rodrigues Paranhos da Costa.

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Nome: Juliana Damasceno

Título: Influência de enriquecimentos ambientais e tamanho do recinto no comportamento de felinos silvestres em cativeiro.

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Dedicated to all non-human animals, in special those present in
the current study, who I have chosen to dedicate my life for.

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“We cannot glimpse the essential life of a caged animal,
only the shadow of its former beauty”.

Julia Allen Field

ABSTRACT

DAMASCENO, J. **Influence of environmental enrichment and enclosure size on the behaviour of wild cats in captivity.** 2016. Thesis (PhD) – Faculty of Philosophy, Science and Literature, University of São Paulo, Ribeirão Preto, 2016.

Environmental enrichment techniques have been shown to be a powerful tool to improve the welfare of captive animals. However, many issues regarding effectiveness and temporality of the effects on behavioural interference require investigations and improvement. This study was conducted to analyse how environmental enrichments classified as intrinsic and extrinsic, as well the enclosure sizes influence the behaviour of wild cats kept in captivity. In the first part of this study the effects of an intrinsic (catnip scent) and an extrinsic (puzzle-feeder) environmental enrichment on the duration of enrichment-directed and pacing behaviours in captive ocelots (*Leopardus pardalis*), considering two application schedules, consecutive (seven days) or intermittent (every two days) was addressed. In the second part it was investigated if the enclosure size affects the duration of pacing behaviour by ocelots kept under enriched (catnip and puzzle-feeder) and non-enriched (baseline) environmental conditions; and in the third part it was tested if three intrinsic enrichments (hay balls without scent, with catnip and with cinnamon) had distinct effects on cheetahs (*Acinonyx jubatus*) and Sumatran tigers' (*Panthera tigris sumatrae*) behaviour regarding pacing, locomotion, inactive, exploratory and enrichment-directed behaviours. In summary, results demonstrated that the enclosure size have a high impact in the duration of pacing behaviour expressed by the ocelots. The extrinsic enrichment (puzzle-feeder) presented a long-term effect on ocelots' behaviour, resulting in a reduction of pacing time. On the other hand, the intrinsic enrichments promoted a short-lived effect in cheetahs and Sumatran tigers, and cinnamon demonstrated a better influence on the reduction of pacing behaviour than catnip. In terms of frequency of exposure the wild cats did not get habituated to the practices between sessions (over experimental days), for either consecutive or intermittent application schedules. The findings presented here elucidated issues related to enrichment science improvement. Among these stand out: 1) different types of practices cause distinct effects on animals' behaviours, and should be applied according to the aim proposed to achieve (long-term, short-term or stereotypy reduction); 2) enclosure sizes can influence the behaviour of wide-ranging animals kept in captivity; and 3) the environmental complexity, provided by the environmental enrichment practices, reduces the negative effect of small sized enclosures in ocelots. The presented outcomes highlight the importance of

investigations focused on the impact of enclosure features and the efficacy of environmental enrichment and methodological design for its application for the welfare of captive animals.

Key-words: animal welfare, intrinsic, extrinsic, pacing behaviour.

RESUMO

DAMASCENO, J. **Influência de enriquecimentos ambientais e tamanho do recinto no comportamento de felinos silvestres em cativeiro.** 2016. Tese (doutorado) – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2016.

É reconhecido que as técnicas de enriquecimento ambiental são importantes ferramentas para a melhoria do bem-estar de animais cativos. No entanto, questões em torno da efetividade e temporariedade dos efeitos das práticas requerem investigação e aprimoramento. O presente estudo foi realizado com o objetivo de analisar a influência de diferentes tipos de enriquecimentos, assim como o tamanho do recinto no comportamento de felinos em cativeiro. O estudo foi dividido em três partes, na primeira, buscou-se analisar se um enriquecimento classificado como intrínseco (catnip) e outro extrínseco (quebra-cabeça alimentar) apresentaram efeitos na expressão de comportamentos direcionados ao enriquecimento e de pacing em jaguatiricas (*Leopardus pardalis*) quando aplicados por meio de frequências de exposição consecutiva (sete dias) ou intermitente (a cada dois dias). A segunda parte do estudo concentrou-se em investigar se jaguatiricas alojadas em recintos maiores apresentaram diferenciada expressão de pacing em comparação àquelas residentes em recintos menores, sob duas condições: sem aplicação (linha de base) e com aplicação de enriquecimento (catnip e quebra-cabeça alimentar). Por fim, a terceira parte investigou se três enriquecimentos intrínsecos (bola de feno sem odor, com catnip e canela) influenciaram de forma distinta os comportamentos de guepardos (*Acinonyx jubatus*) e tigres de Sumatra (*Panthera tigris sumatrae*) em termos da expressão dos comportamentos de pacing, locomoção, inatividade, exploração e comportamento direcionado ao enriquecimento. Em síntese, os resultados indicaram que as dimensões dos recintos influenciaram no comportamento de pacing em jaguatiricas, apresentando maior ocorrência de pacing nos indivíduos alojados em cativeiros pequenos. O enriquecimento extrínseco utilizado (quebra-cabeça alimentar) demonstrou efeito de longa duração em comparação ao intrínseco (catnip), em termos de tempo de interação, assim como, indicaram maior influência na redução da expressão da estereotipia. Por outro lado, os enriquecimentos intrínsecos aplicados no estudo demonstraram um efeito de curto prazo para os comportamentos direcionados ao enriquecimento, contudo, a essência de canela reduziu significativamente os níveis de pacing em guepardos e tigres de Sumatra. Em termos de frequência de exposição, os felinos não demonstraram efeitos de habituação aos estímulos

apresentados entre sessões (ao longo do período experimental), para ambas as condições de apresentação (consecutiva e intermitente). As descobertas do presente estudo contribuíram com a resolução de questões relacionadas ao aprimoramento da ciência do enriquecimento. Dentre estas destacam-se: 1) tipos diferentes de enriquecimentos demonstraram causar efeitos distintos no comportamento dos animais, sugerindo que as práticas devem ser aplicadas para atingir objetivos específicos de acordo com os efeitos provocados (longo-prazo, curto-prazo e/ou redução de estereotipias); 2) características ambientais, como o tamanho do recinto, podem influenciar o comportamento de animais que ocupam grandes extensões em relação a comportamentos anormais; e por fim 3) a complexidade proporcionada pelo enriquecimento pode minimizar esses efeitos maleficientes. Os resultados apresentados nesta pesquisa destacam a importância de investigações focadas no impacto das características do recinto, eficácia do enriquecimento nas interferências comportamentais e no design metodológico, a fim de atender às necessidades comportamentais dos animais em cativeiro.

Key-words: bem-estar animal, intrínseco, extrínseco, pacing.

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1. Introduction.

Photo: Juliana Damasceno

1.1. Felidae family.

Represented by 37 species, the Felidae family is recognized for its beauty and grandeur (SUNQUIST; SUNQUIST, 2002, 2014). The common ancestral origin of cats is still debated, records show that *Proailurus* would be the oldest, dating from about 23 to 33.9 million years ago (PEIGNÉ et al., 2005; ROTHWELL, 2003; PIRAS et al., 2013). According to Johnson et al. (2006) the modern cats existing today have a relatively recent origin, the common ancestor of *Panthera* lineage persisting for 10.8 million years. However, many of speciation events end up becoming unstable taxonomic classifications (JOHNSON et al., 2006). For this reason there are different ways of classifying the family, the most recent being composed of eight lineages (or groups) and 14 genera (Table 1) (SUNQUIST; SUNQUIST, 2014).

Composed of a large number of species, the family has a wide range body sizes, with weights ranging from less than 2 kg, as in the case of the black-footed cat (*Felis nigripes*) and tigrinus (*Leopardus tigrinus*), up to 250 kg in the case of the bengal tiger (*Panthera tigris tigris*) (SUNQUIST; SUNQUIST, 2014). The body size is also used for the categorization of cats, separating two groups: large (> 25 kg) and small cats (up to 5 kg) (CUFF et al., 2015). According to Cuff et al. (2015) the body size is closely related to the phylogeny of the group, as well as the size of the prey.

Excellent hunters, the felids are the most specialized terrestrial carnivores, named as “hyper carnivores” (LEGRAND-DEFRETIN, 1994; BRADSHAW, COOK, 1996; STURGESS; HURLEY, 2007; BRADSHAW, 2012). Due the restricted diet based on meat, cats have a morphology adapted for this type of food (PIRAS et al., 2013). The combination of small forelimbs, though strong and mobile, large paws and claws, as well as well-developed hind leg muscles, provide strength, agility and explosiveness during a hunt (SUNQUIST; SUNQUIST, 2002). Dentition is especially adapted to break the preys’ vertebrae, cut the meat and sever the tendons (BRADSHAW, 2006; PIRAS et al., 2013). The long tail in most species aids balance when climbing vertical surfaces, such as trees, or in the case of species such as the cheetah, leopard and puma, enhances speed and cornering during chasing prey (SUNQUIST; SUNQUIST, 2002).

Communication between cats can occur by vocalizations or olfactory signals. Vocalization is used for both short and long distances, with calls varying between species. While the lion has its strong and thunderous roar, cheetahs and pumas have a thin, high-pitched meow. One of the most common vocalizations between cats is purr, also the most commonly

performed call between mothers and cubs (SUNQUIST; SUNQUIST, 2002). All Felidae species communicate through the use of olfactory signals, using pheromones for territorial marking. Cats have glands in the facial, interdigital, subcaudal and anal region (SUNQUIST; SUNQUIST, 2002) and utilize them to scent mark their territory, as a signal for other conspecifics. These scent marks can be performed as urine spray, scratches or even by rubbing the facial glands (called "cheek rubbing"). Information contained in sensory demarcation can be very specific, detailing identity, status, gender, reproductive condition and when the marking was carried out (SUNQUIST; SUNQUIST, 2002).

The association of cats with humans is related to several factors that originated thousands of years ago, many of which perpetuated until today, among them are: hunting for the use of their skins and "medicinal purposes", association with gods and magic, domestication to have them as pets or for status, or for keeping them for exhibition in zoos, etc. (MATTERN; MCLENNAN, 2000).

Much research related to cats has been developed in recent decades. The results have provided a significant increase in our knowledge of the biology of various species, which remained unknown just over two decades, such as: puma, lynx, tiger, cheetah and ocelots (SUNQUIST; SUNQUIST, 2002).

Table 1. Felidae family taxonomic classification adapted from Sunquist & Sunquist, (2014).

Lineage	Genus	Species	Common name
Panthera	<i>Panthera</i>	<i>Panthera lion</i>	Lion
		<i>Panthera pardus</i>	Leopard
		<i>Panthera onca</i>	Jaguar
		<i>Panthera tigris</i>	Tiger
		<i>Panthera uncia</i>	Snow leopard
	<i>Neofelis</i>	<i>Neofelis nebulosa</i>	Clouded leopard
		<i>Neofelis diardi</i>	Sunda clouded leopard
Pardofelis	<i>Pardofelis</i>	<i>Pardofelis temminckii</i>	Asian golden cat
		<i>Pardofelis badia</i>	Borneo bay cat
		<i>Pardofelis marmorata</i>	Marbled cat
Caracal	<i>Caracal</i>	<i>Caracal caracal</i>	Caracal
		<i>Caracal aurata</i>	African golden cat
	<i>Leptailurus</i>	<i>Leptailurus serval</i>	Serval
Leopardus	<i>Leopardus</i>	<i>Leopardus geoffroyi</i>	Geoffroy's cat
		<i>Leopardus guigna</i>	Guina
		<i>Leopardus tigrinus</i>	Tigrinus
		<i>Leopardus jacobita</i>	Andean mountain cat
		<i>Leopardus colocolo</i>	Pampas cat
		<i>Leopardus wiedii</i>	Margay
		<i>Leopardus pardalis</i>	Ocelot
Lynx	<i>Lynx</i>	<i>Lynx pardinus</i>	Iberian lynx
		<i>Lynx lynx</i>	Eurasian lynx
		<i>Lynx canadensis</i>	Canada lynx
		<i>Lynx rufus</i>	Bobcat
Puma	<i>Puma</i>	<i>Puma concolor</i>	Cougar
		<i>Puma yagouaroundi</i>	Jaguarundi
	<i>Acinonyx</i>	<i>Acinonyx jubatus</i>	Cheetah
Prionailurus	<i>Prionailurus</i>	<i>Prionailurus bengalensis</i>	Leopard cat
		<i>Prionailurus viverrinus</i>	Fishing cat
		<i>Prionailurus planiceps</i>	Flat-headed cat
		<i>Prionailurus rubiginosus</i>	Rusty-spotted cat
	<i>Otocolobus</i>	<i>Otocolobus manul</i>	Pallas's cat
Felis	<i>Felis</i>	<i>Felis catus</i>	Domestic cat
		<i>Felis silvestris</i>	European wildcat
		<i>Felis margarita</i>	Sand cat
		<i>Felis nigripes</i>	Black-footed cat
		<i>Felis chaus</i>	Jungle cat

1.2. General aspects of felids involved in the study.

1.2.1. Ocelot (*Leopardus pardalis*).

Among felid species, eight of them are present in Brazilian territory: oncilla (*Leopardus tigrinus*), margay (*Leopardus wiedii*), ocelot (*Leopardus pardalis*), jaguarundi (*Puma yagouarondi*), pampas cat (*Oncifelis colocolo*), Geoffroy's cat (*Leopardus geoffroyi*), cougar (*Puma concolor*), and jaguar (*Panthera onca*) (NOWELL; JACKSON, 1996). The ocelot, (Fig. 1) is the most recognised felid of America because of its beautiful coat made of rosettes and markings parallel to the body (NOWELL; JACKSON, 1996). The lineage to which they belong (*Leopardus*), which includes all of the cats in the genus *Leopardus* are different from other cats, in that instead of having 19, they have only 18 pairs chromosomes (SANDERSON; WATSON, 2011). They have a wide distribution, occurring from southern Texas and Arizona, through Central America to Argentina (SUNQUIST; SUNQUIST, 2014).

The ocelots are considered generalists, highly adaptable, being the predominant felid amongst Neotropical mesopredators (OLIVEIRA et al., 2008). They inhabit various habitats such as mangrove forests, coastal wetlands, savannas and grasslands, thorny shrubs and tropical forests of all kinds (NOWELL; JACKSON, 1996). They are solitary and nocturnal, with adult body weights ranging between 10 to 11.5 kg for males and 8.8 to 9.4 kg for females. Being territorial, the females occupy areas from 0.8 to 15 km², and males 3.5 to 46 km², which can increase during dry periods (DILLON; KELLY, 2008; NOWELL; JACKSON, 1996; SUNQUIST; SUNQUIST, 2002). In Brazil, Troll and Keri (2003), using 'trap cameras', recorded 10 individuals in an area of 17.71 km², giving a density of 2.82 per 5 km² in the Pantanal, Mato Grosso do Sul. Similar to the vast majority of cats, they mark their territories using body scents (spraying urine) and form latrines (always defecating in a particular location). They often take shelter in the tops of trees, shrubs or fallen tree trunks, and are also known to be excellent swimmers (SUNQUIST; SUNQUIST, 2002, 2014). Sympatrically they divide the living area with other Neotropical cats, however, little is known about the extent of interaction, having only recently been investigated by Oliveira et al. (2008).

Figure 1. Adult subject representative of the species *Leopardus pardalis*, belonging to the Jaguar Breeding Project institution, Campina Grande do Sul, Parana, Brazil. Photo: Juliana Damasceno.

They feed on small and medium-sized mammals, especially rodents, but also small birds, reptiles and fish, most of their prey being terrestrial and nocturnal (NOWELL; JACKSON, 1996; SUNQUIST; SUNQUIST, 2002). They are easily adaptable in small forests due to their wide range of potential prey, feeding even on primates, such as *Alouatta guariba*, (BIANCHI; MENDES; JÚNIOR, 2010). Rodents from the family Cricetidae constitute a large percentage of the diet, with the ocelots consuming about 600 to 800 grammes of food per day (SILVA-PEREIRA et al., 2011; SUNQUIST; SUNQUIST, 2002). According to Emsens et al. (2014), ocelots patrol agouti (*Dasyprocta punctata*) nests. Despite having increased activity at night, they can also hunt during the day, especially on cloudy and rainy days, with a total daily activity period of 12 to 14 hours, usually resting in late afternoon (SUNQUIST; SUNQUIST, 2002).

Female ocelots give birth to just one cub per litter, once a year. The juveniles follow the mother and learn to hunt until their eighth month, occupying the same area for their two first years of life (SUNQUIST; SUNQUIST, 2014). Over their entire life female ocelots are able to produce just nine cubs, which makes them a slow recovery population, from a conservation perspective (SUNQUIST; SUNQUIST, 2014). Despite the growth of research on topics related

to density and diet of these animals, knowledge about the distribution and occupancy of the species remains poorly understood (OLIVEIRA et al., 2008).

Although the species is protected in most of its range, with a ban on hunting in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Suriname, Trinidad, United States, Uruguay and Venezuela, and regulations that limit hunting in Peru, the ocelots are still being illegally hunted, captured and kept as pets or killed (CASO et al., 2008). Deforestation and roadkill also have a major impact in reducing populations of this species (DI BITETTI et al., 2008; HAINES et al., 2006).

According to the Red list of the “International Union for Conservation of Nature” (IUCN) the species is considered as LC (“least concern”), despite the fact that the population is decreasing. However, in Brazil, ocelots are considered to be in a “vulnerable” state of extinction in all regions, despite the Amazon (CASO et al., 2008). In the United States it is threatened with extinction, with just two breeding populations in southern Texas (HAINES et al., 2006).

1.2.2. *Cheetah (Acinonyx jubatus).*

Cheetahs are the only species belonging to the *Acinonyx* genus, presenting a distinct and specialized morphology (MARKER-KRAUS, 1997). Classified in the Puma lineage, cheetahs are composed of five subspecies: *A. j. hecki* (HILZHEIMER, 1913), *A. j. jubatus* (Fig. 2) (SCHREBER, 1775), *A. j. raineyi* (HELLER, 1913), *A. j. soemmeringii* (FITZINGER, 1855) and *A. j. venaticus* (GRIFFITH, 1821; MEESTER, 1971). Molecular evidence suggests that cheetahs originated in North America, then during a period of low sea level they migrated to Asia and from there went to Africa (SANDERSON; WATSON, 2011). Phylogenetically, they diverged from other cats’ lineages between 8.2 to 16.2 thousand years ago (O’REGAN, 2002).

They are the same size as big cats, like leopards and pumas, but weigh much less, with males weighing about 42 kg and females around 35 kg (KRAUSMAM; MORALES, 2005). In contrast to other cats, rather than stalk their prey they are fast chasers (GUGGISBERG, 1975). Adapted to this type of hunt, they are considered the fastest land mammals in the world, reaching up to 113 km/h while chasing prey (SUNQUIST; SUNQUIST, 2014). This speed specialization is reflected in their morphology and physiology. Some of these characteristics which make them different from other cats are given below: large lungs, heart and nasal fossa allow breathing at a rate of 60 to 150 times per minute, allowing their acceleration to be

explosive, reaching up to 72 km/h in two seconds. They have a small head, slender body and long legs (KRAUSMAM; MORALES, 2005). Small, flat and strong claws help in dealing with the ground, increasing their contact in the chase. They have a protective film around the claws which make them visible, even when retracted. A characteristic that has led to the misconception that cheetahs do not have retractable claws, like dogs (SUNQUIST; SUNQUIST, 2014). During running, each step covers about 7 meters and they can reach up to 25 meters per second with increased acceleration. In a recent study, using collars with GPS, attached to five free-living cheetahs in Botswana, Wilson et al. (2013) analyzed 364 hunting chases. The researchers found that the average distance travelled each chase is about 173 m, with an average of 1.3 runs per day, with the fastest speed displayed being 25.9 m/s. Although fast, they are not able to pursue prey over long distances. Their prey are gazelles and antelopes, usually less than 40 kg, however, when the hunt involves more than one cheetah, they can catch larger prey items. Feeding quickly, they ingest about 9 kg of meat in less than two hours. They are very careful with the carcass, sometimes leaving the bones connected (SUNQUIST; SUNQUIST, 2014).

Figure 2. Two individuals of the species *Acinonyx jubatus* belonging to one of the institutions collaborating in this study, Fota Wildlife Park, Cork, Ireland. Photo: Juliana Damasceno.

Cheetah have excellent eyesight, which allows them to locate prey and settle on which target to attack (KRAUSMAM; MORALES, 2005). Another morphological characteristic that differentiates them from other big cats are their short teeth and small canines, specializations developed for the capture and killing of their prey, which occurs by tracheal strangulation (O'REGAN, 2002).

In comparison with other big cats, they are less aggressive. In the Serengeti they hunt between late morning and early afternoon to avoid stronger predators like lions (*Panthera lion*), leopards (*Panthera pardus*) and hyenas (*Parahyaena brunnea*), who could take their prey from them, kill adults and their cubs (SUNQUIST; SUNQUIST, 2014). Called the “hunter leopard” the cheetah is the second animal used by humans in terms of hunting, just losing out to dogs (*Canis familiaris*) (SUNQUIST; SUNQUIST, 2014). Because they are not aggressive such as lions and leopards, they are considered possible to domesticate, and were used in the past as pets for royalty and trained to assist in hunting (MARKER-KRAUS, 1997; GUGGISBERG, 1975).

The cheetah coat is short and they have a pattern of marks, in the form of a line of black dots (like a tear), which starts at the eyes until the end of the mouth on both sides of the face (KRAUSMAM; MORALES, 2005; SUNQUIST; SUNQUIST, 2014). The pattern of stripes on the tail is characteristic of each animal, which facilitates the individual identification of animals for research (SUNQUIST; SUNQUIST, 2014). The cubs have long grey hairs on the head, neck and back with small visible spots, a pattern that makes it difficult for the cubs to be seen by predators (KRAUSMAN; MORALES, 2005). The vocalization is quite different from a roar, being thin and short and can be heard up to two kilometers away (SUNQUIST; SUNQUIST, 2014). Like domestic cats, cheetahs purr after meals or when they are resting (SUNQUIST; SUNQUIST, 2014). Of the wild cats, they have the greatest number of offspring, reaching up to eight cubs in each litter. The large number of cubs is a survival strategy, since the predation rate of these animals by lions and hyenas is high. In the Serengeti only 5% of cubs survive to adulthood (SUNQUIST; SUNQUIST, 2014).

Cheetahs are distributed throughout Africa and western Asia, with the largest concentration in South Africa (MARKER-KRAUS, 1997). There is evidence of population declines since the nineteenth century, when the population had already reduced in regions of Asia Minor and Arabia (MARKER-KRAUS, 1997). The factors, that resulted in the reduction of the population, range from environmental, such as competition with other predators (including in reserve areas), intensity of rainfall, or are related to human action, such as habitat fragmentation and poaching (DURANT; KELLY; CARO; 2004; MARKER-KRAUS, 1997).

Another aggravating factor, in the population decline, is wildlife traffic, mostly due to the sale of cubs to be used as pets by the higher echelons of society in the Middle East (WARCHOL, 2007).

Cheetahs have great difficulty in reproducing in captivity, developing uncommon diseases such as veno-occlusive liver disease, glomerulosclerosis, gastritis, and systemic amyloidosis (TERIO; MARKER; MUNSON, 2004). With a small population, and the difficulty in captive breeding, the genetic variability of this population is compromised, presenting a bottleneck effect in recent years (MARKER-KRAUS, 1997; O'BRIEN et al., 1985). The IUCN Red List consider the species to be in a vulnerable state, citing a 30% population decrease over 18 years. The subspecies that occupy Iran (*A. j. venaticus*) and North Africa (*A. j. heckii*) are regarded as critically endangered (DURANT et al., 2008).

An attempt to start the conservation of the species in captivity began between 1829 to 1994, when over 1,567 cheetahs were captured from the wild and 373 of those taken captive were put on display. During this period there were 2,517 births and 3,472 deaths (MARKER-KRAUS, 1997). Between 1955 and 1994, 2,517 cubs were born in 96 institutions as a result of cooperation between the institutions and the evolution of the management process in to a kind of conservation project (MARKER-KRAUS, 1997). According to the latest edition of "International Cheetah Studbook" (MARKER, 2013), the captive cheetah population (up until 31st December 2013) was 1,689 animals housed in 248 institutions, in 48 countries. Of this total, 30% cheetahs are housed in South Africa, 22% in North America, 21% in Europe, 9% in North and Central Africa, 9% in India and the Far East, 5% in the UK and Ireland, 3% in Australia and New Zealand and 1% unknown (MARKER, 2013). Currently, institutions that keep cheetahs in captivity have breeding programs, aiding in conservation and genetic diversity, as well as preventing more animals from being collected from the wild. Institutions in North America and South Africa are responsible for 30% of cubs' births (MARKER-KRAUS, 1997). In the wild "The Cheetah Conservation Fund" is the institution responsible for their conservation (SUNQUIST; SUNQUIST, 2014).

1.2.3. Sumatran tiger (*Panthera tigris sumatrae*).

According to Luo et al. (2004), tigers are classified into six subspecies: *P. tigris tigris* (Bengal tiger, Indian subcontinent), *P. tigris corbetti* (Indochinese tiger, occurring in Indo-China and Northern Peninsular Malaysia), *P. tigris altaica* (Siberian tiger, occurring in Russia, the Far East and Northeast China), *P. tigris jacksoni* (Malaysian tiger, occurring in Peninsular Malaysia), *P. tigris amoyensis* (South China tiger, occurring in South China) and *P. tigris sumatrae* (Sumatran tiger, occurring on the island of Sumatra in Indonesia) (Fig. 3). There are three more species, considered extinct: *P. tigris balica* (Bali tiger, Bali island in Indonesia) extinct in 1940, *P. tigris virgata* (Persian tiger, Turkey and West Asia and Central) extinct in 1970, *P. tigris sondaica* (Java tiger, on the island of Java in Indonesia) which became extinct in 1980 (NOWELL; JACKSON, 1996).

This species is the largest cat, measuring 3 meters on average (from nose to the tip of the tail), and can hunt prey up to four or five times heavier than themselves (LUO et al., 2004; SUNQUIST; SUNQUIST, 2014). Male tigers can weigh up to 225 kg and can capture a gaur (*Bos gaurus*) which weigh up to 910 kg (SUNQUIST; SUNQUIST, 2014). Among all of the tiger species, the Sumatran are the smallest with males weighing 100 to 140 kg and with a length from 2.2 to 2.55 m and females 75 to 110 kg at 2.15 to 2.30 m long (NOWELL; JACKSON, 1996).

The stripes existing along the body differ from one side to the other, both in quantity and position, that makes an individual as identifiable as fingerprints. The lines that outline the eyes are usually symmetrical, but the face can differentiate the animal, as well as the body (NOWELL; JACKSON, 1996). They are generally flexible in adapting to different types of environment and temperature, hot or cold areas, tolerating severe temperatures down to -34 °C (SUNQUIST; SUNQUIST, 2014).

A tigress gives birth to three to four cubs per litter and breeds every two years, a condition which contributes to the recovery of the population, when there is available habitat and prey in sufficient numbers (SUNQUIST; SUNQUIST, 2014). Males become hunters at 18 months of age, while females develop more slowly and tend to stay longer with their mothers. As independent adults, they occupy territories that can cover up to 280 km² (SUNQUIST; SUNQUIST, 2014). Although they are considered solitary in habit, there are reports of small groups of males or males remaining with females and cubs (NOWELL; JACKSON, 1996).

Figure 3. Subject of *Panthera tigris sumatrae*, belonged to Fota Wildlife Park, Cork, Ireland. Photo: Juliana Damasceno.

Different from cheetahs, tigers are not good runners, hardly chasing prey. They hunt silently, stalking and attacking explosively (SUNQUIST; SUNQUIST, 2014). Generally the main prey are deer and wild pigs, but they can also hunt young elephants, rhinos (in extreme cases), birds, reptiles and primates (NOWELL; JACKSON, 1996). In general they are more nocturnal, hunting when the prey is active, travelling up to 30 km per night, sometimes along pre-established trails (SUNQUIST; SUNQUIST, 2014). They are good swimmers, using rivers and lakes for locomotion, hunting and also to cool off during the summer (NOWELL; JACKSON, 1996). Generally they slaughter small prey by attacking behind the neck, and crush the windpipe of larger prey (SUNQUIST; SUNQUIST, 2014). They feed on the carcass, eating between 14 and 27 kg in 24 hours (SUNQUIST; SUNQUIST, 2014). Regarding Sumatran tigers, little is known about their preference for prey, because of the difficulty of recording the species in the wild. Linkie and Ridout (2011) recorded them by using camera traps, showing the temporal overlap between tigers and deer and sambar (*Cervus unicolor*) and muntjac (*Muntiacus muntjac*) in four different reserve areas in Sumatra, indicating that they are their potential prey.

By occupying a secluded island, the Sumatran tigers have several unique characteristics that differentiate them from other subspecies. According to Masak and Groves (2006), after analysis of 111 skulls of tigers belonging to Southeast Asia, they found that, based on craniometric analysis, the Sumatran tiger differ with 100% accuracy, from the neighboring subspecies of the island of Java (*Panthera tigris sondaica*). Based on morphological and genetic evidence, Masak and Groves (2006) suggested that they should be considered as different species: *Panthera sondaica* and *Panthera sumatrae*. Cranial analyses also indicate that sexual dimorphism in Sumatran tigers is stronger than in the other subspecies. The male has a larger skull and the maxila than the female (MAZAK, 2004). Genetic analysis by Luo et al. (2004) identified several unique features, compared to other subspecies, which shows an isolation of the continent's population during the speciation process resulting in a highly restricted gene flow (LUO et al., 2004). Another prominent morphological feature in the Sumatran tiger is the tuft of hair around the face (NOWELL; JACKSON, 1996).

Many year ago tigers use to occupy all Indonesian islands, but unfortunately the species from Bali (*Panthera tigris balica*) and Java (*Panthera tigris sondaica*) are extinct (WIBISONO; PUSPARINI, 2010). Currently only Sumatran tigers (*Panthera tigris sumatrae*) remain, however, with only a few isolated populations, most occupying 12 preserved areas covering about 88,000 km² (SANDERSON et al., 2006; WIBISONO; PUSPARINI, 2010).

They are considered as critically endangered of extinction (CR) by the IUCN Red List (LINKIE et al., 2008). Since 1970 the population of Sumatrans has drastically decreased from 1.000 to approximately 250 individuals in 2007 (WIBISONO; PUSPARINI, 2010). Although several projects and actions have been developed by the Indonesian Government for the tigers' conservation, there is still a lack of accurate information about the distribution of the species (WIBISONO; PUSPARINI, 2010).

The population reduction is due to factors ranging from the poaching of tigers and their prey, habitat fragmentation, death due to conflict with residents, to the sale of body parts for medicinal purposes and to be used as pets (LINKIE et al., 2003; WIBISONO et al., 2009; WIBISONO; PUSPARINI, 2010). The trade of tigers for pets has been an aggravating factor for the conservation of the species, with there being now more tigers in American backyards than living in the wild in Asia (SUNQUIST; SUNQUIST, 2014). According to Sunquist and Sunquist (2014) it is a type of commerce which is common and easy in North America, where is possible to buy a tiger cub for \$500, less than the price of a cow.

To maximize the management plan for the conservation of tigers in Indonesia it is necessary to develop research that addresses more accurately the surveying of the tiger

population and its existing prey in forest fragments, as well as factors that directly cause a reduction in habitat (LINKIE et al., 2003). Projects such as "Sumatran tiger trust" funded by WAZA (World Association of Zoos and Aquariums) support projects related to the management and protection of Sumatran tigers in the wild and in captivity.

1.3. Felids in captivity.

Due to the degradation and fragmentation of habitat by human action, the vast majority of cat species have fragmented populations and many of them are at risk of extinction, which has stimulated captive breeding for their conservation (GENARO; ADANIA; GOMES, 2001; OLIVEIRA, 1994). For many cat species, such as tigers, there are now more individuals living in captivity than in the wild (SUNQUIST; SUNQUIST, 2014).

In Brazil there are several institutions concerned with the conservation of Neotropical cats in captivity such as: Institute for the Conservation of Neotropical Carnivores (Pro Carnivores), Wild Animal Conservation Center (Itaipu Binacional), No Extinction (NEX), Brazilian Center for Neotropical Feline Conservation of Riparian Forest Association (Associação Mata Ciliar, AMC), Research and Conservation of Wildlife Association (Jaguar Breeding Project), among others. In Europe, a large number of zoos has undertaken to carry out research and participate in projects aimed at captive breeding and, to make this possible, they have exchanged animals between the institutions to ensure the genetic diversity of captive populations. Examples of participating zoos and financial projects aimed at conservation of felines are London Zoo Foundation, Fota Wildlife Park in Ireland and several zoos in the UK, all belonging to EAZA (European Association of Zoos and Aquariums).

Although a large number of institutions are involved in wild cat conservation, several challenges are present with respect to their satisfactory management, among these are: low conception rates, high mortality rate of cubs, behavioural problems, various diseases, artificial selection and other genetic issues (QUIRKE; O'RIORDAN; ZUUR, 2012).

1.4. Chronic stress and abnormal behaviours.

In the wild animals are exposed to a challenging environment of constant change, where physical and cognitive efforts are continuously required, such as avoiding predators, finding and having access to food, moving through a difficult habitat, defending their territory from other species and/or conspecifics, socializing, mating, etc. (POWELL, 1997). In the natural environment these challenges result in physiological and behavioural responses that allow animals to confront and solve situations. That challenge is characterized, for example, by escape and fight reactions. Adverse stimuli are called stressors, and stress would be the state developed by the individual when facing a threat to its homeostatic state (MOBERG, 2000). The physiological stress response is an adaptive mechanism that helps the individual to adjust to environmental changes and unpredictability, as well as to quickly respond to various stimuli that occur in the environment (WIELEBNOWSKI, 2003). Confronting stimuli, such as these, elicits a hypothalamic response by synthesizing and eliminating the corticotropin-releasing factor, which activates the release of ACTH by the pituitary gland. This in turn stimulates the release of glucocorticoids (cortisol and corticosterone) by the adrenal cortex, the so-called stress hormones (WIELEBNOWSKI, 2003). Other hormones such as prolactin and somatotropin (growth hormone), thyroid-stimulating and gonadotropins (luteinising hormone and follicle-stimulating) are also affected directly and indirectly by stress (MOBERG, 2000). The activation of the HPA axis (hypothalamic, pituitary and adrenal) can occur in several situations that will depend on the intensity of the stressor stimulus. When these stressors are short-term, stress is called acute and often is beneficial, helping the individual to cope with the stimulus quickly, by displaying responses such as increased heart rate and blood pressure (WIELEBNOWSKI, 2003). However, when the adverse stimulus has a long-term effect, not allowing the animal to adjust or recover physiologically, stress becomes chronic, also called distress, damaging the homeostasis of the individual (BROOM, 2001, MOBERG, 2000). According to the model proposed by Moberg (1985, 2000), the stress response is divided into three general states: recognition of the stressor, biological defence and the consequences of the stress response. After recognizing the threatening stimulus (stressor), the nervous system activates biological systems for defense, they are: behavioural, autonomic, endocrine and immune. When the threat is eliminated, the biological system returns to normal operation. However, in the case of chronic stress, this system is still in operation, which generates a change in the biological functioning, resulting in physical and psychological pathologies. The effects of prolonged stress (chronic)

may vary between growth suppression, changes in metabolism and immune response, failure in reproduction and behavioural changes (MOBERG, 2000). Moberg (2000) emphasised that the differentiation between stress and distress results in biological costs to the animal. Short-term stressors activate the HPA axis eliciting the fight or flight response. If an animal is being chased by a predator, it needs to run away or it dies. However, this cost is not without benefits to the animal, since survival is important. On the other hand, when the long-term stress suppresses the growth of a young animal or impairs the efficiency of reproduction, for example, the cost is high. According to Wielebnowski (2003), both distress and stress are present in the wild in situations such as: hunting, being hunted, mating, fighting, establishing hierarchies, looking for shelter, pathologies, parasites and temperature changes. In contrast, in captivity, animals react differently even to the same stressful sources, due to their enclosure. Restricted and poor space (with lack of structural complexity), the regular management routines, as well as contact with humans, are examples of stressors that make the captive environment predictable, with no option for the captive animal to control key variables for their comfort, and welfare (SWAISGOOD et al., 2003; MCPHEE, 2002; HILL; BROOM, 2009). Restrictions imposed by the captive environment limit the opportunities for the animals to express behaviours at appropriate levels, such as foraging (HILL; BROOM, 2009). Chronic stress (or distress) may result due to a failure to adjust to the captive environment (BROOM, 2008). The absence of required stimuli, such as the presence of adverse stimuli and unnatural captive environments can contribute to its occurrence. Morgan and Tromborg (2007) suggested that sources causing stress for captive animals may be related to the environmental structural conditions (e.g. lighting, temperature, sounds at high frequencies or adverse, uncomfortable substrates, containment or immobility), social characteristics (forced contact with humans, abnormal formation of groups or social isolation, for example), stable diet and feeding regimes, or that they may be related to various restrictions in behavioural expressions. The static conditions of the captive environment, as well as the absence of appropriate stimuli, may result in boredom, inability to deal with (natural) stress agents, lack of motivation as well as absence of opportunities to express natural behaviours (MCPHEE, 2002). Under these conditions, behaviours considered as "normal" may be replaced by abnormal (or maladaptive) behaviour, these being classified as behaviours that differ in shape, frequency and/or context to those presented by most members of a species in the wild (CARLSTEAD, 1996; BROOM; JOHNSON, 1993). Examples of abnormal behaviour include: apathy, excessive aggressiveness, hypersexuality, poor socialisation, self-mutilation and stereotypies, among others (BOERE, 2001; SHEPHERDSON, 1989).

Stereotypies within the science of animal welfare, are considered as repetitive behavioural patterns without purpose or apparent function (MASON, 1991). They are expressed by invariable sequential behaviour in a particular context, considered outside the standard of normal behaviour displayed by the species (BROOM, 1983). These types of repetitive abnormal behaviours occur frequently in captive carnivores, and can develop, for example, in situations of stress and fear (MASON; CLUBB, 2003). Mason et al. (2007), affirmed that stereotypies can originate from motivational and/or cerebral disfunction, and secondarily by habit formation and copy effects. The frustration effect may result from an inability to escape from a negative stimulus, for example (MASON, 1991; CARSTEAD; BROWN; SELDENTISCKER, 1993; SHEPHERDSON; CARLSTEAD; WIELEBNOWSKI, 2004). Examples of stereotypies include head or body shaking, constant chewing, swallowing air, repetitive movements of limbs or organs such as the tongue, excessive licking, fixed swimming patterns and pacing (SHYNE, 2006). Pacing, consists of walking from one side to the other or completing the same route at a high frequency with no apparent function, which is often displayed by captive cats (BRETON; BARROT, 2014; QUIRKE, O'RIORDAN; ZUUR, 2012). The expression of stereotypy leads to a gradual inability to interact with the environment (stress), developing in the animal under the influence of external factors, combined with the pre-disposition of an individual and history (LAWRENCE; RUSHEN, 1993). Although the execution of pacing is the most common stereotypic behaviour in captive carnivores, studies involving analysis of this behaviour have not focused on the causes of expression (MASON; RUSHEN, 2006). Clubb and Vickery (2006) proposed three hypotheses related to the motivation for the development of stereotypic-like pacing. The first involves the participation of various types of motivation, including food, the second is related to the lack of stimuli in the environment, and finally the third suggests that non-motivational factors make species that in the wild occupy vast areas, more prone to developing locomotor stereotypies such as pacing. Presenting several possible origins and causative factors in captivity, these stereotypies are considered as an indicator of low welfare and affect about 85 million animals around the world, including farm, laboratory and wild animals in zoos (MASON; LATHAM, 2004).

1.5. Animal welfare.

According to Broom (1986) welfare consists of the individual's status, in relation to attempts to adjust to their environment, and the levels of this state can vary between very good and very bad. Adjustment, in this sense, refers to mind control, body stability, as well as to the animal's ability to respond to a range of environmental stimuli, including those that may be harmful and adverse (BROOM; FRASER, 2007; HILL; BROOM, 2009). With respect to the science of welfare, the environment includes external stimuli which have direct effects on the animal (BROOM, 2008). Thus, failure in adjusting to these stimuli can lead to stunted growth, reproduction and even the death of the individual.

Considering that welfare of an individual is a state that can vary between very good and very bad, when stress is present welfare is compromised, which characterizes these conditions as closely related (BROOM, 1988; BROOM; MOLENTO, 2004). Thus, stress indicators may also be used to measure an individual's welfare (VOLPATO et al., 2009). Welfare is compromised when there is a failure in adjusting to the environment, resulting in chronic stress (BROOM, 2008; BROOM; MOLENTO, 2004). Moberg (1985), affirmed that despite the fact that stress indicators are valid to assess health conditions, an individual living in a stress, free environment does not necessarily have a high welfare level. Thus, it is necessary to evaluate how animals understand and cope with the environment.

The definition of welfare can be applied to humans and animals (wild, farm, zoo, laboratory or pets) and is directly related to terms such as: necessity, freedom, adaptation, feelings, stress and health (BROOM; MOLENTO, 2004). Attempts to adapt to an environment could be measured utilizing a scale that begins with a very good welfare condition, wherein strategies related with satisfaction and necessities could be applied, up to a very poor welfare state, when intense intervention needs to be undertaken, to recover stable levels of physiology and the physical state become satisfactory (BROOM, 1988; BROOM, 2008).

Welfare evaluation is a challenge, which requires combined measures which encompass psychological, biological and the natural life of the individual (BROOM; FRASER, 2007; DAWKINS, 2006; SANTANA; PARANHOS DA COSTA, 2010). It should be realized that apart from any ethical issue, after evaluation, the results can be utilised to make decisions about the ethics of the animal's situation (BROOM, 2008). For the welfare assessment of a species to be reliable, first it is necessary to have a broad knowledge about the biology of the species in question, including the strategies that it uses to cope with the natural environment (SANTANA;

PARANHOS DA COSTA, 2010). According to Broom (1988), the welfare condition of an individual should be analysed over the whole day, taking into account any changes in stimuli that could occur. In addition to the environmental variables, individual differences must be considered, because each individual reacts in a particular way to stimuli (HILL; BROOM, 2009).

Among the measures to assess the welfare levels are: a) physiological indicators of pleasure; b) behavioural indicators of pleasure; c) extension of expressed preferred behaviours; c) range of normal behaviours, whether present or absent; d) extension of physiological and anatomical developmental processes; e) physiological adaptability; f) immunosuppression; g) the existence of diseases; h) behavioural attempts to adapt; i) behavioural disorders; j) brain alterations; k) prevalent physical damage; l) low capacity for growth; and finally, m) reduced life expectancy (BROOM, 2000). Broom (1988) affirmed that measures used to identify welfare levels depend on whether the problem is short term (during the management process, such as containment or transport, for example) or long term (intensive confinement such as gestation cages for pigs). When the individual's health is impaired with respect to animal health measures, such as detecting the presence of diseases, injuries or deformities can be recognized by means of scores indicating, for example, the proportion of injury (DAWKINS, 2006). However, when the goal is to assess the psychological health of the animal, physiological and behavioural measures can be used.

Regarding the use of physiological measures, high concentrations of salivary or blood glucocorticoids or an increased heart rate are examples of possible short-term low welfare indicators (BROOM; FRASER, 2007). Despite being an alternative, the use of physiological indicators becomes difficult to interpret in terms of well-being, as many factors and changes can influence the answers regarding the adaptation of the animal to the environment (DAWKINS, 2006). On the other hand, the use of behaviour as a measure, despite being inexpensive, consists of a practical and valid indicator, to identify situations for both short- and long-term conditions (HILL; BROOM, 2009). According to Dawkins (2006), behavioural measures have an advantage in comparison to other types, as they are not invasive and are a direct reflection from the animal's perspective.

For proper behavioural assessment, in relation to welfare levels, it is necessary to accumulate an extensive knowledge of the behavioural repertoire of the species, respecting the limitations for each species (HILL; BROOM, 2009). Through behavioural evaluation it is possible to discover what the animals need in the environment. Dawkins (2004, 2006) suggests that by utilizing behavioural analysis it is possible to answer two main questions, which are:

“How is the animal's health?” and “What does the animal want?” According to Dawkins, these questions could be answered through the application of preference tests, or by the quantification of many behaviours, such as vocalization or escape from adverse stimuli, for example. Applying questions such as “What do the animals want?” increases the chance of understanding an animal's necessities, related to resources and opportunities to express particular behaviours (HILL; BROOM, 2009). Utilizing motivational strength tests, as a means to evaluate the energetic value and the effort which the animal expends to access an important resource, it is possible to identify how important this resource is for the individual (BROOM; FRASER, 2007). An example of this type of measurement is demonstrated in a study conducted by Manser et al. (1998), in which laboratory rats needed to lift a door weighing 429 g, to access another cage compartment, which contained a bed and a nest. Another method related to behavioural measures is qualitative analysis, which evaluates an animal's temperament utilising scores, stipulated for adjectives related to emotional states, such as: confident, nervous, calm, etc. (WELMESFELDER et al., 2001). Examples of behavioural measures that identify harmful welfare states can be applied through an analysis of an inability to execute normal behaviours, conspecific attacks, expression of disorientation, such as bar biting or the sucking of body parts or objects, or by frequency and duration of pacing (BROOM, 1988).

The concern with animal welfare started in the 1980's, after the emergence of the intensive system of raising animals for consumption in the 1970's, which grouped large numbers of individuals in small spaces. These type of systems facilitated disease proliferation and inappropriate environmental settings (BROOM; FRASER, 2007). As a result of commercial interest, much of the literature involving animal welfare issues is focused on livestock, followed by pets and lastly wild animals (HILL; BROOM, 2009; WHITHAM; WIELEBNOWSKI, 2003). However, in recent years, in the interest of wild animals, institutions and universities have conducted increased research, focused on the development and testing of techniques to measure and improve welfare levels (HILL; BROOM, 2009; KAGAN; CARTER; ALLARD, 2015; WHITHAM; WIELEBNOWSKI, 2003).

Strategies utilised to assess welfare and preference strength must be adapted to the animal housing conditions (BROOM, 2008). In institutions, such as zoos and conservation sanctuaries, it can be difficult to apply measures using physiological indicators, mainly due to the invasive management and difficulties in performing them, also unreliable results may arise as a consequence of low sample numbers (WHITHAM; WIELEBNOWSKI, 2003). In those cases, adjustments, using non-invasive methods may be performed as an alternative to physiological measurement, e.g. measuring cortisol metabolites in feces. However, even with

the use of non-invasive methods, by conducting repeated measurements on the same animal, it may be difficult to differentiate between current effects (acute stress) and long term (chronic stress) (WHITHAM; WIELEBNOWSKI, 2003).

Organizations such as AZA (Association of Zoos and Aquariums), BIAZA (British and Irish Association of Zoos and Aquariums), EAZA (European Association of Zoos and Aquariums) and Detroit Zoological Society are developing and designing strategies and standards of welfare measures for zoo animals. Among the viable measures for evaluation of wild animal welfare in captivity, institutions have focused on use of behavioural indicators of the animals, as well as training keepers and other employees to be able to detect and sample behaviours. Kagan et al. (2015) proposed an universal model of animal welfare for institutions such as zoos. This model is based on four main points, namely: 1) philosophy and policy of the institution, which should be focused on the quality of life of each animal, caring for the individual needs and providing for the welfare of animals comprehensively; 2) structure and a program of resources focused on sensory ecology and the natural history of the species, making the environment similar to the natural situation and promoting activities that take into account the routine of the species over a 24 hour period; 3) implementation, which involves the training of people working directly and indirectly with the animals, so that they are able to analyse and provide what they require; and finally 4) evaluation, which implies a rigorous scientific analysis of the techniques used to improve the welfare conditions.

Future directions for improving the evaluation of zoo animal welfare are based on the preparation of the institution as a whole, combined with scientific research through behavioural analysis, combined with other possible measures to be applied depending on the conditions and type of containment, such as non-invasive methods, e.g. (KAGAN; CARTER; ALLARD, 2015; WHITHAM; WIELEBNOWSKI, 2003).

1.6. Environmental enrichment.

1.6.1. Concept and history.

According to Shepherdson (1998, 2003), environmental enrichment consists in the identification and subsequent provision of stimuli, previously absent, that are necessary for the physical and psychological welfare of the animal. It is a dynamic process, which include changes in environmental structure and management practices, aimed at an increase in the opportunities for choice and species-specific behaviours (SHEPHERDSON, 2003). Hoy, Murray and Tribe (2010), considered environmental enrichment as any husbandry practice aimed at improving animals' welfare. Despite this there is no consensus in the definition of environmental enrichment (YOUNG, 2013), all concepts involve practices which focus on an improvement in animal welfare for captive animals. Thus, enrichment techniques will stimulate behaviours depending on the goal for which they were designed. These behaviours could be such as those performed in the wild, or promote opportunities to learn behaviours and perform tasks with cognitive demands (MELLEN; SHEPHERDSON, 1997).

To promote animal welfare, environmental enrichment practices reduce stress caused by the captive environment, identifying and minimising sources causing chronic stress and/or assistance in raising the animals' ability to deal with acute stress (MELLEN; MACPHEE, 2001; VASCONCELLOS, 2009). The use of enrichment has shown to be an effective tool for reducing abnormal behaviours such as stereotypy in mammals (SWAISGOOD; SHEPHERDSON, 2005; SHYNE, 2006). In a meta-analysis review, Shyne (2006) analysed 54 scientific articles involving the application of environmental enrichment for mammals, focused on the reduction of stereotypy. Among the studies, 90% were effective in decreasing abnormal behaviours during the application period, when compared to data collected during the baseline period (prior to enrichment introduction). Besides a reduction in abnormal behaviours, enrichment techniques can assist in reproductive success (DÍEZ-LÉON, et al, 2013; CARLSTEAD; SHEPHERDSON, 1994; PIZZUTTO, 2003), the reintroduction process of wild animals (READING; MILLER; SHEPHERDSON, 2013), activity and exploratory behaviours increase (MACHADO; GENARO, 2010), as well as species-specific behaviours (VAN DE WEERD et al., 2006), a reduction in aggression (MELLOTTI, et al., 2011; VAN DE WEERD et al., 2006; MORK; BJERKENG; RYE, 1999), and other benefits to animals' welfare.

Despite having originated from the ideas proposed by Yerkes (1925) and Hediger (1950, 1969), which pointed out the importance of the physical and social environment, as well as the impact of the management regime and diet on welfare animals, environmental enrichment practices only began to be applied by institutions from the 1980s (MELLEN; MACPHEE, 2001). In terms of the literature, research using the term "environmental enrichment" showed publications in international journals only starting after 1985, and since then studies focused in this area have been increasing (HOY; MURRAY; TRIBE, 2010; DE AZEVEDO; CIPRESTE; YOUNG, 2007). In 1993, the first conference on environmental enrichment was held, resulting in the publication of the book "Second Nature: Environmental Enrichment for Captive Animals" (SHEPHERDSON et al., 1998), which comprises a compilation of studies addressing enrichment application for captive animals. Until this point, there was little perspective on the future directions of environmental enrichment activities (MELLEN; MACPHEE, 2001). From 1985 to 2004, 744 articles were published utilising the keywords "environmental enrichment" (DE AZEVEDO; CIPRESTE; YOUNG, 2007). In a recent study, Alligood and Leighty (2015), reviewed publications in 12 journals in the enrichment field, during the period from 2002 to 2014, and found 94 articles evaluating the application of environmental enrichment. According to that study, 51% of research was aimed at the reduction of stereotypies, 36% in an increase in activity, 28% in the stimulation of species-specific behaviour and play, 16% in the use of space and exploration, 14% in the reduction of aggression, 11% with respect to choice tests, 6% in the promotion of social interactions and only 2% was aimed at increasing reproductive success. The vast majority of studies related to primates (34%) and felids (21%).

1.6.2. Enrichment for cats in captivity and the classification of techniques.

Enriching the captive cats' environment implies a previous knowledge about behavioural aspects of these carnivores, such as those related to foraging, based on hunting, and connected to the extent of territorial occupation (QUIRKE, O'RIORDAN, DAVENPORT, 2013; SKIBIEL, TREVINO, NAUGHER, 2007). Studies demonstrate that small enclosures and with those low complexity (BRETON; BARROT, 2014; MOREIRA et al., 2007), as well as the proximity and view of conspecifics in adjacent enclosures (QUIRKE; O'RIORDAN; ZUUR, 2012), increase stress levels of captive felids, expressed by high blood cortisol levels and pacing occurrence. The stereotypy performed as pacing is frequently shown by captive

felids and can represent up to 23% of their activity (MOHAPATRA; PANDA; ACHARYA, 2014). The motivation for cats to execute abnormal behaviour has been discussed by some authors as a need to travel over long distances (BRETON; BARROT, 2014; CLUBB; VICKERY, 2006). On the other hand, enrichment practices applied to these animals have proven to be effective, in promoting a reduction in pacing expression (QUIRKE, O'RIORDAN, 2011; RESENDE et al. 2009), inactivity reduction and an increase in natural behaviours related to exploration and activity (CARLSTEAD, BROWN, SELDENSTICKER 1993; ELLIS; WELLS, 2008; WELLS; EGLI, 2004), as well as an improvement in reproductive rates (CARLSTEAD; SHEPHERDSON, 1994; MOREIRA et al., 2007). Mellen and Shepherdson (1997) suggested that the preparation of enrichment techniques for felids should consist of practices which provide conditions similar to those present in the natural environment (e.g. introduction of the same substrate type, vegetation and elevated areas for them to climb and occupy). They should also provide opportunities for the animals to receive food, as a result of their responses and stimulate cognition, through techniques involving problem solving and learning. Enrichment practices can be classified according to the behaviours elicited by them and that classification can differ according to the author. The most utilised classification divides the practices into five categories: cognitive (occupational), feeding, sensory, social and structural (physical) (DE AZEVEDO; CIPRESTE; YOUNG, 2007; YOUNG, 2013; BLOOMSMITH et al., 1991).

- *Cognitive*: practices that stimulate cognition, generally involve solving problems followed by a food reward, access to a conspecific or another type of positive reinforcement. The application, of this category for felids, can be applied through the production of a food puzzle, in which the animal needs to solve a problem to get food. Aiming to reduce pacing in small cats, Resende et al. (2009), designed a "surprise package", which consisted of paper bags containing meat wrapped in alfalfa introduced in to the environment, which resulted in a reduction of the stereotypic behaviour after the application of these devices. Jenny and Schmid (2002) introduced boxes containing meat to Siberian tigers (*Panthera tigris altaica*). The device could be opened only after the animals learned how to slide a door on one side of the box and withdraw the food. Through the application of food puzzles, animals reduced their pacing and increased their active and resting behaviour. Enrichments that stimulate cognition promote greater environmental control by the animal, which may reduce aggression and increase positive emotions (ZEBUNKE; PUPPE; LANGBEIN, 2013). Training animals through operant conditioning can also be considered a type of cognitive enriching (WESTLUND, 2014). Training may benefit animal welfare conditions, given that the aim is reduce stressful and

invasive management methods, increase environmental control by the animal, promote choice options and stimulate positive human-animal relationships through the use of positive reinforcement (MAPLE, 2007; MELFI, 2013). Powell (1997) reported about the training of ocelots in Asheboro Zoo in North Carolina, United States, aimed at performing veterinary procedures, such as close inspection screening and injections. However, while many institutions also train cats for public display and other management procedures, there is still the need for scientific recording that proves the benefits of the techniques, for the welfare of these animals (MELFI, 2013; SZOKASKI; LITCHFIELD; FOSTER, 2012).

- *Feeding*: despite the fact that the captive environment requires the expression of different behaviours than those executed in the wild, animals maintain those which were moulded by natural selection during their evolutionary history (NEWBERRY, 1995). Thus, an adaptation of stimuli related to natural foraging animal is necessary for captivity. Law, Graham and McGowan (2001) suggest that making feeding time more complex encourages the animal to be more active and interested in the environment. Feeding enrichment can be accomplished by changing the animal's diet by introducing new items, varying the time of meals or an innovation in the display of the food (scattered in the enclosure, inside boxes, bags or hanging, in the form of carcasses, shakes or frozen food etc.) (WOOSTER, 1997). Cats are the most specialised carnivores, adapted to be excellent predators, which means that the behaviours related to hunting are essential for these animals (BASHAW et al. 2003; SZOKALSKI; LITCHFIELD; FOSTER, 2012; WOOSTER, 1997). Methods that include the introduction of new items, changes in routine and food provision can stimulate hunting behaviour, when they are designed for that purpose (SZOKASKI; LITCHFIELD; FOSTER, 2012). Examples of these practices can be cited as meat hidden in the environment (SHEPHERDSON et al., 1993; WOOSTER, 1997), frozen blood (WOOSTER, 1997), food suspended by cables or vegetation (DAMASCENO; GENARO, 2014; LAW; GRAHAM; MCGOWAN, 2001), intact carcasses (BASHAW et al. 2003; MCPHEE 2002; RUSKELL et al, 2014), among others. Aside from stimulating the behaviours involved in hunting, these enrichments also help reduce stress and expression of abnormal behaviours, as well as increase activity and exploratory behaviours (ELLIS, 2009). McPhee (2002), reduced the stereotypical behaviour of leopards (*Panthera pardus*) and lions (*Panthera leo*) after introducing intact carcasses of calves. Ruskell et al. (2014), by offering white-tailed deer carcasses (*Odocoileus virginianus*) for Bengal tigers (*Panthera tigris tigris*) and pumas (*Puma concolor*), identified a reduction of pacing, as well as increased locomotor behaviour. Shepherdson et al., (1993) noted an increase in exploratory behaviour in leopard cats (*Felis bengalensis*) from 5.5% to 14%, after providing hidden food

and alternating meals portions. Studies have shown that if given the choice between food freely available and an opportunity in which food is a result of their responses, there will be a tendency for the animals to choose the second option, a phenomenon termed as contrafreeloading (MCGOWAN et al. 2010; VASCONCELLOS, ADANIA, ADES, 2012). An alternative for cheetahs is to make them work for their food, in the practice called the "cheetah run". This consists of a device that suspends food by a steel cable running at a high speed, which encourages the animal to chase it, mimicking the natural chase (QUIRKE, O'RIORDAN, DAVENPORT, 2013). Changing the feeding time is also an alternative to feeding enrichment, through the reduction of predictability. Quirke and O'Riordan (2011), identified a pacing decrease from 13.7% to 7.7% in cheetahs, when the feeding schedule was altered from always being at 16h to randomly from 12h to 14h.

Feeding enrichment strategies are considered to be the primary and most utilised enrichment category for institutions, according to research conducted in 25 zoos in Australia, New Zealand, Singapore and the United Kingdom (HOY; MURRAY, TRIBE, 2010).

- *Sensory*: this category can be subdivided into four others: olfactory, auditory, visual and tactile. Considered as the most important sensory enrichment (HOY; MURRAY, TRIBE, 2010), olfactory stimulation can be applied by introducing odours originating or not from the natural environment of the animals (WELLS, 2009). Typical examples are the application of herbs, spices, aromatic oils, urine/feces and body odor of prey or conspecifics, other scents derived from animals, commercial perfumes and artificial essences (CLARK; King, 2008; HOY; MURRAY, TRIBE, 2010; WELLS, 2009). Smell plays an important role in the feline universe for intraspecific communication (e.g. tracing mates, recognition of reproductive status, territorial marking) and for the prey location (POWELL, 1997; SUNQUIST; SUNQUIST, 2002). The introduction of odours into the cats' environment encourages behaviours involved in communication and territoriality, such as patrolling and urine spray scent marking (MELLEN; SHEPHERDSON, 1997; SKIBIEL, TREVINO, NAUGHER, 2007; SZOKALSKI; LITCHFIELD; FOSTER, 2012). Despite this, research involving olfactory enrichments are few, 46% of existing studies have applied scents for captive felines (CLARK; KING, 2008). The introduction of herbs is used in order to cause different reactions in animal. Certain scents have relaxing and calming effects, such as chamomile and lavender, while others are stimulants such as cinnamon, catnip, nutmeg and pepper (WELLS, 2009). Skibiell, Trevino and Naugher, (2007), identified an increase in active behaviour and pacing reduction after application of cinnamon, chili powder and cumin in the environment of six species of cats (ocelots, tigers, cheetahs, jaguars, lions and pumas). Through the introduction of cinnamon essence in the

tigrinus environment, Resende et al. (2011) found a reduction of pacing when compared with the levels expressed before the introduction of this enrichment odour. The use of catnip (*Nepeta cataria*) is known to cause positive interactive reactions in domestic cats, such as sniffing, playing, rubbing and rolling (ELLIS, 2009; ELLIS; WELLS, 2010), and has also been used as an olfactory enrichment alternative for domestic cats of other wild species such as black-footed cat (WELLS; EGLI, 2004), tigrinus (RESENDE et al, 2009), lions (POWELL, 1995), snow leopards (ROSANDHER, 2009) and ocelots (POWELL, 1997). The use of catnip causes effects such as a reduction in pacing and an increase in activity and exploration, as well as scent marking. Body odours or prey faeces/urine are efficient alternatives related to the natural environment of the animal. Quirke and O'Riordan (2011) observed an increase in exploratory behaviour when they offered oryx (*Oryx dammah*) faeces to cheetahs. Van Metter, Haddiger and Bolen (2008) added zebra faeces to the environment of Sumatran tigers, resulting in increased diversity of animal behaviours.

As an artificial option, the use of synthetic pheromones has been used in situations to reduce anxiety, fear, and promote positive interactions (ELLIS, 2009). Spielman (2000) not in refs utilised Feliway (*Ceva Sante Animale*, France), similar to cats facial pheromone, in the environment of tigers and noted an increase in behaviours such as rubbing and urine spray marking.

A more complex alternative to promote olfactory enrichment is the rotation of the animals' enclosures (SZOKALSKI; LITCHFIELD; FOSTER, 2012). Stelvig (2002) identified an increase in active and exploratory behaviour after changing snow leopards between enclosures, which allowed a snow leopard to smell the odours of the previous resident.

The application of odours requires one to be especially careful about the risks that the chosen essence can bring to the animal's health in terms of toxicity, contamination or stress (WELLS, 2009). In zoo environments it is common to use cleaning products for the hygiene of the enclosure. This practice can be harmful to animals, either by changing the characteristic odor of the species, prejudicing the animals' health (CLARK; KING, 2008). As an alternative, Powell (1997) recommended that aromatic essences could be diluted in vinegar for cleaning ocelots' enclosures, functioning as a disinfectant and olfactory enrichment at the same time. Given the difficulty experimentally, in applying scents in a different environment than the laboratory (where it is possible control variables), Clark and King (2008) recommended that methodological strategies, to promote environmental enrichment, should be taken into account when choosing the type and the amount of the essence.

Vision can be stimulated by adding mirrors, televisions or reflectors in the animals' enclosures (ELLIS; WELLS, 2008; HOY; MURRAY, TRIBE, 2010). These stimulation practices have been utilised for a long time in the area of animal experimentation for cognition tests in abstract activities and recently started to be used as an enriching method (WELLS, 2009). Ellis and Wells (2008) introduced televisions showing videos related to the natural environment of animals (such as birds, rodents, fish and other cats), images of humans moving and still images of objects. The images caught the attention of animals, resulting in 6% of behavioural activities with respect to the total observation time. The animals spent more time looking at the moving images and those of biological relevance containing images of birds and rodents. As an alternative to reducing stress, limiting the animals' view of keepers, visitors and conspecifics is also a visual enrichment practice. The provision of visual barriers is recommended as an essential element for captive cats, when housed in places such as laboratories, clinics and adoption shelters (ROCHLITZ, 1998) and has also been indicated for wild cats in zoos (BASHAW et al., 2007, QUIRKE; O'RIORDAN; ZUUR, 2012).

Classical music and sounds of conspecifics' vocalizations or from the natural environment are options to stimulate the audition of captive animals (HOY; MURRAY, TRIBE, 2010; WELLS, 2009). In the case of cats, typical sounds of the natural habitat can have a positive impact on individuals' welfare. Markowitz, Aday and Gavazzi (1995) detected increased activity and reduction of pacing after introducing prey sounds (birds) for a female African leopard (*Panthera pardus pardus*). Kelling et al. (2012) presented eight kinds of sounds of a roar of a male lion for a couple of lions in Atlanta zoo, Georgia, United States. That method resulted in an increase in roars often expressed by a captive male and the animals spent more time in the outside environment. In a recent study, Snowdon, Teie and Savage (2015), developed two specific compositions for cats (in the same frequency of vocalizations). The researchers played cats' songs to 47 neutered domestic cats, followed by presentation of classical music (control). After analysing behaviours of orientation and approach, as well as the latency to respond to the sound, the cats demonstrated a preference to music produced specifically for the species instead of classical music. This result showed how the features of the music can differently influence the behaviour of animals. The use of music as an enrichment must be appropriate for the specie, as well as for the proposed objectives (SNOWDOWN; TEIE; SAVAGE, 2015). Although the introduction of sounds like classical music and radio stations have proven to be an effective environmental enrichment, promoting relaxation and a reduction in aggression for non-human primates and farm animals (for review see WELLS,

2009), there is still need for further investigations of the effects of this technique and its nuances in cats.

- *Social*: social environment may be enriched with practices that alter the formation of groups (or introduction of a new conspecific, if the animal is in solitary confinement), or promoting positive human-animals interactions (HOY; MURRAY, TRIBE, 2010). Changing the social environment should be enforced through the knowledge and management that respects any hierarchical structures, taking into account species-specific aspects. Although the vast majority of members belonging to the Felidae family have a solitary habit in the wild, in captivity they are usually housed in pairs or trios (MELLEN; SHEPHERDSON, 1997). The grouping in captivity forces the development of sociality, for access to the existing resources in the environment. Thus, the positive social interaction, with the expression of affiliative behaviour rather than agonistic, becomes an environmental enrichment tool for individually housed cats (CROWELL-DAVIS; CURTIS; KNOWLES, 2004). De Rouck et al. (2005) found that tigers housed in pairs perform more natural behaviours compared to those housed singly. Mellen, Hanes and Shepherdson (1998) observed a lower frequency of pacing in cats housed in groups, in comparison to those individually housed. Quirke, O'Riordan and Zuur (2012) recommended housing cheetahs in groups, since individuals are compatible with each other, and express affiliative behaviours. Positive social behaviours (affiliative) among cats can be identified by expression of "allo-grooming" (an individual licks the conspecific body), "allo-play" (the animals exhibit play behaviour with each other), "allo-rubbing" (animals rub their heads and the flanks on each other), and by direct body contact when resting, touching noses or tails (CROWELL-DAVIS; CURTIS; KNOWLES, 2004). The introduction of other types of environmental enrichment can simultaneously stimulate the animals' sociability. The combination of physical and cognitive enrichment, for example, can promote intraspecific relationships, resulting in social enrichment (MCCUNE, 1995). Through the introduction of a food puzzle (cognitive enrichment), Dantas-Divers et al. (2011) identified social interactions in a colony of 27 domestic cats with no aggressive events associated with access to the item. Stimulation of sociability in captive animals goes beyond intraspecific contact, positive interaction with other species, including humans, can also be enriching. The positive human-animal contact such as the "handling" (positive manipulation) can reduce fear and aggression in pigs and other farm animals (PARANHOS DA COSTA, et al., 2012). Operant conditioning by positive reinforcement, consists of a social enrichment when it promotes positive contact between the trainer and the animal (WESTLUND, 2014).

- *Structural*: Finally, structural enrichment (physical) ranges from the design of the enclosure, including vegetation, substrate, housing, introduction of objects and furniture, that allow the animals to perform movements and activities, that would be present in the natural environment, such as climbing, jumping, crawling, digging, trotting, running, amongst others (HOY; MURRAY, TRIBE, 2010). The application of the techniques can be realised by the systematic change of the environment, displacement of already known objects, or by introducing new items, ensuring the maintenance of novelty, within a previously predictable, environment (GENARO, 2005). Physical enrichment has been great applied in many published studies, especially for rodents in laboratories, because of the easy application in these environments (DE AZEVEDO; CIPRESTE; YOUNG, 2007). However, despite being considered as important as food enrichment, these techniques have not been applied with the same frequency in zoos (HOY; MURRAY, TRIBE, 2010). For animals that occupy large territorial areas in the wild, such as felids the size and complexity of the environment can impact on their welfare (MASON et al., 2007). According to Mellen, Hayes and Shepherdson (1998), small cats, kept in captivity with greater complexity, spend less time pacing compared to animals housed in a less complex captive environment. Restricted space reduces a cats' abilities to perform behaviours related with territoriality extent, that it would perform free-living, as well as the proportion of the time that it would more naturally spend (SZOKALSKI; LITCHFIELD; FOSTER, 2012). Moreira et al. (2007) revealed that small cats, like margay (*Leopardus wiedii*) and oncilla (*Leopardus tigrinus*), when moved from large to smaller enclosures, demonstrated stress responses, characterised by the appearance of altered behaviours and increased corticosteroid concentration in fecal samples. In the same study, the authors showed that small captive environments enriched with objects such as tree trunks, plants and hiding places decreased the stress response of cats. Lyons, Young and Deag (1997) analysed the behaviour of nine species of felines in captivity and found that the animals housed in larger environments showed greater frequency of active behaviour and preferred to occupy high places such as trees and shrubs. In relation to environmental structure, quality is as important as the size for the welfare (MORGAN; TROMBORG; 2007). Leopard cat (*Prionailurus bengalensis*) reduced pacing expression by 50% after the introduction of apparatus that increased environmental complexity (CARLSTEAD; BROWN; SEIDENSTICKER, 1993). Structural enrichments comprise also modifications which add variation to a space, that cannot be expanded (DE OLIVEIRA; TERÇARIOL; GENARO, 2015). Increasing the complexity of cats' enclosures can be made by the introduction of substrates, vegetation, shelters for animals to hide and rest,

platforms and elevated pathways, tree trunks, hammocks and swings, swimming pools, objects like balls and toys, among others (NEWBERRY, 1995; ROCHLITZ, 1999).

The classification of enrichment techniques illustrates the possibilities to enrich the animals' environment and which stimuli can elicit specific behaviours, resulting in welfare improvement. However, in practice, it is difficult to stratify the stimulation caused by each class of techniques. For example, by introducing a puzzle-feeder, an enrichment classified as cognitive, the enriching item stimulates at the same time, the animals' cognition (need to solve a particular task to get the reward), feeding (positive reinforcement, food), visual (the object that the device is made of), olfactory (the food and object odour) and social (if more than one animal will use the enrichment). Regardless of the strategy utilised, the application of enrichment needs to have a specific goal to satisfy the biology of the animal. So that environmental enrichment develops as a science, it is necessary to define the existing practices and then identify the limiting factors, of quality and quantity of application, as well as effectiveness evaluation (HOY; MURRAY, TRIBE, 2010).

1.6.3. Challenges and strategies for improvement of the environmental enrichment.

The science of environmental enrichment remains in the development phase, requiring adjustments to different aspects. Issues surrounding the application of the methodology, including factors such as evidence of stimulus effectiveness, attested through systematic evaluation, monitoring and by designing practical measures (HOY; MURRAY, TRIBE, 2010; TAROU; BASHAW, 2007).

Studies published in the last fifteen years highlight the development need, in terms of application evaluation and effectiveness of enrichment practices. Mellen and MacPhee (2001) suggested a protocol aiming at a systematic implementation and evaluation of enrichment practices named SPIDER ("Setting goals, Planning, Implementation, Documentation, Evaluation and Re-adjustment"). This method works like a spider web, connecting the six steps. These consist of: 1) the establishment of the goals that the enrichment aims to achieve (e.g. reduction of abnormal behaviour or activity increase); 2) the planning and approval of the technique by a team of employees, who consider aspects such as the natural history and the individual animal; 3) implementation of the approved techniques through the provision of time and materials for the application of the enrichment; 4) documentation of the enrichment,

through the registration of interactivity with enrichment and other behavioural changes; 5) evaluation of the technique effectiveness or not, by data collection during the implementation; and finally, 6) re-adjustment if it has not reached the proposed objective or needs some improvement. Continuing the ideas of Mellen and MacPhee (2001), Alligood and Leighty (2015) reviewed the progress in enrichment publications, subsequent to the publication of the protocol (2002 to 2014). Discussing the progress of the SPIDER method and needs for advances in this area of knowledge, the authors searched for topics related to species, target behaviours, strategies and analytical techniques. After the analysis of 94 publications, in peer-review journals, the authors highlighted the growth of publications focused on improving the effectiveness of the techniques. However, they also emphasised the need for studies focused on the importance of daily planning practices, individual aspects, approach to a greater number of species and application of techniques in specific areas such as reintroduction. In addition, the improvement of the science of enrichment also needs to confront issues, involved in quantity and quality of practices applied in institutions such as zoos, especially which those lacking time for implementation, financial support and technical preparation by employees (HOY; MURRAY, TRIBE, 2010).

According to Mellen and MacPhee (2001) planning of environmental enrichment activities should be based on the natural history of animals, guided by the existing knowledge about their activity when free-living, leading to the encouragement of species-specific behaviours. Although, the natural behaviour should serve as a guide for the development of techniques, it should not be the only principle to be followed. Newberry (1995) affirmed that the most useful approach for developing enrichment techniques should consider behavioural plasticity, as the functionality and adaptation to captive environment, rather than the natural origin. Behaviours considered as normal in the natural environment must be adapted to the captive situation, requiring thus attention to the assessment of how able the animals are to adjust to a different housing condition (NEWBERRY, 1995). In wild animals that express behaviours such as avoidance of predators, fear of humans, masking signs of injuries and illnesses, as well as a large accumulation of parasites, these are not wanted in the captive environment (MELLEN; MACPHEE, 2001). Regarded as natural, adaptive and related to the learning process (STADDON; ETTINGER, 1989; THORPE 1956), stimuli habituation is detrimental to environmental enrichment effectiveness and requires further investigation (ANDERSON; ARUN; JENSEN, 2010; MURPHY et al., 2003).

The habituation process is represented by a response decrease, due to monotony presentations of stimuli (SATO, 1995; STADDON; ETTINGER, 1989; THORPE 1956). The

monotony occurs when the same stimulus is presented in a repetitive, consecutive or at fixed intervals (unvaried) of use (SATO, 1995). The habituation effect has been a frequently mentioned topic in research involving enrichment application to non-human primates (VICK; ANDERSON; YOUNG, 2000; PLATT; NOVAK, 1997), swine (TRICKETT, GUY, EDWARDS, 2009), canids (PULLEN; MERRILL; BRADSHAW, 2012; INGS et al., 1997), bears (ANDERSON; ARUN; JENSEN, 2010), felids (HARE; JARAND, 1998; ELLIS; WELLS, 2010; WELLS; EGLI, 2004), and other species. The habituation process could happen within a session or between sessions of stimuli exposure (TAROU; BASHAW, 2007). The habituated response has been shown in environmental enrichment research in distinct ways: after minutes, hours or days of exposure. Vick, Anderson and Young (2000), identified habituation after 30 minutes of exposure of a feeder device, containing peanuts and fruit for two primate species (*Macaca arctoides* and *Macaca sylvanus*). In another study, Van de Weerd et al. (2006) recorded a decrease in interaction with sisal ropes, exposed for pigs over five days, from 8.4% in the first day to 4.6% on the fifth day. Despite long-term effects, the habituated response is not irreversible. Spontaneous recovery happens after a period of the absence of the habituated stimulus. When the same stimulus is exposed again the response may reoccur (MURPHY et al., 2003; TOMPSON; SPENCER, 1966). In addition to a recovery of response, a phenomenon known named as dishabituation, which may occur, consists of a response restoration, mediated by a different stimulus (THOMPSON, 2009). This effect is caused by a presentation of a new stimulus that contains different characteristics from that previously presented (PULLEN; MERRILL; BRADSHAW, 2012). Up to now, little enrichment research has systematically investigated the habituation process. Among the exponents, Pullen, Merrill and Bradshaw (2012), aiming to find habituation and dishabituation patterns, identified an average of four consecutive presentations of 10 min (or less) for dogs to get habituated to exposures of toys. However, the authors did not detect dishabituation effects over exposure intervals. Anderson, Arun and Jensen (2010), utilised logs with holes containing honey to study the habituation effect in sloth bears (*Melursus ursinus*) by consecutive and intermittent exposure schedules. The study demonstrated that the bears get habituated to both exposure schedules. In the case of felids, Hall, Bradshaw and Robinson (2002), investigated the habituation and dishabituation role in play behaviour related to hunting in domestic cats. The cats get habituated after three exposures of the same toy, and dishabituation happened after the exposure of a new toy which was a different colour and odour. Although habituation is a natural and common process, investigations of the phenomenon of occurrence patterns assists in the

attempt to extend the positive effects, caused by enriching stimuli, for the improvement of animal welfare.

Highlighting issues surrounding enrichment techniques, Tarou and Bashaw (2007) proposed some predictions based on experimental behaviour analysis (experimental psychology), aiming for the maximization of behavioural changes. According to the authors, the enrichment reinforcement effect influences the intensity of the animal's response and in terms of how fast or slow will be the habituation effect. Based on these concepts they classified enrichment as intrinsic and extrinsic. An intrinsic enrichment is one in which the animals' response elicited by the stimulus which is itself the reinforcement, and this effect regulates the probability of the same response happening again. For example, a new scent or object in the environment stimulates the novelty seeking. The exploratory response, by itself, is the reinforcement in that case. On the other hand, extrinsic enrichments are those in which the behavioural response results in a reinforced consequence, such that will regulate the probability of the same response happening again. A puzzle-feeder, for example, stimulates the animal to execute a task to gain a reward (food). It is that reward which reinforces the execution of the behaviour. Other enrichments considered as extrinsic are: hidden food in the environment and other cognitive devices. Based on that classification Tarou and Bashaw (2007) suggested predictions related to the effects caused by these types of enrichment. According to them, extrinsic enrichment have prolonged effects resulting in a lower habituation process. Hidding food for 11 bush dogs (*Speothos venaticus*) Ings, Waran and Young (1997) did not identified a significant decrease in the animals' response for searching behaviour, over 19 experimental days. In contrast, intrinsic enrichments may present short-term effects. In a study conducted by Wells and Egli (2004), habituation was found to occur after the third day of exposure of olfactory enrichments (nutmeg, catnip and body odour of prey) to black-footed cats. Groves and Thompson (1970) affirmed that aspects, such as frequency and intensity of stimulus response, influence the extent of the habituation phenomenon. Quirke and O'Riordan (2011b) recommended randomised application of different enrichment devices to minimise habituation effects.

In general, enrichment practices are in the development and improvement phase, in relation to a range of aspects, from systematic planning and evaluation to issues involving financial support. Practices concentrating on application improvement and behavioural impact analysis are required at the moment (DAMASCENO; GENARO, 2014; DE AZEVEDO; CIPRESTE; YOUNG, 2007; TAROU; BASHAW, 2007; HOY, MURRAY; TRIBE, 2010; QUIRKE; O'RIORDAN, 2011b). Therefore, based on the necessity for research examining the

evolution of enrichment practices, the current study investigated how enrichments, both intrinsic and extrinsic and environmental features, influenced captive wild cats.

1.7. Objectives.

The main objective of the current study consisted in verifying the influence of enrichments, classified as intrinsic and extrinsic, as well as environment at features influence the behaviour of three species of captive wild cats. In relation to intrinsic and extrinsic enrichments, the study hypothesis was based on predictions proposed by Tarou and Bashaw (2007) which suggested that those enrichment types have different effects on animals' behaviours in terms of how long they sustain an influence. Regarding the effects of enclosure feature the hypothesis stated that the environment size influences on stereotypy in the carnivores, such as ocelots.

To attempt the main objective, specific ones were conducted, which were:

- Analyse if different exposure schedules (consecutive and intermittent) diverge on behavioural influence.
- Identify if distinct intrinsic enrichment items diverge on behavioural influence in terms of enrichment-directed and pacing behaviour.
- Inquire if felids expressed a reduction in response to stimuli to characterize habituation.
- Examine the effectiveness of the used enrichment items as potential tools to enrich captive wild cats' environment.
- Analyse if the enclosure size influenced the time spent pacing in captive ocelots.
- Identify if after introduction of the enrichment ocelots housed in different sized enclosures, diverge in levels of pacing reduction.

2. Chapter 1.

Photo: Juliana Damasceno

Intrinsic *versus* extrinsic environmental enrichment: effects on the behaviour of captive ocelots (*Leopardus pardalis*) under consecutive and intermittent schedule exposure.

This chapter is under the submission process to the journal *Applied Animal Behaviour Science*.

Abstract

Environmental enrichment is an efficient tool to improve the welfare of captive animals. However, as a recent science, which is still under development, research is needed into the method of application and the resulting effectiveness in changing behaviour. The animals' response to different types of enrichment in relation to the frequency of exposure, habituation and the influence of enrichment on pacing behaviour, were investigated in the present study. Dividing enrichments into either intrinsic or extrinsic, the aim was to identify how these forms of enrichment influenced enrichment-directed behaviours and pacing behaviours, for both consecutive and intermittent frequencies of exposure. A puzzle-feeder with wet cat food inside, representing an extrinsic enrichment, and catnip scent, representing an intrinsic enrichment, were introduced to 20 captive ocelots (*Leopardus pardalis*) according to different schedules for seven days. Results revealed that the two forms of enrichment had different effects in relation to enrichment-directed behaviours. Extrinsic enrichment produced a longer-lasting effect for the consecutive schedule (median IQR 17.52, 6.421-35.338, minutes) and for the intermittent schedule (median IQR 6.50, 2.988-26.296, minutes) when compared to the intrinsic enrichment effect for the consecutive (median IQR 0.68, 0-2.921, minutes) and for the intermittent schedule (median IQR 1.68, 0.238-4.058 minutes). Both forms of enrichment resulted in decreased levels of pacing, with no evidence of habituation over the 24 days of the experiment (between sessions), for the two different schedule treatments. These findings revealed that the enrichments utilised in this study are effective for this species and could be introduced according to different schedules. Both forms of enrichment impacted positively upon the animals' welfare, however, the extrinsic enrichment promoted greater behavioural changes.

2.1. Introduction.

Research involving enrichment applications for captive animals has increased in the last two decades (e.g. DE AZEDO; CIPRESTE; YOUNG, 2007; HOY; MURRAY; TRIBE, 2010; MELLEN; MCPHEE, 2001). Aiming to improve animal welfare by the stimulation of natural behaviours (CARLSTEAD; SHEPHERDSON, 1994; ELLIS, 2009; LAW; GRAHAM; MCGOWAN, 2001), environmental enrichment techniques have proved to be a helpful tool, which can positively influence felid reproduction (CARLSTEAD; SHEPHERDSON, 1994; MOREIRA et al., 2007), social contact (DE ROUCK et al., 2005; GARDIÁNOVÁ; KOCOURKOVÁ, 2014), feeding (BASHAW et al., 2003; BOND; LINDBURG, 1990; DAMASCENO; GENARO, 2014; DANTAS-DIVERS et al., 2011; MCPHEE, 2002; SHEPHERDSON et al., 1993), reduction of abnormal behaviours (QUIRKE; O' RIORDAN, 2011a; RESENDE et al. 2009; SHERWIN et al., 1999) and an increase in activity levels (CARLSTEAD et al., 1993; ELLIS; WELLS, 2010; WELLS; EGLI, 2004). However, there are still issues regarding the methodology of applying enrichment and habituation to enrichment stimuli (ANDERSON et al., 2010; DE AZEDO; CIPRESTE; YOUNG et al., 2007; HOY; MURRAY; TRIBE et al., 2010; MURPHY et al., 2003; TAROU; BASHAW, 2007).

Tarou and Bashaw (2007) proposed a classification for enrichment based upon the experimental analysis of behaviour. According to them, enrichment can be classified as intrinsically and extrinsically reinforcing. Intrinsic enrichment includes those in which a response elicited by the stimulus improves, by itself, the probability of the same response happening again. Examples of this type include the introduction of novel objects or scents into the captive environment. Extrinsic enrichments are those in which a reward, as a consequence of a response to the stimulus, improves the probability of the same response occurring again. Puzzle-feeders, food hidden in the environment and social access to a conspecific, are examples of extrinsic enrichment. As the animals are challenged, extrinsic enrichment offers a degree of mental stimulation and may encourage the expression of natural behaviours (MEEHAN; MENCH, 2007). Tarou and Bashaw (2007) also presented some predictions about how these types of enrichments could influence an animal's behaviour and how to evaluate the short- and long-term effectiveness of these techniques. The authors postulated that extrinsic enrichments produce greater and more prolonged effects on the animal's behaviour, in contrast to the intrinsic ones, which result in short-term effects.

Felids, as the most specialized carnivores, can benefit from food-based enrichment (BASHAW et al., 2003; SZOKALSKI; LITCHFIELD; FOSTER, 2012; WOOSTER, 1997). This gives felids an opportunity to express natural behaviours involved in hunting (ELLIS, 2009). Bashaw et al. (2003) observed increased levels of feeding behaviours after they gave horse leg bones and live fish to African lions (*Panthera leo*) and Sumatran tigers (*Panthera tigris sumatrae*). Powell (1995), who introduced ice balls containing fish to African lions, observed higher levels of locomotion, sniffing, licking and paw manipulation behaviours. The use of enrichments, such as new scents, in the environment improves the sensory novelty into the animal's environment and can stimulate behaviours such as scent marking, sniffing, rubbing, grooming and exploration (ELLIS; WELLS, 2010; MACHADO; GENARO, 2014; POWELL, 1995; RESENDE et al., 2011; WELLS; EGLI, 2004).

Food and olfactory enrichment are helpful tools to reduce the occurrence of abnormal behaviours, such as pacing (BASHAW et al., 2003; POWELL, 1995; RESENDE et al., 2011). However, these positive effects can be limited due to habituation of the animals to the enrichment stimuli. Habituation is defined as a decrease in the animals' response, after repeated presentation of the stimuli (QUIRKE; O'RIORDAN, 2011b; STADDON; ETTINGER, 1989).

In order to examine how intrinsic and extrinsic enrichment influence animals' behaviour, the present study introduced one of each type to captive ocelots (*Leopardus pardalis*). The aims of the present study were to: 1) investigate how the two different forms of enrichment (intrinsic and extrinsic) affected enrichment-directed and abnormal (pacing) behaviours; 2) investigate if different frequencies of exposure to each form of enrichment affected the ocelot's response to the enrichment, and 3) determine if habituation to the two forms of enrichment was evident, based upon levels of enrichment-directed and pacing behaviours.

2.2. Methods.

This study was approved by the Ethics Committee on Animal Use of the Campus of the city of Ribeirão Preto, University of São Paulo (USP), Brazil, and it complied with the ethical principles of animal experimentation (protocol number 13.1.1234.53.0) (Appendix A).

2.2.1. *Subjects and Study site.*

The subjects of this study were 20 ocelots (13 males and seven females) between four and 16 years old, six originally from the wild and 14 born in captivity (Table 2). They belonged to two conservation institutions located in Brazil: Centro de Felinos, Associação Mata Ciliar (AMC), Jundiaí, São Paulo and the Jaguar Breeding Project (JBP), Campina Grande do Sul, Paraná. Both institutions are non-profit making and are dedicated to the conservation of neotropical species. The ocelots were housed individually in enclosures comprising between 9 to 100 m², with a height of two to four meters. Each enclosure had vertical areas for the ocelots to occupy, elevated pathways, shelters (one or more), an elimination area, brushes, vegetation, more than one type of substrate (cement and sand, for example) and a management area (cage). The diet was composed of beef, chicken with a mineral supplement and dead rodents, being alternated over seven days of each week. Water was available *ad libitum*, being replaced once per day. The feeding time was from 16:00h to 17:00h every day and each animal received a specific amount according to its nutritional needs. Professionals (biologists and veterinarians) and caretakers provided veterinary care and daily sanitary monitoring.

2.2.2. Enrichment.

To identify if two different types of enrichment (intrinsic and extrinsic) could have different effects on the ocelots' behaviour, one of each type was chosen. Both enrichments were novel to the animals, having never been presented to them before.

2.2.2.1. Intrinsic Enrichment (I).

For intrinsic enrichment, the herb *Nepeta cataria*, commonly known as catnip, was applied. This scent has been utilised in previous studies as enrichment for domestic and wild cats (ELLIS; WELLS, 2010; RESENDE et al. 2011; WELLS; EGLI, 2004), as well as, specifically for ocelots in the wild (WEAVER et al., 2005) and in captivity (POWELL, 1997; WOOSTER, 1997). Following the methodology of Wells and Egli (2004), 5 ml of liquid herb, brand Pet Mais® was utilised (Fig. 4). The scent was applied to a 20 cm² wooden square attached inside each enclosure. To ensure that any behavioural reactions would be the result of the scent and not the object, the wooden square had been put in position 20 days prior to the start of the study.

2.2.2.2. Extrinsic Enrichment (E).

For extrinsic enrichment, a puzzle-feeder was designed. It was a wooden box measuring 45x30x30 cm (length, width and height, respectively) with two apertures of eight cm in diameter (Fig. 5, 6). Inside the wooden box the reward consisted of 580 g of wet cat food (fish flavoured, Whiskas®). To increase the difficulty in accessing the food, grass was also added inside the wooden box. In order to avoid injury due to a possible displacement of the box, a granite stone weighing 4 kg was inserted inside the box. The reward (food) was obtained when the animal performed a behavioural response of inserting its paw through the upper opening of the box and it removed the cat food contained inside.

Table 2. Informations about the ocelots, according to each institution, Associação Mata Ciliar (AMC) and Jaguar Breeding Project (JBP).

Subject	Institution	Gender	Origin	Age
Google	AMC	F	Captivity	5
Guria	AMC	F	Captivity	10
Jussara	AMC	F	Captivity	16
Ilha	AMC	F	Wild	12
Tererê	AMC	F	Wild	14
Bah	AMC	M	Captivity	6
Duma	AMC	M	Captivity	13
Maleado	AMC	M	Captivity	7
Pedro	AMC	M	Captivity	14
Piá	AMC	M	Captivity	10
Pig	AMC	M	Captivity	8
Tchê	AMC	M	Captivity	6
Zoletil	AMC	M	Captivity	2
Geléia	AMC	M	Wild	8
Filhote	JBP	F	Wild	4
Nissa	JBP	F	Wild	14
Leo	JBP	M	Captivity	6
Lotus	JBP	M	Captivity	7
Rick	JBP	M	Captivity	8
Titico	JBP	M	Wild	13

Figure 4. Demonstration of catnip (*Nepeta cataria*) spray Pet Mais® brand deposited on the 20 cm² wooden square in an ocelot enclosure in AMC (Associação Mata Ciliar, Jundiaí, São Paulo, Brazil). Photo: Juliana Damasceno.

Figure 5. A sketch of the puzzle-feeder consisting of a wooden box 45x30x30 cm (length, width and height, respectively) with two openings on top with a diameter of 8 cm. Photo: Juliana Damasceno.

Figure 6. Picture of the puzzle-feeder designed as an extrinsic enrichment. Comprising of a wooden box, containing cat food fish flavor Whiskas®. Placed inside the box there is a 4kg granite stone positioned to impede the displacement of the box, in order to prevent possible injuries to the animals. Photo: Juliana Damasceno.

2.2.3. Procedure.

Enrichments were applied for two hours for each ocelot, always from 14:00h to 16:00h, before feeding time, as recommended by Tarou and Bashaw (2007). The puzzle-feeder was removed from the enclosure after the experimentation time. It was not possible to remove the scent from the enclosure due to the fact that the wooden square was fixed in the environment. The ocelots were divided into two groups: consecutive (C) and intermittent (I). For the consecutive group (C), 11 ocelots received both forms of enrichments for seven days, always Monday to Sunday, every day. For intermittent group (I) nine ocelots received the enrichment three days out of the seven days (1, 4 and 7), always Monday, Thursday and Sunday. The schedule of experiments (Table 3) started with seven days of baseline observations, without enrichment, followed by intrinsic enrichment application for seven days (group C), and three days (group I). After that, there was a break of seven days without enrichments, followed by

another intrinsic enrichment application, as explained above. Before extrinsic enrichment application, 14 days of no enrichment introduction was applied, in order to avoid overlapping effects due to the preceding enrichment. Following this interval, the extrinsic enrichment was applied in the same manner as highlighted above.

A CCTV (closed-circuit television) programme (Fig. 7), including 16 Sony® cameras and two HDRs (Stand Alone®), recorded images in real time (30 frames/s) (Fig. 8). The cameras were installed in each enclosure (Fig. 9), with a view of the entire enclosure, to register all of the animals' behaviour expressed within the two hours of each experiment. This behavioural observation method was selected for the study in order to prevent any possible interference with the ocelots' behaviour by the presence of the observer.

The experiments thus took over of 42 days, excluding the intervals, totalling 1,248 hours of behaviour data. Observations were performed according to Altmann (1974), utilizing the focal sampling method, with continuous registration of behaviours during the two hours of each session. Behaviours were registered utilizing an ethogram adapted from Quirke and O'Riordan (2011a, 2011b) and Stanton, Sullivan and Fazio, (2015) (Table 4).

Figure 7. Demonstration of the central CCTV installed in the ocelots' enclosures in Associação Mata Ciliar (AMC) institution. Photo: Juliana Damasceno.

Figure 8. Demostration of the screen recording ocelots' behaviours by CCTV circuit in Associação Mata Ciliar (AMC).

Figure 9. Example of the camera installed in an ocelot enclosure in Associação Mata Ciliar (AMC) institution. Photo: Juliana Damasceno.

Table 3. Experimental schedule of enrichment application.

Group	Experiment Sessions							
	7days	7days	7days	7days	14days	7days	7days	7days
Consecutive	BL	1IC	----	2IC	----	1EC	----	2EC
	7days	1,4,7	7days	1,4,7	14days	1,4,7	7days	1,4,7
Intermittent	BL	1II	----	2II	----	1EI	----	2EI

BL – baseline (without enrichment); IC – intrinsic enrichment group consecutive; EC – extrinsic enrichment group consecutive; II - intrinsic enrichment group intermittent; EI – extrinsic enrichment group intermittent; 1 – first week; 2 – second week; (---) – means interval without observations and enrichment application.

Table 4. Ethogram related to behaviours recorded during the observation period adapted from Quirke and O’Riordan (2011a, 2011b) and Stanton, Sullivan and Fazio. (2015).

Behaviour	Definition
Enrichment directed	Interacting with the enrichment item, any kind of direct contact as touching, rolling, sniffing, playing, rubbing, and others.
Exploration	Urine spray, scratching or rubbing objects or enclosure substrate.
Groom	Cat cleans itself by licking, scratching, biting or chewing the fur on its body. May also include the licking of a front paw and wiping it over its head.
Inactive	Lying down, sitting or standing still.
Locomotion	Running, walking, climbing or jumping.
Pacing	Repetitive locomotion in a fixed pattern, such as back and forth along the same route. Can include walking, trotting and running. Movement seems to have no apparent goal or function. Must be performed at least twice in succession before qualifying as stereotypic.

2.2.4. *Data Analysis.*

Firstly, the total number of seconds in which each animal spent in enrichment-directed behaviours for each introduction, was utilised in the analysis. As data were non-normally distributed and highly positively skewed, a $\log_{10}(x+c)$ (x = enrichment-directed behaviours in seconds, $c = 1$ second) transformation was carried out prior to statistical analysis. A generalized least squares model, which can take into account the heteroscedasticity of dependent variables, was carried out for each of the consecutive and intermittent treatment schedules in order to test the significance of a number of factors, namely; days since first introduction of enrichment, week, sex, enrichment type (intrinsic or extrinsic), in combination with a number of two-way and three-way interactions between factors. This was in order to determine which factors or interactions of factors significantly influenced duration of enrichment-directed behaviours. Model selection was carried out using a backwards stepwise procedure with likelihood ratio testing in order to determine which explanatory variables were significant. The accepted alpha level for significance was taken to be 0.01.

Secondly, in order to determine if the intrinsic and extrinsic enrichment treatments significantly reduced pacing behaviour during the two weeks of each enrichment treatment, the average number of seconds in which each of the 20 ocelots engaged in pacing behaviour was calculated across all baseline observations and the first week and second weeks of intrinsic and extrinsic enrichment introductions. As the data were non-normally distributed, a Friedman ANOVA was conducted in order to determine if there was a significant difference in time spent pacing between baseline observations and observations in the first week and second weeks of introduction of both the intrinsic and extrinsic enrichment. If the Friedman ANOVA was significant, Wilcoxon signed rank tests with a Bonferroni correction, applied for multiple tests were performed to determine significant pair-wise relationships. The alpha level for significance for the Wilcoxon signed rank tests was taken to be 0.008.

2.3. Results.

2.3.1. Enrichment-directed behaviours.

The enrichment-directed behaviours performed by the ocelots with intrinsic and extrinsic enrichment, can be seen in Figures 10 (A and B) and 11 (A and B), respectively.

2.3.1.1. Consecutive schedule treatment (Group C).

The final model retained one significant explanatory variable (coefficient value= 1340.73, SE= 111.129, $t=12.064$, $p=<0.0001$). The duration of interaction with enrichment was significantly influenced by the type of enrichment, with ocelots spending more time on enrichment-directed behaviours with the extrinsic enrichment item (median, IQR in minutes) (17.52, 6.421-35.338) compared to the intrinsic enrichment (0.68, 0-2.921) (Table 5). No significant differences were observed in the duration of enrichment-directed behaviours in terms of days since first introduction, week, sex or the two-way interactions between week and enrichment type, days since first introduction and enrichment type, sex and enrichment type, or the three-way interaction between days since first introduction, week and enrichment type (Fig. 12, 13) (Table 5).

2.3.1.2. Intermittent schedule treatment (Group I).

The final model for the intermittent schedule also retained one significant explanatory variable (coefficient value = 739.57, SE= 143.451, $t=5.155$, $p=<0.0001$). The duration of enrichment-directed behaviours was significantly influenced by enrichment type with ocelots spending more time interacting with the extrinsic enrichment (median, IQR in minutes) (6.50, 2.988-26.296) compared to the intrinsic enrichment (1.68, 0.238-4.058) (Table 6). No significant differences were observed in duration of interaction with enrichment in terms of

days since first introduction, week, sex or the two-way interactions between week and enrichment type, days since first introduction and enrichment type, sex and enrichment type, or the three-way interaction between days since first introduction, week and enrichment type (Fig. 14, 15) (Table 6).

2.3.2. Pacing behaviour.

There was a significant difference ($\chi^2 = 31.765$, d.f. = 4, $p < 0.001$) in the amount of time (median, IQR in minutes) that the 20 ocelots spent pacing between baseline (70.30, 5.774-107.055), intrinsic week 1 (43.62, 8.129-84.969), intrinsic week 2 (56.50, 7.863-107.059), extrinsic week 1 (27.92, 0.836-54.879) and extrinsic week 2 (22.41, 3.092-59.129) phases. Ocelots spent significantly more time pacing during the baseline phase compared to intrinsic week 1 ($V=148$, $p=0.0069$), extrinsic week 1 ($V=148$, $p=0.0007$) and extrinsic week 2 ($V=146$, $p=0.0011$). Ocelots spent significantly more time pacing during the intrinsic week 2 phase when compared with the intrinsic week 1 phase ($V=27$, $p=0.0066$). There was no significant differences observed between the baseline phase and intrinsic week 2 ($V=93$, $p=0.761$). Nor between extrinsic week 1 and extrinsic week 2 phases ($V=49$, $p=0.338$).

Figure 10. Illustration of ocelot expressing enrichment-directed behaviour with intrinsic device (catnip, *Nepeta cataria*) sprayed on a wooden square fixed in the environment in the Jaguar Breeding Project (JBP). A- Sniffing the catnip, B – executing “cheek-rubbing”, when the animal rubs its face to scentmark. Photo: Juliana Damasceno.

Figure 11. Ocelot expressing enrichment-directed behaviour with puzzle-feeder in Associação Mata Ciliar (AMC). A- Removing the cat food from the box, B- eating the cat food. Photo: Juliana Damasceno.

Table 5. Median (inter-quartile ranges) number of minutes spent performing enrichment-directed behaviours for different factors for overall consecutive enrichment treatment schedule and schedule split by enrichment type.

Schedule Treatment	Enrichment type	Sessions	Week	Sex
Group C overall	<i>Intrinsic</i>	<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
		0.68 (0-2.921)	3.59 (0.221-37.621)	3.59 (0.304-15.929)
	<i>Extrinsic</i>	<i>Day 1</i>	<i>Week 2</i>	<i>Female</i>
		17.52 (6.421-35.338)	3.60 (0.429-18.604)	3.40 (0.150-18.150)
		<i>Day 2</i>		
		4.47 (1.358-19.263)		
		<i>Day 3</i>		
		2.74 (0-13.213)		
		<i>Day 4</i>		
		3.04 (0-14.354)		
		<i>Day 5</i>		
		3.13 (0.392-9.304)		
		<i>Day 6</i>		
		5.20 (0.563-14.446)		
Group C	<i>Intrinsic</i>	<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
		1.03 (0-2.958)	0.57 (0-2.883)	0.78 (0-2.971)
	<i>Extrinsic</i>	<i>Day 1</i>	<i>Week 2</i>	<i>Female</i>
		0.86 (0.1-2.508)	0.70 (0-2.950)	0.16 (0-1.854)
		<i>Day 2</i>		
		1.43 (0.113-2.933)		
		<i>Day 3</i>		
		0.15 (0-2.754)		
		<i>Day 4</i>		
		0.08 (0-2.367)		
		<i>Day 5</i>		
		0.77 (0.017-2.933)		
		<i>Day 6</i>		
		0.71 (0-3.429)		
Group C	<i>Extrinsic</i>	<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
		37.81 (14.375-61.363)	16.10 (7.366-32.783)	17.93 (6.20-36.625)
	<i>Extrinsic</i>	<i>Day 1</i>	<i>Week 2</i>	<i>Female</i>
		19.06 (5.308-28.017)	18.15 (4.566-38.783)	16.81 (6.942-32.408)
		<i>Day 2</i>		
		19.29 (11.817-32.946)		
		<i>Day 3</i>		
		14.18 (1.150-31.996)		
		<i>Day 4</i>		
		14.71 (7.867-22.258)		
		<i>Day 5</i>		
		9.34 (4.467-28.646)		
		<i>Day 6</i>		
		14.53 (7.829-33.308)		

Group C – consecutive schedule treatment.

Table 6. Median (inter-quartile ranges) number of minutes spent interacting with enrichment for different factors for overall intermittent enrichment treatment schedule and schedule split by enrichment type.

Schedule Treatment	Enrichment type	Sessions	Week	Sex
Group I overall	<i>Intrinsic</i>	<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
	1.63 (0.238-4.058)	1.63 (0-6.054)	3.60 (0.242-7.863)	3.34 (0.279-7.750)
	<i>Extrinsic</i>	<i>Day 3</i>	<i>Week 2</i>	<i>Female</i>
	6.50 (2.988-26.296)	3.60 (0.229-8.296)	3.69 (0.329-7.517)	3.77 (0.246-7.863)
Group I	<i>Intrinsic</i>	<i>Day 6</i>		
		4.86 (1.725-7.963)		
		<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
		1.27 (0.033-3.163)	1.65 (0.250-4.642)	1.26 (0.121-2.992)
Group I	<i>Extrinsic</i>	<i>Day 3</i>	<i>Week 2</i>	<i>Female</i>
		1.84 (0.188-3.621)	1.62 (0.192-3.708)	2.79 (0.70-4.304)
		<i>Day 6</i>		
		2.68 (0.663-6.704)		
Group I	<i>Extrinsic</i>	<i>Day 0</i>	<i>Week 1</i>	<i>Male</i>
		5.98 (0.071-26.296)	5.98 (1.65-22.942)	6.38 (3.404-20.979)
		<i>Day 3</i>	<i>Week 2</i>	<i>Female</i>
		9.44 (3.533-20.096)	7.21 (3.733-27.392)	6.63 (0-28.175)
Group I	<i>Extrinsic</i>	<i>Day 6</i>		
		7.21 (3.321-28.70)		

Group I – intermittent schedule treatment.

Figure 12. Boxplots and scatterplots highlighting the duration of interaction with enrichment (minutes) by the ocelots in terms of enrichment type (top left), days since first introduction of enrichment (top right), week (bottom left) and sex (bottom right) for the consecutive schedule treatment.

Figure 13. Boxplots and scatterplots highlighting the duration of interaction with enrichment (minutes) by the ocelots in terms of week (top left), sex (top right) and days since first introduction of enrichment (bottom left), all split by enrichment type (intrinsic or extrinsic) for the consecutive schedule treatment.

Figure 14. Boxplots and scatterplots highlighting the duration of interaction with enrichment (minutes) by the ocelots in terms of enrichment type (top left), days since first introduction of enrichment (top right), week (bottom left) and sex (bottom right) for the intermittent schedule treatment.

Figure 15. Boxplots and scatterplots highlighting the duration of interaction with enrichment (minutes) by the ocelots in terms of week (top left), sex (top right) and days since first introduction of enrichment (bottom left), all split by enrichment type (intrinsic or extrinsic) for the intermittent schedule treatment.

2.4. Discussion.

The ocelots' behaviours were affected by the intrinsic and extrinsic enrichments in different ways in terms of enrichment-directed behaviours. The puzzle-feeder (extrinsic enrichment), engaged the animals for more time than catnip (intrinsic enrichment), for both of the schedule treatments. Extrinsic enrichments, which submit the animals to complete a task to win a reward, tend to be more powerful in response magnitude than intrinsic, and promote longer-lasting effects on animal behaviours (ANDERSON; ARUN; JENSEN, 2010; SCOTT; FARAH; PODSAKOFF, 1988; TAROU; BASHAW, 2007). In the case of the present study, the ocelots needed to remove wet food contained inside a wooden box, which required time and cognitive efforts. To obtain food in an extrinsic enrichment device, requires the expression of multiple behaviours involved in manipulation (SCHNEIDER; NOGGE; KOLTER, 2014). Although, the intrinsic enrichment was shown to be an effective short-term enrichment, influencing the cats' behaviour, it was for a short period of time only, which has been demonstrated in previous studies also utilizing scents for cats (ELLIS; WELLS, 2010; RESENDE et al., 2011; WELLS; EGLI, 2004). In the present study, the catnip was shown to have a short-lived effect on the ocelots' behaviours within-session, resulting in a lower duration in enrichment-directed behaviours due to the scent, by both treatment schedules. Pullen and collaborators (2011), who also found a short-lived effect by an intrinsic device. Introducing animal shaped toys to 16 captive dogs (*Canis familiaris*) they found a decrease in response to the objects of 11 dogs after 10 seconds of presentation, between the second and tenth exposure. Intrinsic reinforcements increase behaviours, such as exploration and novelty seeking, but also promote a low influence on an animal's response (TAROU; BASHAW, 2007).

There was no significant difference in the two-way analysis for enrichment type comparing with week, days and sex. In addition, there was no significant difference in the three-way analysis comparing week, days and enrichment type. Those results mean that there was no habituation in the response of the ocelots with respect to enrichment-directed behaviours, for either type of enrichment between sessions. This means that the animals interacted, in general, during every enrichment session. Furthermore, there was no evidence of habituation between sessions of exposure for the two different applied treatment schedules (consecutive and intermittent). This finding contradicted the prediction proposed by Tarou and Bashaw (2007), suggesting that lower exposures of the same enrichment could result in slow habituation when compared with frequent exposure to the same. However, is important to point out that the

prediction was for different enrichment items and for different species of animal. A possible explanation why the ocelots in the present study did not habituate to the scent can be explained by the stimulant effect of catnip on cats' behaviour, which has been seen in ocelots in previous studies also (POWELL, 1997; WEAVER et al., 2005; WOOSTER, 1997). Intrinsic enrichments, such as olfactory ones, are an easy way to incorporate novelty into the captive environment (QUIRKE; O'RIORDAN, 2011b). Having introduced intrinsic enrichments (new objects such as scented squash, zebra dung and frozen blood balls) to two lions and four Sumatran tigers, Van Metter et al. (2008), identified an increase in natural and active behaviours, and the animals were not found to habituate to the stimulus, over the ten weeks of their study. As proposed by Tarou and Bashaw (2007), in the present study, there was no habituation over exposures to the extrinsic enrichment which was applied (the puzzle-feeder). According to them, habituation to extrinsic enrichments can occur for this type of reinforcement in a single exposure, but not for the device itself. In a recent study, applying extrinsic (food hidden, such as fruits, carrots and larvae) and intrinsic enrichments (the scent from a pinch of cinnamon in water), Schneider, Nogge and Kolter, (2014) also did not find habituation, in the response of five Malayan sun bears (*Helarctos malayanus*) when those enrichments were applied for 12 consecutive days. In an other study, Ings, Waran and Young, (1997) detected only about a 20% decrease in the response of bush dogs (*Speothos venaticus*) over 30 exposures of a scattered feeding enrichment.

In terms of abnormal behaviour, both enrichment types were shown to be effective in reducing pacing, for both treatment schedules and for the two weeks during which each enrichment type was applied, when compared to baseline observations. However, there was an increase in pacing behaviour for the catnip, comparing week 1 with week 2, but it was still less than that expressed during baseline observations. This result implies a short-term effect of intrinsic enrichment in decreasing abnormal behaviours. In relation to extrinsic enrichment, it was effective in reducing pacing for the two weeks of exposure, when less passing occurred in week 2 than in week 1. Zebunke, Puppe and Langbein, (2013) reduced antagonistic behaviour in domestic pigs by the introduction of a cognitive enrichment, in which the animals needed to complete a task (press a button after an auditory signal) to win a food reward. Skibieli et al. (2007), found a reduction in pacing behaviour after the introduction of spices (-21.25%) and frozen fish (-26.58%) for five species of captive large cats. Utilizing a similar schedule treatment to that applied in the present study, Anderson et al. (2010) also found a reduction in stereotypies, when logs with holes containing honey, were introduced to 14 captive sloth bears, for five days in consecutive and intermittent frequencies. Enrichments involving challenges,

such as feeding devices, stimulate animals' cognition, allowing them to express behaviours similar these performed in the wild, which promote intense activity, increasing environmental interaction. In addition, these techniques could reduce and/or prevent the development of stereotypies (MEEHAN; MENCH, 2007).

Research that investigates a variety of schedules for enrichment application allow more flexibility for institutions to apply enrichments with certain efficiency (QUIRKE; O'RIORDAN, 2011b). Utilizing consecutive and intermittent schedules, the present study proved that extrinsic and intrinsic enrichment affected ocelots' behaviours in different ways, in terms of enrichment-directed behaviours, but both of them were equally efficient in decreasing pacing, for the two treatments schedules. In summary, the designed puzzle-feeder and catnip are efficient tools for improving the welfare of captive ocelots, with no evidence of habituation in the animals' response to the devices, for both schedules of introduction. Further investigations about how different devices could affect the response of the captive animals are essential for the development of enrichment practices.

2.5. Conclusion.

Extrinsic and intrinsic reinforcements affect animals' behaviours in distinct levels. Extrinsic enrichment promotes longer-lasting effects on animals' behaviours, as demonstrated in the present study, by ocelots spending more time performing enrichment-directed behaviours with the puzzle-feeder than with the catnip, for consecutive and intermittent treatments schedules. In addition, the utilised extrinsic and intrinsic enrichments decreased pacing behaviour, for both enrichment schedules and with no evidence of habituation to the applied stimulus. However the puzzle-feeder was more effective than catnip for reducing levels of stereotypy.

3. Chapter 2.

Photo: Juliana Damasceno

Effect of enclosure size on pacing behaviour in captive ocelots under enrichment and non-enrichment conditions.

Abstract

Wide-ranging carnivores tend to express high levels of stereotypies when in captivity, and that trend seems to be related to their high frequency of locomotion in the wild. The current study investigated if the enclosure size influences the expression of pacing behaviour in ocelots, kept under either enriched or non-enriched conditions. The study was conducted with twelve ocelots, housed in two conservation breeding institutions located in Brazil, six of them were housed in small enclosures (9-15 m²), and six in large enclosures (80-100 m²). All ocelots were exposed to three environmental conditions, the non-enriched condition (baseline), without making any changes in their enclosures, and the enriched conditions, firstly using an olfactory device (with catnip inside) that was followed by the use of a puzzle-feeder (a wooden box with cat food inside). All observations were carried out from 14:00h to 16:00h. The baseline observations were carried out during three days, being followed by three more days after the application of the olfactory device, which were repeated after a week-long interval for three more days. After a two week-long interval, the puzzle-feeder enrichments were introduced into the enclosure, recording the ocelots' behaviour during three days, repeating this procedure after a weeklong interval. The results revealed that ocelots housed in small enclosures spent more time pacing than those housed in larger enclosures, and that the introduction of the puzzle-feeder significantly decreased the time spent pacing by the ocelots housed in small enclosures, when compared to the baseline observations. The olfactory enrichment did not influence the time spent in pacing behaviour for either group of ocelots. Based on these results it is concluded that the amount of space and complexity of the enclosures has an important role in the expression of pacing behaviour by ocelots kept in captivity; and that the adoption of environmental enrichment, that stimulates foraging behaviour, is effective in reducing pacing behaviour, even when it is not possible to expand the space and the complexity of the enclosures.

3.1. Introduction.

Ocelots (*Leopardus pardalis*) are considered to be generalists, highly adaptable, and the predominant feline in the group of neotropical mesopredators (OLIVEIRA et al., 2008); they live in a wide spectrum of habitats, such as: mangrove forests, coastal wetlands, savannah and grasslands, thorny bushes and tropical forests of all types (NOWELL; JACKSON, 1996). Territorial and nocturnal, *Leopardus pardalis* is considered a wide-ranging species, occupying from 0.8 to 15 km² for females, and from 3.5 to 46 km² for males, although their range increases during dry periods (DILLON; KELLY, 2008; NOWELL; JACKSON, 1996; SUNQUIST; SUNQUIST, 2002). However, when kept in captivity, the size of their environment is reduced greatly; the legal minimum space for ocelot enclosures is approximately 8 m² for a single animal in Scotland (DANGEROUS ANIMAL ACT, 1976), and 5 m² in Brazil (IBAMA, 2015).

Besides being restricted, the space in captivity is usually poor, lacking structural complexity and opportunities for social interactions, creating a very predictable environment where the animals have no control of the situation (SWAISGOOD et al. 2003; MCPHEE, 2002; HILL; BROOM, 2009). Studies have shown that a limited enclosure size can have a negative impact on reproduction and growth of captive animals, and leads to behavioural abnormalities, such as stereotypies (MASON, 2010).

Stereotypy is defined as abnormal behaviours, characterized by the expression of repetitive behaviour, without any objective or function (BROOM, 1983; MASON, 1991). Stereotypies signal the incapacity of an animal to cope with its environment, being developed in the animal due to a range of factors regarding external stimuli, pre-disposition and life history (LAWRENCE; RUSHEN, 1993). It may also reflect an environment which lacks the conditions required for normal brain development (MASON; LATHAM, 2004). Examples of stereotypies include head or body shaking, constant chewing, swallowing air, repetitive movements of limbs or tongue, excessive licking, fixed swimming patterns and pacing (SHYNE, 2006). Pacing consists of a constant and repetitive walk (or trot), around the same route, without any apparent objective, and it is the stereotypy most expressed by captive carnivores (BRETON; BARROT, 2014; SHYNE, 2006; QUIRKE; O'RIORDAN; ZUUR, 2012).

According to Mason et al. (2007), stereotypies can evolve primarily from frustration and/or brain dysfunction, they can could also start due to a habit formation and copy effect. Clubb and Vickery (2006) proposed three hypotheses for the motivational factors responsible for the development of stereotypies in carnivores: the first is comprised of multiple factors,

including deprivation of foraging opportunities; the second is the inability to escape; and the third is that non-motivational factors lead wide-ranging species to persist in abnormal behaviours. Species with large home ranges, such as felids, have a greater tendency to develop stereotypies in captivity (SWAISGOOD; SHEPHERDSON, 2005). According to a study conducted by Clubb and Mason (2007) the levels of stereotypies in carnivores are related to the lack of opportunities to express ranging behaviours, due to the extensive reduction in their home-range size.

Strategies aimed at to improving animal welfare, such as environmental enrichment, have been shown to reduce stereotypies in captive animals. A meta-analysis study conducted by Shyne (2006) found that 90% of studies utilizing environmental enrichment were successful in reducing stereotypies. Feeding, olfactory (as a sensory technique) and structural changes in the captive environment are considered the most important techniques in environmental enrichment (DE AZEVEDO; CIPRESTE; YOUNG, 2007; HOY; MURRAY; TRIBE, 2010). Enriching the environment is an alternative to increasing the complexity and the novelty in the enclosure (ELLIS, 2009; GENARO, 2005).

The current study aims to investigate how enclosure size can influence pacing behaviour in ocelots, kept in captivity, under environmental and non-environmental enrichment conditions, by comparing ocelots housed in large and small enclosures during three different conditions: baseline (without enrichment), olfactory and puzzle-feeder environmental enrichments.

3.2. Methods.

This study was approved by the Ethics Committee on Animal Use of the Campus of the city of Ribeirão Preto, University of São Paulo (USP), Brazil, and it complied with the ethical principles of animal experimentation (protocol number 13.1.1234.53.0) (Appendix A).

3.2.1. *Subjects and Study sites.*

Twelve ocelots (six males and six females), housed individually in twelve enclosures, in two different animal conservation breeding institutions (named here A and B), located in Brazil were observed. The animals were aged between 4 and 16 years old, and three of them were born in the wild and nine in captivity, as described in Table 7.

Their diet was composed of beef, chicken with a mineral supplement and dead rodents, being alternated over seven days of each week. Water was available *ad libitum*, being replaced once per day. The feeding time was from 16:00h to 17:00h every day and each animal received a specific amount of food, according to its requirements. Professionals (biologists and veterinarians) and caretakers provided veterinary care and daily sanitary monitoring.

3.2.2. *Enclosures.*

The ocelots' enclosures were classified according to their size, as small (from 9 to 15 m²) and large (80 to 100 m²) with a height of two to four meters (Fig. 16 A, B). Both types of enclosures had furniture as recommended for felids (ROCHLITZ, 1999), such as vertical areas to occupy, elevated pathways, shelters (one or more), vegetation, more than one type of floor substrate (cement and sand, for example) and a management area (cage). The large enclosures were more complex in terms of the quantity, and this was due to the space available. Thus, the large enclosures were also more complex, and due to this, termed large/complex.

Table 7. Information about the 12 studied ocelots, to which institution they belonged, gender, origin, age and enclosure size.

(*) For reasons of confidentiality the institutions were labelled by the letters A or B.

Name	Institution*	Gender	Origin	Age (years)	Enclosure size
Google	A	F	Captivity	5	Small
Guria	A	F	Captivity	10	Small
Jussara	A	F	Captivity	16	Small
Tererê	A	F	Wild	14	Small
Piá	A	M	Captivity	10	Small
Pig	A	M	Captivity	8	Small
Filhote	B	F	Captivity	4	Large
Nissa	B	F	Wild	14	Large
Leo	B	M	Captivity	6	Large
Lotus	B	M	Captivity	7	Large
Rick	B	M	Captivity	8	Large
Titico	B	M	Wild	13	Large

3.2.3. *Environmental enrichment.*

Two types of environmental enrichments were applied for the ocelots: *olfactory* - catnip scent (*Nepeta cataria*) (5mL) sprayed on a wooden square pre-fixed in the enclosure (Fig. 4); and *puzzle-feeder* - composed of a wooden box of 45x30x30 cm (length, width and height, respectively) with two apertures of 8 cm in diameter on the top (for the animal to access), containing 580 g of wet cat food of fish flavour (Whiskas®) and grass inside; a granite stone of 4 kg was also placed inside the wooden box to avoid injury to the animal by possible box movement (Fig. 6).

Figure 16. Examples of enclosures structures. A - Small (institution A) measuring 9 m²; B – Large/complex measuring 80 m² (institution B).

3.2.4. *Procedures*

The behaviour of the ocelots was recorded by using a CCTV (closed-circuit television), with cameras installed inside each enclosure. Two hours of observations were carried out for each ocelot, always from 14:00h to 16:00h per day. This time was chosen as they were the hours before feeding time, as recommended by Tarou and Bashaw, (2007) when applying environmental enrichments containing food, and the time which stereotypies are highly expressed (CLUBB; VICKERY, 2006; SWAISGOOD; SHEPHERDSON, 2005). Baseline (B) observations were conducted for three days, without any environmental enrichment. Following that, olfactory enrichment (O) was applied for three days and repeated for three more days after a week-long interval. After that, there was a two week interval, followed by three days of a puzzle-feeder (P), which was also repeated three times more after another week-long interval. Thus, the schedule of the experiments consisted: BO-O--P-P. The environmental enrichment conditions were considered O and P, and the non-enrichment B.

The recording of pacing behaviour was performed using the animal focal sampling method, in accordance with Altmann (1974), by continuous recording of this behaviour during the two hours of each observation session. *Pacing* was defined as repetitive locomotion in a fixed pattern, for example, back and forth along the same route. This behavioural category includes walking, trotting and running, where movement seems to have no apparent goal or function, but must be performed at least two times in succession before qualifying as a stereotypy. The definition was adapted from Quirke and O’Riordan (2011a, 2011b) and based on a categorical definition for cats’ behaviour by Stanton et al. (2015).

3.2.5. *Data analysis.*

The data violated the assumptions of normality and homogeneity of variance. The non-parametric Mann-Whitney test was utilised to compare the duration time spent pacing (in min.) when kept in small and large enclosures under non-enriched (baseline) and enriched conditions.

The Friedman ANOVA test was utilised to compare whether the groups within the small and large enclosures differed in the amount of time spent pacing between baseline and

enrichment conditions. When the Friedman's tests was significant, Wilcoxon signed rank tests with a Bonferroni correction were performed to determine significant pair-wise relationships.

All analyses were conducted using SPSS version 22.0 and the alpha level for statistical significance was taken to be $P < 0.05$.

3.3. Results.

The time spent pacing during the baseline condition differed significantly between the animals housed in large ($\bar{x} = 8.36 \pm 6.32$) and small ($\bar{x} = 78.81 \pm 17.87$) enclosures (Fig 1A, Mann-Whitney test: $Z = -2.20$, $P = 0.02$). When the olfactory enrichment was applied no significant difference was observed in the amount of time spent pacing ($Z = -1.76$, $P = 0.09$) (Fig. 1B) between the ocelots housed in large ($\bar{x} = 9.94 \pm 3.75$) or small ($\bar{x} = 63.55 \pm 19$) enclosures. However, when the puzzle-feeder was applied, there was a significant difference between the time spent pacing for ocelots housed in large ($\bar{x} = 1.61 \pm 1.06$) *versus* small ($\bar{x} = 41.46 \pm 11.55$) enclosures ($Z = -2.58$, $P = 0.009$) (Fig. 17 C). Comparing the behaviour of the ocelots within each enclosure condition, the animals housed in large enclosures did not differ in the amount of time spent pacing according to the treatments (baseline, and puzzle-feeder and olfactory environmental enrichment) (Friedman ANOVA test: $\chi^2 = 10.46$, d.f. = 5, $P = 0.063$) (Fig. 18 A). In contrast, ocelots housed in small enclosures differed significantly in the amount of time spent pacing ($\chi^2 = 11.95$, d.f. = 5, $P = 0.035$), and this difference was between the baseline and puzzle-feeder enrichment conditions (Wilcoxon signed rank test: $Z = -1.99$, $P = 0.046$), as shown in Figure 18 B.

Figure 17. Mean $\pm$ SE of minutes spent in pacing by ocelots housed in large *versus* small enclosures under A- non-enriched (Baseline) and enriched conditions (B- Puzzle-feeder and C- Olfactory). Asterisk (*) indicates them to be significantly different ($P < 0.05$) according to the Mann-Whitney test.

Figure 18. Mean $\pm$ SE of minutes spent in pacing expression by ocelots under non-enriched (Baseline) and enriched conditions (Puzzle-feeder and Olfactory) within each housing condition (A- Small and B- Large). Asterisk (*) indicates a significantly difference ($P < 0.05$) according to Friedman ANOVA test.

3.4. Discussion.

This study examined how enclosure size can influence pacing behaviour expression in ocelots under enriched and non-enriched environmental conditions. During the baseline period (non-enriched environmental condition) the ocelots housed in large enclosures expressed significantly less pacing than individuals housed in small enclosures. This result corroborates the findings of another multi-institutional study, conducted by Breton and Barrot (2014), who identified that tigers housed in smaller enclosures paced longer distances than those housed in larger ones. The authors also found that the distance covered in captivity (including pacing, walking, running and jumping) was related to the proportion covered in the wild. According to Clubb and Mason (2003, 2007) wide-ranging animals show more evidence of stress and abnormal behaviours when in captivity, probably due their naturally high level of activity in wild. Captive ocelots can present lower activity levels when compared to that expressed in the wild, but they can perform a higher frequency of stereotypies, such as pacing (WELLER; BENNETT, 2001).

In the current study, the high pacing levels expressed by ocelots housed in small enclosures significantly decreased through the application of the puzzle-feeder enrichment. Enrichments strategies such as puzzle-feeders, increases the complexity in the environment by requiring the animals to work for their food, stimulating the expression of natural food related behaviours and improving behavioural indicators of animal welfare, such as pacing reduction (MORGAN; TROMBORG, 2007). Enrichment devices, which create foraging challenges, are considered to be the most effective way to reduce stereotypic behaviour in a review conducted by Shyne (2006). Specifically, puzzle-feeders similar to that designed for the current study, have been shown to be an effective tool to reduce pacing in wild cats, such as Amur tigers (JENNY; SCHMID, 2002), Sumatran tiger (BASHAW et al., 2003), leopard cat (SHEPHERDSON et al., 1993) and in other carnivores such as bears (CARSTEAD; SEIDENSTICKER; BALDWIN, 1991; RENNER; LUSSIER, 2002) and foxes (CARSTEAD, 1991).

On the other hand, the addition of a new scent (catnip) did not reduce the time spent pacing in ocelots, kept in both small and large enclosures. This result may reflect the short-lived effect caused by the olfactory stimulus and the chosen scent. Catnip (*Nepeta cataria*) is an herb which stimulates active behaviours in cats (ELLIS, 2009), however its effects are more related to increase of exploratory, play and scent marking behaviours (ELLIS; WELLS, 2010; WELLS;

EGLI, 2004) than reducing pacing. For example, Resende et al. (2011) applied cinnamon and catnip to Oncilla cats (*Leopardus tigrinus*) but they observed a decrease in pacing only when cinnamon was introduced. Although the introduction of scents in the captive environment is an effective tool for stimulating natural behaviours, such as exploration, activity, behavioural diversity and social affiliations (WELLS, 2009), few studies have found a consistent influence on pacing behaviour. Research which identified relevant results, by applying scents aimed at decreasing stereotypies, utilised scents such as cinnamon, chilli powder and cumin (RESENDE et al., 2011; SKIBIEL; TREVINO; NAUGHTER, 2007). In the previous chapter of this thesis the use of catnip demonstrated a decrease in pacing behaviour when compared the baseline in the first week of introduction, but that decrease did not happen for the second week of exposure. This previous result demonstrates that catnip was effective in reducing pacing, but the influence was not prolonged. Further investigations regarding the reason why other stimulating scents are more efficient, than catnip, in terms of pacing reduction, are required.

In the case of the present study, only the food/cognitive related enrichment was demonstrated to have a significant impact in alleviating captive stress consequences (represented by pacing) in ocelots housed in small environments. The larger enclosures were also more complex, containing more brushes, shelters and pathways than the small ones. In addition to foraging challenge enrichments, other practices also could benefit wild cats' welfare such as structural complexity improvement. For example, Moreira et al. (2007), found that after transferring female margay (*Leopardus wiedii*) and oncilla cats (*Leopardus tigrinus*) females from large to small enclosures, high levels of corticoid concentrations and frequency of pacing were identified. However, when the small enclosures were enriched with tree trunks, plants, and a nest box, the corticoid levels declined in the oncilla females. Improving the complexity in small enclosures represents an alternative when size extension is not possible (DE OLIVEIRA; TERÇARIOL; GENARO, 2015).

The findings presented elucidate the necessity of providing effective enrichment strategies to minimize abnormal behaviours. Environments for wide-ranging animals, such as ocelots, should provide more space, multiple den sites, frequent novelty and more control opportunities over adverse and rewarding stimuli (CLUBB; MASON, 2007). The introduction of enrichments, which promote foraging challenges, as well as improving the structure and complexity of enclosures, have been shown to be efficient and are recommended tools to reduce stereotypies in felids kept in captivity, improving the welfare of these animals.

3.5. Conclusion.

The physical structure of enclosures (size and complexity) influences the expression of pacing behaviour in captive ocelots and the negative effects of small/less complex enclosures can be reduced by the application of environmental enrichment. The results indicate that enrichment practices that involve foraging challenges or structural improvement features are effective strategies to reduce pacing in captive felids. Further studies aimed at investigating how captive environmental features and provision of complexity by enrichment practices could influence and benefit animals' welfare are required.

4. Chapter 3.

Photo: Juliana Damasceno

The effect of intrinsic enrichments applied to cheetahs and Sumatran tigers in captivity.

A modified version of this chapter has been accepted with revision by to the Journal *Zoo Biology*.

Abstract

Environmental enrichment is a well-known technique, aimed at improving captive animal welfare. In terms of an animal's response, intrinsic forms of environmental enrichment (such as new objects and scents) are those which increase the probability of observing a response happening again by itself, thus being considered intrinsically reinforcing. The aim of this study was to investigate how three forms of intrinsic enrichment, namely, a hay ball without scent, a hay ball with catnip and a hay ball with cinnamon, influenced the behaviour of six cheetah and two Sumatran tigers at Fota Wildlife Park, Ireland. Enrichment-directed behaviours, pacing behaviour, locomotion, inactive and exploratory behaviours were investigated. The results indicated that the three forms of enrichment had similar effects in terms of enrichment-directed behaviour, with cinnamon showing the highest levels. Cinnamon also significantly reduced pacing behaviour when compared with baseline observations. No evidence of habituation, in the form of significantly reduced enrichment-directed behaviours, was observed meaning that these forms of enrichment could be applied frequently for these species and still elicit a response. These three forms of enrichment are a simple, practical, and effective enrichment method.

4.1. Introduction.

Environmental enrichment includes a range of techniques, aimed at improving the physical and psychological welfare of animals in captivity, in addition to encouraging the performance of natural behaviours (CARLSTEAD; SHERPHERDSON, 1994; ELLIS, 2009; LAW; GRAHAM; MCGOWAN, 2001). Research surrounding issues for the design and implementation of environmental enrichment are common. Issues concerning the area of environmental enrichment include a lack of variety of effective techniques for certain species, as well habituation to the applied stimuli (TAROU; BASHAW, 2007). Habituation is defined as a decrease in response to a stimulus after repeated exposure to that stimulus (THOMPSON; SPENCER, 1966). This can occur within the same session (intra-session) or between sessions (inter-session) (LEUSSIS; BOLIVAR, 2006). The habituation process to novelty is an evolutionary strategy in animals, as it is related to exploration and consequent adaptation to the environment (THOMPSON; SPENCER, 1966; WONG et al., 2010). However, when incorporating environmental enrichment techniques, aimed at stimulating natural behaviours, through the creation and maintenance of environmental novelty in captivity (CARLSTEAD; SHERPHERDSON, 1994; ELLIS, 2009; LAW; GRAHAM; MCGOWAN, 2001), the habituation process can eventually reduce the effectiveness of the enrichment (TAROU; BASHAW, 2007).

Enriching captive felids is challenging, due to the complexity of behaviours required for predation and exploration in these species (QUIRKE; O'RIORDAN; DAVENPORT, 2013; SKIBIEL; TREVINO; NAUGHER, 2007). The absence of stimuli, which elicit such behaviours, can cause stress and lead to the development of abnormal behaviours, such as stereotypical pacing behaviour, best described as repetitive, maladaptive and apparently functionless behaviour (MASON, 1991). The existing literature surrounding enrichment for captive felids has focused upon the stimulation of natural active behaviours such as hunting and exploration (QUIRKE; O'RIORDAN, 2011a; SZOKALSKI; LITCHFIELD; FOSTER, 2012). However, there is a relative lack of research highlighting novel effective forms of enrichment for some species, such as the cheetah (QUIRKE; O'RIORDAN, 2011a).

Enrichment techniques can be classified in different ways, as animate and inanimate (ELLIS, 2009), or as cognitive (occupational), food (nutritional), structural, social, and sensorial (BLOOMSBITH; BRENT; SCHAPIRO, 1991; DE AZEVEDO et al, 2007; YOUNG, 2003), or even as physical, social and temporal (CARLSTEAD; SHEPHERDSON, 2000).

However, there is another classification proposed by Tarou and Bashaw (2007) based upon the animal's response to enrichment and how those stimuli could influence its behaviour. According to Tarou and Bashaw (2007), enrichment can be classified as intrinsically or extrinsically motivating. Intrinsic enrichments are those in which the response elicited by the stimuli improves the probability of this same response happening again. Examples of these techniques include new objects or new scents in the environment. In contrast, extrinsic forms of enrichment are those in which a reward, received through interaction with the enrichment, improves the probability of this same response happening again. In other words, the animal(s) need to solve a problem to gain a reward, which could be food or contact with a conspecific, but by doing so, the animal would be motivated to engage with the enrichment again later on. Examples include puzzle-feeders or hiding food in the environment.

The introduction of intrinsic enrichments, such as new objects and scents to the captive environment, can increase exploration and stimulate territorial behaviours, such as scent marking, improving psychological well-being (QUIRKE; O'RIORDAN, 2015; TAROU; BASHAW, 2007; WELLS, 2009). Wells and Egli (2004) introduced three different types of odors to black-footed cats (*Felis nigripes*) and observed an increase in activity and exploration. However, over the five days of testing, a decrease in the animal's response to the odors was observed, demonstrating evidence of habituation. Clark and King (2008) highlight the importance and the difficulty in designing, implementing and evaluating the application of olfactory enrichments for zoo animals. They suggest important points to consider before applying scents for animals, including the relevance of that scent in motivating an expected behaviour, how to present the scent to the animal in terms of duration, individual differences in response, and any health implications involving the chosen scent. Hoy, Murray and Tribe, (2010) evaluated the effectiveness of enrichment applied in zoos for mammals and found that olfactory enrichment was considered the most important sensory enrichment.

The present study applied three types of intrinsic enrichment to captive cheetahs (*Acinonyx jubatus*) and Sumatran tigers (*Panthera tigris sumatrae*). The aims of the study were to: 1) assess the effectiveness of these forms of enrichment in terms of levels of interaction with enrichment, and changes in the levels of pacing, locomotion, exploratory and inactive behaviours, 2) evaluate if habituation occurred over the seven days of introduction for each form of enrichment. These investigations will add to the current literature in terms of providing evidence related to the effectiveness of intrinsic enrichment for these two species of captive felid.

4.2. Methods.

The current research is fully compliant with the ethical principles of animal experimentation, according to Fota Wildlife Park, Carrigtwohill Co. Cork, Ireland (see Appendix B).

4.2.1. Study Site and animals.

The study was carried out at Fota Wildlife Park (51.8992°N, 8.2982°W), Carrigtwohill Co. Cork, Ireland, between January and May 2015. The subjects of the study were a male and a female Sumatran tiger (*Panthera tigris sumatrae*), and two female and four male cheetahs (*Acinonyx jubatus jubatus*). All cats were adults and captive born, between two and seven years old (Table 8). The eight animals were housed, singly or in pairs, in six enclosures measuring between 420 m² to 3500 m² (Fig. 19). The enclosures contained vertical areas (Fig. 20), trees, refuges and raised areas. Individuals were fed with rabbit, chicken, horse meat and beef steak. The cheetahs are fed six days a week (from 14:00h to 16:00h) with approximately 1.8kg of food while the tigers are fed five days a week (about 16:00h) with 5 kg in each serving.

Table 8. The subjects of this study. Information regarding species, enclosure, gender, origin and age.

Individual	Species	Enclosure	Gender	Origin	Age
Gimpy	<i>Acinonyx jubatus jubatus</i>	A	F	Captivity	6
Topaze	<i>Acinonyx jubatus jubatus</i>	B	F	Captivity	7
Pompom	<i>Acinonyx jubatus jubatus</i>	C	M	Captivity	6
Claude	<i>Acinonyx jubatus jubatus</i>	C	M	Captivity	6
Marvin	<i>Acinonyx jubatus jubatus</i>	D	M	Captivity	2
Bandy	<i>Acinonyx jubatus jubatus</i>	B	M	Captivity	6
Dourga	<i>Panthera tigris sumatrae</i>	E	F	Captivity	4
Denar	<i>Panthera tigris sumatrae</i>	F	M	Captivity	3

Figure 19. One of the cheetahs' enclosures in Fota Wildlife Park, Cork, Ireland. Photo: Juliana Damasceno.

Figure 20. Vertical areas in one of the Sumatran tigers' enclosures in Fota Wildlife Park, Cork, Ireland. Photo: Juliana Damasceno.

4.2.2. *Intrinsic enrichment.*

Each form of enrichment utilised in this study had not been previously introduced to any of the animals at Fota Wildlife Park. The items utilised in this study were:

- *Hay ball (HB)*: ball made with hay (approximately 30 cm diameter) tied using natural jute string (used in gardening and safe in case of animal ingestion) (Fig. 21).
- *Catnip (CA)*: using the Hay ball as the vector, 5 ml of liquid catnip herb (*Nepeta cataria*), (the Kong® brand Kong Company), was deposited onto the Hay ball (Fig. 22 A).
- *Cinnamon (CI)*: using the Hay ball as the vector, 5 ml of cinnamon (*Cinnamomum zeylanicum*), (Colony™ brand, Wax Lyrical Ltd.), was deposited onto the hay ball (Fig. 22 B).

For the first application, HB enrichment is characterized as an intrinsic enrichment as it is a new object in the environment.

Figure 21. Hay ball (approximately 30 cm of diameter) made of hay tied with natural string and applied to cheetahs and Sumatran tigers. Photo: Juliana Damasceno.

Figure 22. Scents which were sprayed in the hay ball. A - Liquid catnip herb (*Nepeta cataria*), Kong® brand Kong Company; B – Liquid cinnamon (*Cinnamomum zeylanicum*), Colony™ brand, Wax Lyrical Ltd.

4.2.3. Procedures.

The experiments were conducted over seven consecutive days (Monday to Sunday) from 9:00h to 14:00h. This period of day was chosen as no feeding or cleaning was taking place at this time, therefore minimising any possible interference with the enrichment introduction. Behaviour data were recorded directly, comprising one hour per enclosure per day, by randomized order on each day of observation. Focal animal sampling or all occurrence sampling (ALTMANN, 1974) was utilised to collect behaviour data according to the categories of behaviour shown in Table 9.

The enrichment items were placed into the enclosure over the fence. In order to minimise the possibility of conflict between the animals, and to reduce the probability of monopolization (DAMASCENO; GENARO, 2014), one item was introduced for each cat when more than one cat was housed in the same enclosure. Baseline (BL) observations were carried out for one week (seven days) prior to the start of the enrichment, followed by HB for seven days, then seven days of interval (without any data collection or introduction of enrichment), then CA for seven days, another seven days of interval and, finally, CI also for seven days (see Table 10). According to Clark and King (2008) it is necessary to leave a gap between the introduction of

different scents to allow the dissipation of the previous scent and avoid accumulation of scent. Seven days of interval are used also to minimize the effect of one enrichment on another. Each enrichment treatment was applied for seven continuous days, in order to analyse whether habituation to each form of enrichment occurred and also to provide replicate treatments for behavioural data analysis.

Table 9. Ethogram utilised in animals' observations. The described behaviour categories were adapted from, Quirke and O'Riordan (2011).

Behaviour	Definition
Enrichment	Interacting with the enrichment item, any kind of direct contact such as touching, rolling, sniffing, playing, catching, rubbing, carrying, and other.
Exploration	Urine spray, sniffing, scratching or rubbing objects or enclosure substrate.
Self-Grooming	Licking own body.
Inactive	Lying, sitting or standing still.
Locomotion	Running, walking, climbing or jumping.
Negative social	Aggression, striking with a paw, biting, showing teeth with vocalization, chasing other cat.
Pacing	Repetitive locomotory movement along a given route (up/down fence line, around enclosure or object in enclosure) uninterrupted by other behaviours.
Positive social	Playing together, allogrooming or allorubbing.

Table 10. Experimental design.

Experimental days	7 days	7 days	7 days	7 days	7 days	7 days
	BL	HB	----	CA	----	CI

Legend: BL – Baseline, behavioural observations with no enrichment introduction; HB – Hay ball with no scent; ---- - interval with no enrichment introduction; CA –hay ball with catnip; CI – Hay ball with cinannamon.

4.2.4. *Data analysis.*

Data from tigers and cheetahs were combined due to the fact that there was no difference among the species for the observed behaviours. The duration (in minutes) that each animal spent interacting with each form of enrichment during the first hour after each introduction was utilised in the analysis. The duration (in minutes) that each animal spent performing pacing, inactive, exploratory and locomotion behaviours was also considered during data analysis. Since the data violated normality and homogeneity of variance assumptions, a Friedman ANOVA test was used for data analysis. The average duration in which each animal interacted with each form of enrichment over the seven days of introduction was calculated. A Friedman ANOVA ($n=8$ animals) was then conducted in order to determine if there was a significant difference in the interaction time between the three different forms of enrichment (Hay ball, Catnip and Cinnamon). This procedure was repeated using the average duration (in minutes) that each animal spent in pacing, inactive, exploratory and locomotion behaviours, during baseline and each enrichment phase, in order to determine if there was a significant difference between the different phases of the study. The average latency of each animal to start interacting with enrichment was calculated across the seven days of introduction for each type of enrichment. A Friedman ANOVA was conducted in order to determine if there was a significant difference in latency between enrichment types. Secondly, in order to determine if habituation to enrichment occurred over the seven days of introduction, three Friedman ANOVAs ($n=8$), one for each enrichment type, were conducted using the duration each animal spent interacting with each enrichment on each day of introduction. The same procedure was carried out using pacing data. As a number of tests were conducted, the alpha level for statistical significance was taken to be $p < 0.01$ for all Friedman ANOVAs. If the Friedman tests were significant, Wilcoxon signed rank tests with a Bonferroni correction, applied for multiple tests were performed to determine significant pair-wise relationships. All analyses were conducted using R version 3.2 and SPSS version 22.0.

4.3. Results.

4.3.1. Interaction time.

The interaction of Sumatran tigers and cheetahs with the enrichment can be seen in Figures 23, 24 and 25. The mean interaction time (minutes $\pm$ SE), across all treatments was $\bar{x} = 5.15 \pm 0.42$ minutes. There was no significant difference in interaction time between the three different forms of enrichment ($\chi^2 = 3.25$, d.f. = 2, $p > 0.05$) (Fig. 26). The longest mean interaction time (mean in minutes $\pm$ SE) across seven days of introduction was observed for cinnamon ($\bar{x} = 5.43 \pm 0.85$) followed by the hay ball ($\bar{x} = 4.16 \pm 0.76$) and catnip ($\bar{x} = 3.11 \pm 0.64$) (Fig. 26). There was no significant difference ($\chi^2 = 6.00$ d.f. = 6, $p > 0.05$) in interaction time between the seven days of introduction for the hay ball enrichment (Fig. 27). The longest interaction time was observed on day 1 of introduction ($\bar{x} = 7.89 \pm 3.285$) with the shortest interaction time occurring on day 4 of introduction ($\bar{x} = 2.75 \pm 1.064$) (Fig 27). There was no significant difference ($\chi^2 = 5.52$, d.f. = 6, $p > 0.05$) in interaction time between the seven days of introduction for the catnip enrichment. The longest interaction time was observed on day 6 of introduction ($\bar{x} = 4.36 \pm 2.11$) with the shortest interaction time occurring on day 3 of introduction ($\bar{x} = 1.49 \pm 0.77$) (Fig. 27). There was no significant difference ($\chi^2 = 12.90$, d.f. = 6, $p = 0.05$) in interaction time between the seven days of introduction for the cinnamon enrichment. The longest interaction time was observed on day 6 of introduction ($\bar{x} = 7.51 \pm 2.27$) with the shortest interaction time occurring on day 3 of introduction ($\bar{x} = 2.70 \pm 1.382$).

In terms of latency, the overall mean was $\bar{x} = 18.95 \pm 2.92$ for all enrichment types. There was no significant difference in the latency between the three types of enrichment ($\chi^2 = 3.161$, d.f. = 2, $p > 0.05$). The longest latency was for catnip ($\bar{x} = 24.8 \pm 4.85$), followed by the hay ball ($\bar{x} = 16.15 \pm 5.61$), and the shortest latency was for cinnamon ($\bar{x} = 15.9 \pm 4.01$).

Figure 23. A Sumatran tiger (*Panthera tigris sumatrae*), belonging to Fota Wildlife Park, Cork, Ireland, expressing directed enrichment behaviour to Hay ball (HB). Photo: Juliana Damasceno.

Figure 24. A cheetah (*Acinonyx jubatus jubatus*), belonging to Fota Wildlife Park, Cork, Ireland, expressing directed enrichment behaviour to Hay ball with catnip (CA). Photo: Juliana Damasceno.

Figure 25. A cheetah (*Acinonyx jubatus jubatus*), belonging to Fota Wildlife Park, Cork, Ireland, expressing directed enrichment behaviour to Hay ball with cinnamon (CI). Photo: Juliana Damasceno.

Figure 26. Mean $\pm$ SE of the total cats' interaction time (minutes) with the enrichment forms (Hay Ball (no scent), Catnip and Cinnamon), between January & May 2015.

Figure 27. Mean $\pm$ SE of the cats' interaction time (minutes) with the enrichment forms (Hay Ball (no scent), Catnip and Cinnamon) according to the day of the treatment application, between January & May 2015.

4.3.2. Pacing behaviour.

There was a significant difference ($\chi^2 = 10.35$, d.f. = 3, $p = 0.01$) in time spent pacing between baseline, hay ball, catnip and cinnamon treatments (Fig. 28). The highest level of pacing (minutes $\pm$ SE) was observed for the baseline phase ($\bar{x} = 17.06 \pm 2.75$), followed by hay ball ($\bar{x} = 11.36 \pm 3.77$), catnip ($\bar{x} = 7.35 \pm 4.11$) and cinnamon ($\bar{x} = 7.19 \pm 1.97$) (Fig. 28). Pairwise comparisons between baseline and each enrichment phase highlighted a significant difference between the baseline phase and cinnamon enrichment phase ($W = 56$, $p = 0.0104$) for pacing behaviour (Table 11). There was no significant difference in time spent pacing between each day of introduction for the hay ball ($\chi^2 = 3.17$, d.f. = 6, $p > 0.05$), catnip ($\chi^2 = 8.94$, d.f. = 6, $p > 0.05$) and cinnamon ($\chi^2 = 8.98$, d.f. = 6, $p > 0.05$) (Fig. 29).

Figure 28. Mean $\pm$ SE of time spent (minutes) by the cats (n=8) in pacing behaviour during the application of each type of enrichment (Hay Ball (no scent), Catnip and Cinnamon), during the seven days of each treatment compared with Baseline observations (without enrichment), between January & May 2015.

Figure 29. Mean $\pm$ SE of time spent (minutes) by the cats (n=8) in pacing behaviour during the application of the enrichment forms (Hay Ball (no scent), Catnip and Cinnamon) for baseline period and days during the treatment application.

Table 11 - Results of pairwise comparisons using the Wilcoxon-signed rank test comparing levels of pacing between baseline, hay ball, catnip and cinnamon phases of the study. Accepted alpha level after Bonferroni correction was taken to be 0.0166 (0.05/number of tests (3)).

Phase	Baseline	Hay ball	Catnip	Cinnamon
Baseline	-	W= 47, $p= 0.1304$	W= 51, $p= 0.0498$	W= 56, $p= 0.0104$

4.3.3. Locomotion, inactive and exploratory behaviours.

There was no significant difference in mean time spent in locomotion ($\chi^2 = 1.05$, d.f. = 3, $p > 0.05$), exploratory ($\chi^2 = 2.85$, d.f. = 3, $p > 0.05$), and inactive ($\chi^2 = 8.55$, d.f. = 3, $p > 0.01$) behaviours between baseline, hay ball, catnip and cinnamon treatments (Fig. 30).

Figure 30. Mean $\pm$ SE amount of time (minutes) cats ($n=8$) spent in locomotion, inactive and exploratory behaviour during the application of type of enrichment (Hay Ball (no scent), Catnip and Cinnamon), for the seven days of each treatment compared with Baseline observations (without enrichment), between January & May 2015.

4.4. Discussion.

The response of the cats to the three forms of enrichment highlight that they were effective options as short-term environmental enrichment for these species. As predicted by Tarou and Bashaw (2007), there was habituation to the different forms of enrichment within-sessions, as the cats spent on average around five minutes interacting with the enrichments from the beginning of each session. However, no evidence of habituation was observed between different days (sessions) for each form of enrichment. Ellis and Wells (2010) introduced cloths without odor, with catnip, lavender and prey scent to domestic cats housed in a shelter for three hours over five consecutive days and found, for all experimental conditions, a reduction in the animal's response after the first hour, suggesting that the cats had habituated to the stimuli.

Intrinsic forms of enrichment appear to have short-lived effects but can still function as effective forms of environmental enrichment (TAROU; BASHAW, 2007), as highlighted in this study. The enrichment utilised in this study was easily destroyed through the cat's interaction with it. This contributed to the observed length of interaction time. However, for the catnip and cinnamon enrichment, the scents remained on the hay, even after the hay ball was destroyed, therefore still providing a scent stimulus. Hall, Bradshaw and Robinson, (2002) presented a new object to domestic cats for four sessions on the same day, with two minutes for each session. They found highest frequencies of interaction with the objects for the first session when compared to the fourth, which means that the cats were more interested in the objects when they were novel to them.

In terms of enrichment-directed behaviours there was no significant difference between the cats level of interaction with each of the three intrinsic enrichments applied, meaning that the cats interacted with hay ball as a new object at the same level as when it was infused with catnip and cinnamon scent. Ellis and Wells (2010) also offered cloth with and without scent for domestic cats, and found high levels of interactions with cloths without scent. Despite there being no statistically significant difference in the levels of enrichment interaction, in the current study, there was a trend towards an increased level of interaction with cinnamon, also shown by the shortest latency for interaction. The effects of catnip on felid behaviour are well known (ELLIS, 2009; GROGNET, 1990; TUCKER; TUCKER, 1988). However, other scents, including peppermint, have been shown to have similar effects, such as an increase in activity levels. Similar to the current study, Powell (1995) observed that African lions (*Panthera leo*) spent more time interacting with peppermint compared to catnip.

In the present study, the cats engaged in enrichment-directed behaviours, which were related to novelty seeking, play and exploration. The introduction of new scents into the captive environment can also stimulate scent marking and other exploratory behaviours, while also encouraging greater activity levels (MELLEN; SHEPHERDSON, 1997). After the introduction of various perfumes and colognes to captive and free-ranging cheetahs, leopards and lions, Thomas et al. (2005) found that each species engaged more frequently in exploratory behaviours, such as cheek-rubbing and scent-marking. Wells and Egli (2004), found a reduction in levels of inactivity upon the introduction of nutmeg (*Myristica fragrans*), catnip and prey odor (quail, *Coturnix coturnix*) to the enclosures of captive black-footed cats (*Felis nigripes*). Upon introducing rat scent to 21 domestic cats in a shelter, Machado and Genaro (2014), observed an increase in exploration behaviours such as sniffing, rubbing and urine spraying. In contrast to these previous studies, levels of locomotion, inactive and exploratory behaviours did not show any significant change upon the introduction of the three forms of enrichment in the present study. Similar to our findings, Resende et al. (2011) did not find any significant change in levels of active or resting behaviours after applying catnip and cinnamon to oncilla (*Leopardus tigrinus*) enclosures. However, there was a trend for levels of inactivity to increase for each enrichment treatment when compared to baseline levels. Stereotypical behaviours in cheetahs are influenced by many factors such as enclosure size, feeding time, group membership and ability to view conspecifics in adjoining enclosures (QUIRKE; O'RIORDAN, ZUUR, 2012). A significant reduction in pacing behaviour was observed during the cinnamon treatment, when compared with baseline, with a decreasing trend in levels of pacing behaviour being observed for hay ball and catnip enrichment treatments. Similar to our study, Resende et al. (2011), applied cinnamon and catnip for eight captive oncilla cats (*Leopardus tigrinus*) and also found a decrease in pacing behaviour during their cinnamon treatment. Skibieli, Trevino and Naugher, (2007) identified consistent reductions in levels of pacing behaviour after the introduction of spices such as cinnamon, chilli powder and cumin to five species of felids. The combination of interaction with enrichment and a decrease in pacing behaviour could explain the trend for increased levels of inactivity observed. This can be considered a positive result as abnormal stereotypical behaviour is widely regarded as an indicator of reduced animal welfare.

Considering the lack of available time and money for enrichment practices in some institutions, it is important to ensure that enrichment strategies are practical to apply, inexpensive and effective (BRENT, 1992; ELLIS, 2009; SKIBIEL; TREVINO; NAUGHER, 2007). The three forms of enrichment utilised in the current study are cheap, easy to build and to apply. This, in combination with the observed results, highlighting the lack of a decreased

response to each form of enrichment, over the course of seven days and the observed decrease in pacing behaviour, suggests that these three forms of enrichment are effective and can be incorporated in the enrichment schedules of cheetahs and tigers in captivity, in order to improve their welfare.

4.5. Conclusions.

The main findings of the current research demonstrated that the intrinsic enrichment practices utilised (hay ball without scent, and with catnip and cinnamon scent) were observed to be effective short-term enrichments for cheetahs and Sumatran tigers in captivity in terms of interaction with those items. No habituation was observed across seven days in terms of interaction with enrichment. The animals interacted more with the cinnamon scent than other scents. This scent was also observed to reduce pacing behaviour when compared with baseline levels. Finally, the three enrichments applied are easy to administer, inexpensive, practical and interactive options for enrichment for felids in captivity.

5. General Discussion and Conclusion.

Photo: Juliana Damasceno

The investigations in the current study were employed to address issues surrounding the methodological application and practical/effective environmental enrichment strategies applied to captive wild cats. Through a multi-institutional and transnational study, this research analysed how different types of enrichment and enclosure size could influence the behaviour of wild cats, in terms of stimulating the expression of natural behaviours and by reducing the expression of stereotypies. In general, the main findings identified were: 1) forage challenging enrichments, such as puzzle-feeders, classified as extrinsic practices promoted longer-lasting effects in ocelots than intrinsic enrichments, in terms of duration of the enrichment-directed behaviours; 2) intrinsic enrichments, such as new objects or scents introduced into the environment promoted enrichment-directed behaviours, however they only lasted for a short-term duration for the three studied species, ocelots, tigers and cheetahs; 3) consecutive and intermittent schedules of exposure did not result in a habituated response between sessions (over the experimental period), in either schedule for the ocelots or for the consecutive schedule for the tigers and cheetahs; 4) catnip and cinnamon demonstrated a distinct influence in terms of pacing reduction, with cinnamon shown to be more effective, but both introduced scents were effective for tigers and cheetahs and catnip for ocelots; 5) enclosure size was seen to cause a high impact in pacing expression in ocelots; and finally 6) puzzle-feeders were found to be more effective than catnip in decreasing pacing in ocelots.

In summary, the findings elucidated that environmental enrichment practices could promote benefits for the welfare of animals in distinct ways. Some techniques could be more effective than others, depending on what objectives were trying to be achieved. For example, if the goal is to reduce pacing in wild cats and/or promote longer-lasting interactions, the research showed that the better option is to apply an extrinsic enrichment, such as a puzzle-feeder. However, if the goal is an enrichment which is easy to administer, low cost and short-term, intrinsic enrichments such as hay balls, catnip and cinnamon are recommended. In addition, the results of the research highlighted the high impact of enclosure features on the behaviour of captive animals which in the wild are wide-ranging, corroborating with other studies on wild cats (BASHAW et al., 2007; BRETON; BARROT, 2014; MOREIRA et al, 2007).

The captive environment for wild cats should provide size, complexity, activity and novelty enough to meet their requirements. However, further studies, similar to the current presented research, are needed to identify the ideal conditions. Studies focused on issues associated with enrichment and welfare science with respect to evaluating practices and effective methodological designs, which match the aspired goals, are required to improve and maintain the physical and psychological welfare of the captive animals.

6. Appendix A.

UNIVERSIDADE DE SÃO PAULO
Campus de Ribeirão Preto
Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que o trabalho (Protocolo 13.1.1234.53.0), intitulado "Habituação a diferentes condições e tipos de enriquecimento ambientais para jaguatiricas (*Leopardus pardalis*) em cativeiro", de autoria de **Juliana Damasceno** e **Gelson Genaro**, por estar de acordo com os **Princípios Éticos na Experimentação Animal** adotado pela **Comissão de Ética no Uso de Animais (CEUA)** do *Campus* de Ribeirão Preto – USP foi aprovado em reunião da CEUA de 04/11 /2013.

Colaboradores: Cristina Harumi Adania
Luciano do Valle Sabóia

This is to certify that the work (Protocol number 13.1.1234.53.0), entitled: "Habituação a diferentes condições e tipos de enriquecimento ambientais para jaguatiricas (*Leopardus pardalis*) em cativeiro", by **Juliana Damasceno and Gelson Genaro** is in accordance with the Ethic Principles in Animal Experimentation adopted by Ethic Commission for the Use of Animals (CEUA) of the Campus of Ribeirão Preto – USP, and was approved in an ordinary meeting of CEUA, November, 4 2013.

Ribeirão Preto, 8 de novembro de 2013.

Presidente da CEUA
Profa. Dra. Cláudia Maria Padovan

Secretária da CEUA
Vanessa Bombonato Garcia

7. Appendix B.

To. Juliana Damasceno

Bióloga, Mestre e Doutoranda em Ciências
Programa de Pós-Graduação em Psicobiologia (USP)
University College Cork, Ireland.

15th Feb 2016

RE: Juliana Damasceno's research project on the environmental enrichment in Cheetahs and Sumatran tigers at Fota Wildlife Park, Carrigtwohill, Co. Cork Ireland.

To whom it may concern

The above research project which was carried out at Fota Wild Park focused on understanding how the captive environment may influence Cheetah and Tigers and investigated whether aspects of animal husbandry could be improved through environmental enrichment tools.

Fota has ethical considerations that must be taken into account when undertaking research on animals. The above project was assessed in terms of its ethics as well as other factors such as quality of science and logistics to ensure only the most worthwhile and non-intrusive studies are undertaken. This research project was fully compliant with our research ethical policy.

Yours, sincerely

Sean McKeown
Director

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