Strategies of Environmental Enrichment for ocelot *Leopardus pardalis* (Carnivora, Felidae) at Parque Estadual Dois Irmãos: a study case in Brazil

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Abstract. Environmental enrichment is used by zoos to stimulate the interaction of captive animals with the environment, to elevate welfare and diversifying behavioral opportunities. This case study investigated the effect of environmental enrichment on the behavioral repertoire of an ocelot adult male captive at Parque Estadual Dois Irmãos, Brazil. The experiment was carried out from August 2011 to June 2012 and it was divided into three phases called: Pre-enrichment, Enrichment, and Post-Enrichment. The sampling methods used were Focal-animal and *ad libitum*. The observations suggested an increase of the locomotion behavior and a significant decrease in stereotypic behaviors of the animal. The techniques of enrichment applied in this study were effective for the reduction of stereotypies and increase the behavioral repertoire of the species in captivity.

Key words: animal welfare, behavioural repertoire, felids, stereotypies.

Resumo. Estratégias de enriquecimento ambiental para jaguatirica *Leopardus pardalis* (Carnivora, Felidae) no Parque Estadual Dois Irmãos: um estudo de caso no Brasil. O enriquecimento ambiental é utilizado por zoológicos para estimular a interação dos animais cativos com o ambiente, para elevar o bem-estar e diversificar as oportunidades comportamentais. Este estudo de caso investigou o efeito do enriquecimento ambiental no repertório comportamental do macho de jaguatirica adulto cativo no Parque Estadual Dois Irmãos, Brasil. O experimento foi realizado de agosto de 2011 a junho de 2012 e foi dividido em três fases denominadas: Pré-enriquecimento, Enriquecimento, e Pós-enriquecimento. Os métodos de análise utilizados foram Animal-focal e *ad libitum*. As observações sugerem um aumento do comportamento de locomoção e uma significante redução nas estereotipias do animal. As técnicas de enriquecimento aplicadas nesse estudo foram efetivas para a redução das estereotipias e aumento do repertório comportamental da espécie no cativeiro.

Palavras-chave: bem-estar animal, repertório comportamental, felídeos, estereotipias.

Introduction

Environmental enrichment is largely applied to improve the quality of the captive animal life, to provide incentives for the psychological, physiological and behavioral welfare (Newberry, 1995; Shepherdson, 1998; Swaisgood & Shepherdson, 2005). The promotion of natural be

havior, which is exposed by their conspecifics in the wild and the reduction of stereotypies may be obtained through the administration of simple and low-cost enrichment techniques (Skibiel *et al*., 2007; Castillo-Guevara *et al*., 2012).

Many studies about environmental enrichment procedures for felids have been developed around the world (Lindburg, 1988; Mellen, 1993; Powell, 1997; Wooster, 1997; Weller & Bennett, 2001; Jenny & Schmid, 2002; Mcphee, 2002; Bashaw *et al*., 2003; Wells & Egli, 2004; Rochlitz, 2007; Skibiel *et al*., 2007; Quirke & Riordan, 2011; Resende *et al*. 2011; Normando *et al*., 2014; Manfrim *et al*., 2017). Some of these were carried out on the *Leopardus pardalis* Linnaeus, 1758 (Mellen, 1993; Powell, 1997; Skibiel *et al*., 2007; Normando *et al*., 2014; Manfrim *et al*., 2017). This species is a medium sized felid (11 kg), with nocturnal-crepuscular activity, (Oliveira & Cassaro, 2005; Di Bitetti *et al.,* 2006) and it is widely distributed from United States towards Central and South America, except Chile. *Leopardus pardalis* is listed by IUCN in the least concern category (Paviolo *et al.,* 2015). Currently, the dominant treats for the species are habitat loss, retaliatory killing and illegal trade of ocelots as pets and pelts (Sunquist & Sunquist, 2002).

For felids, a good enrichment program should enhance their sensory environment (Powell, 1997). Some records show the importance of enrichment sensory and food (Wooster, 1997; Wells & Egli, 2004; Skibiel *et al*., 2007; Resende *et al*., 2009, 2011). Studies on enrichment and well-being for captive felids provide information about the biology of these animals and reveal how the improvements in maintenance and welfare management may assist in the conservation plan (Law *et al*., 1997). Thus, the success of our protocol of enrichment can be quantified by the decrease of abnormal behaviors and the increase of exploratory behaviors (olfactory marking and exploration). Herein, our inquiry focuses on the reduction of stereotypies by promoting the welfare and diversifying behavioral opportunities for the ocelot.

Materials And Methods

The study was carried out with the unique ocelot resident at the Parque Estadual Dois Irmãos (PEDI) (8º 9’ 17’’ S e 34º 52’ 05” W) Recife, PE, Brazil – a Conservation Unit of 1157.72 hectares, whose 14 ha corresponds to the zoo. The ocelot’s enclosure has an area of 50 m^2 and a height of 2.6 m. It is made of stone masonry walls and its front part is a wire mesh. The enclosure’s ground was covered with grasses, some plants, small trees, and trunks spread randomly throughout the enclosure.

The animal studied was an adult male ocelot, *Leopardus pardalis*, resident of PEDI (tattoo PEDI 020). He was incorporated into the PEDI zoo on January 14, 2008, from Centro de Triagem de Animais Silvestres/Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis of the Paraíba State, Brazil (Screening Center For Wild Animals/Brazilian Environmental Institute of Renewable Resources). We elaborated an ethogram after eight hours of observation for the

definitions of behavioral categories and identification of behavior using the *ad libitum* method (Altmann, 1974). The study was conducted from August 2011 to June 2012 and it was divided into three phases: Pre-enrichment (Pre-EE, or baseline) summing fifty-one hours of observation, Enrichment (E) comprising twenty-one hours and Post-enrichment (Post-EE) totaling sixty hours of observation.

The behaviors analyzed were based on the ethogram, composed of nine categories and eleven behavioral acts (Table 1), as described previously in the literature (Weller & Bennet, 2001; Wells & Egli, 2004; Resende *et al*., 2009).

The pre-enrichment phase was composed of seventeen sessions (3 hours per session), overall 51 hours of behavioral observations obtained with the Focal-animal sampling (Altmann, 1974). We collected the data in three periods: morning (from 09:00 am to 12:00 pm); afternoon (from 2:00 pm to 5:00 pm), and evening (from 5:00 pm to 8:00 pm). Each Focal-animal lasted five minutes, with 2 minutes of sampling at 3 minutes intervals.

Before the enrichment phase, a questionnaire was given to the zookeeper on his perception regarding the behavior of the evaluated animal. This questionnaire was based on a previous model used for the nebulous panther (*Neofelis nebulosa* Griffiths, 1821) by Wielebnowiski *et al*. (2002). The information compiled was used to choose enrichment techniques according to the animal's needs and to identify its stereotypies.

In the enrichment phase we introduced three enrichment types: food, physical and sensory. The artifacts were materials of easy access and management (Table 2). In total 21 sessions were completed (two or three sessions per week) from December 2011 to May 2012. Each session lasted one hour or immediately after the animal losing interest in the enrichment.

The post-enrichment phase consisted of twenty sessions (3 hours per session) from May 2012 to June 2012, as in Pre-EE phase we used the Focal-animal sampling (Altmann, 1974), in order to analyze behavioral changes after the introduction of enriching stimuli. Our observations occurred during two periods: morning, from 09:00 am to 12:00 pm; and afternoon, from 2:00 pm to 5:00 pm. As the Parque Estadual Dois Irmãos (Dois Irmãos State Park) administration did not allow observations in the evening during this phase. We compared the animal's stereotypic and natural behaviors by taking the sum of pacing and figure eight pacing records versus the sum of all other behavior records between the baseline and Post-EE phases, except the physiological and agonistic categories.

Statistical analyses were performed using the R Program, with a significance level of 0.05. The normality of the data was evaluated with the Shapiro-Wilk test and, according to the structuring of the data, the parametric Student's t-test was used for the normal distribution, and the nonparametric Mann-Whitney test was used

Table 1. Ethogram of the ocelot (*Leopardus pardalis*) behaviors at the Parque Estadual Dois Irmãos, Brazil.

Behavior Category	Description
Inactive	
Resting	Animal lies down, often with eyes closed.
Stand Alert	Animal is standing in whatever body posture, eyes open and responsive to stimuli. In general, the head moves toward objects that claim its attention.
Locomotory	Animal is climbing, walking, running or skipping over.
Self-grooming	Animal licks itself and/or scrubbing the previous limbs licked on the body (mainly head).
Olfactory Exploration	Snout of the animal is moving the nares above the surface of food/object or it is sniff out the surroundings.
Olfactory Marking	
Claws	Animal is marking the surfaces using its forelimbs.
Spray	Animal ejects jets of urine on surfaces (walls, plants, etc).
Hindlimbs	Animal is standing up and it moves its hind limbs alternatively against the ground.
Cheeks	Animal is scrubbing cheeks on surfaces.
Hind limbs/ Cheeks	Animal is scrubbing cheeks on surfaces associated with the hind limbs scratching the ground.
Body	Animal is scrubbing body on surfaces.
Abnormal Behavior	
Pacing	Animal repeats, at least, three times, a locomotor pattern in a straight course, without apparent function.
Pacing figure-of-eight	Animal is showing a locomotor pattern in the 8-shaped without apparent function.
Not Visible	Animal stays inside the trunk or behind the visual barrier, out of sight of the observer.
Physiologic	Animal urinating, drinking, defecating, yawning, eating, lounging around, spitting, scratching and chewing grass.
Agonism	
Growling	Sounds emitted by the animal.

Table 2. Classification and description of the enrichment techniques for the ocelot (*Leopardus pardalis*) at the Parque Estadual Dois Irmãos, Brazil.

Type of Enrichment	Description
Food	Beef tied by a string hanging on the enclosure's ceiling
Food	A hollow and drilled pumpkin filled with slices of chicken meat
Food	Live mice inside hay's ball plus trail of blood on the substrate
Food	Live fishes swimming in the pool
Food	Cardboard box stuffed with chicken pieces
Food	Live quail
Food	Liana ball with beef slices inside
Food	Blood trail plus live quail
Food	Pieces of meat were hidden in strategic locations of the enclosure
Sensory	Panache hanging on the enclosure's ceiling with blood
Sensory	Jaguar feces (*Panthera onca*) in two points of the enclosure
Sensory	Bromeliads and sweet basil plus change of the loft trunks
Sensory	Blood Popsicle
Sensory	Mint leaves spread on the enclosure substrates
Habitat	Visual barrier

for the data that not exhibit a normal distribution. These tests were employed to compare variables between the baseline and Post-EE phases.

Results

In general our observations showed a large reduction of stereotypes after implementation of the environmental enrichment. However, exploratory behaviors such as olfactory marking also decreased in the Post-EE phase.

Behaviors like pacing and growling counted 13% of the observation efforts in the Pre-EE (n = 17). In contrast, these behaviors showed 2% of the observation efforts in the Post-EE phase (n = 20). The enrichment showed statistical differences between Pre-EE and Post-EE phase (df = 36, Mann-Whitney, p = 0.01 and Mann-Whitney, p = 0.002, respectively; Figures 1a and 1b). Moreover, the association between these behaviors reduced during Post-EE phase. Most of the time, the animal emitted growling agonist behavior simultaneously while performed pacing and observed visitors.

The stereotype behavior figure eight pacing decreased from 13% in the Pre-EE phase (n = 17) to 0.15% in the Post-EE (n = 20) but the difference between phases was not statistically significant (df = 36, Mann-Whitney, p= 0.208).

Self-grooming behavior had a decrease

of approximately 17% between Pre-EE (n = 17) and Post-EE (n = 20) and showed a statistical difference between phases (df = 36, Mann-Whitney, p = 0.009; Figure 1c). The stand alert behavior changed from 12% in the Pre-EE (n = 17) to 14% in the Post-EE (n = 20) and showed statistical difference between phases (df = 36, Mann-Whitney, p = 0.009; Figure 1d).

The specimen increased locomotor behavior frequency from 2.75% at baseline (n = 17) to 10% in the post enrichment phase (n = 20), but did not show statistical differences between phases (df = 36, t-test, p = 0.099). The percentage of resting behavior during the Post-EE (33% of the observations) (n = 20) was twice higher than observations carried out in the Pre-EE conditions (13%) (n = 17) and statistical differences were detected between phases (df = 36, t-test, p = 0.035; Figure 1e).

The ocelot spent nearly 4% and 3% of the registration time on olfactory and on environment exploration during Pre-EE (n = 17) and Post-EE (n = 20) phases, respectively, and it were observed statistical differences (df = 36, t-test, p = 0.008 and t-test, p = 0.005, respectively; Figure 1f).

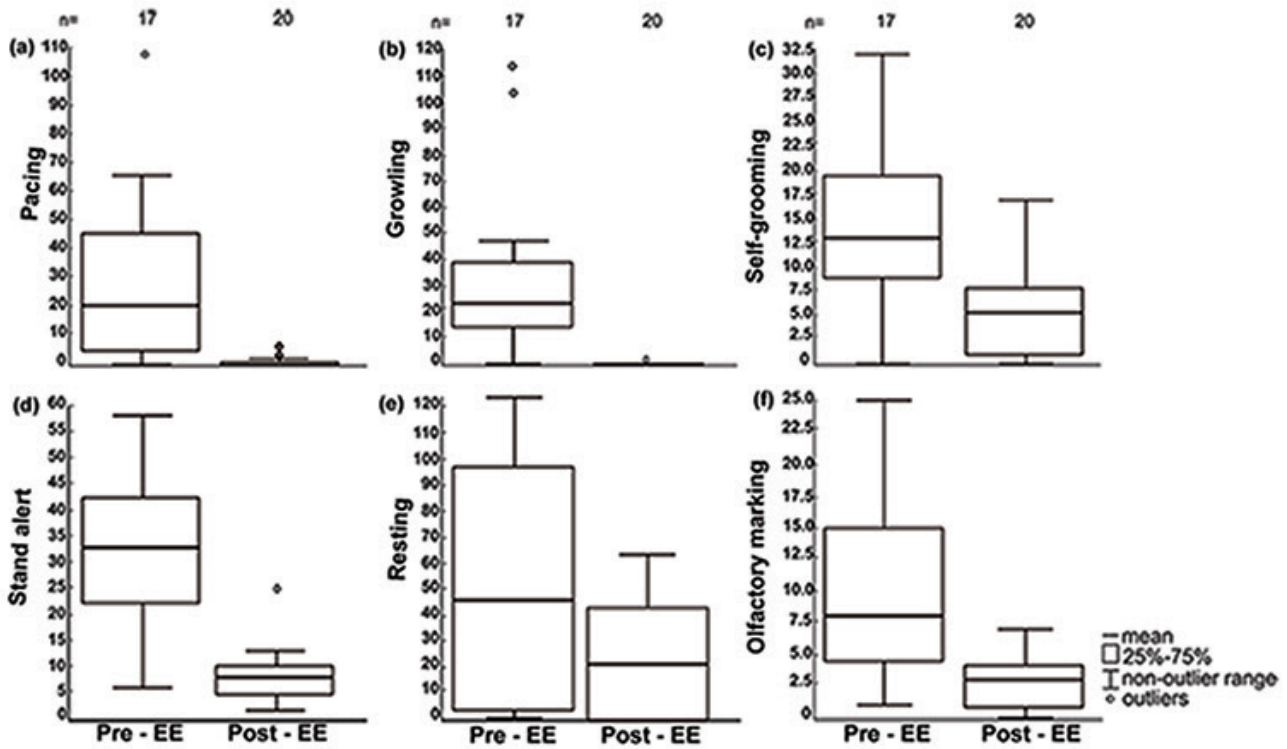

Figure 1. Variation of the frequency of behaviors of an ocelot *Leopardus pardalis* during the pre- and post-environmental enrichment phases, between 2011 and 2012 at the Parque Estadual Dois Irmãos, Brazil: a, 'pacing'; b, 'growling'; c, 'self-grooming'; d, 'stand alert'; e, 'resting'; f, 'olfactory marking'.

The ocelot spent 6% of the observation efforts not visible in the Pre-EE (n = 17), and 23% in the Post-EE (n = 20) but no differences were found between phases (df = 36, Mann-Whitney, p = 0.716). The not visible category (Table 1) had no significant difference identified between the phases. However, there was a change in hiding place. At baseline, the animal used a hollow trunk located at the upper side of the enclosure to hide. In contrast, during the Post-EE phase, the ocelot used the visual barrier, implemented during the enrichment phase, as its main hiding spot. This behavior was observed mainly in the morning when it was less active.

Discussion

Overall, the present study confirmed that the application of our enrichment protocol influenced the pattern of the animal's activities and increased its welfare. This enrichment also worked for the reduction of stereotypic behaviors, since the pacing was the most frequently observed behavior in the Pre-EE phase, associated with feeding time. Our data corroborated those found by Mason *et al*., (2007) and Castillo-Guevara *et al*. (2012) Which argued that zoos are important research centers, where you can test hypotheses on how to control or to eliminate the abnormal behavior.

Weller & Bennett (2001) obtained a similar result in their study with captive ocelots in two zoos and a center of wildlife of Texas, USA. The diversity in food presentation of our protocol was important to reduce mainly pacing, whereas this stereotype is a consequence of the frustrated appetitive foraging behavior (Weller & Bennett, 2001; Jenny & Schmid, 2002). We recorded significant reduction of pacing and our research confirmed the adequacy of the enrichment procedures applied. In addition, variation in feeding times of the zoo and the distribution of food in different places may contribute to the pacing reduction (Quirke & Riordan, 2011). According to Clubb & Mason (2003), this stereotype is the most abnormal behavior observed within the carnivores, and our enrichment protocol reduced it.

The decrease of self-grooming in the Post-EE phase was also a positive outcome since the high frequency of this behavior is often associated with idleness in cats. This habit can cause skin lesions due to the long time the cats spend licking themselves (Del-Claro, 2004).

In the present study, there was a reduction in exploratory behaviors, in contrast with previous studies that have shown the environmental enrichment protocol would increase the frequency of these behaviors (Bashaw *et al*., 2003; Skibiel *et al*., 2007; Castillo-Guevara *et al*., 2012). Our enrichment protocol (Table 2) did not stimulate exploratory behaviors in this male ocelot. Although, there was a reduction in inactive behaviors and stereotypies while active behaviors increased.

The sensory enrichments, such as the blood popsicle caused an immediate response in the animal. As previously reported by Resende *et*

al. (2011) and Skibiel *et al*. (2007), our sensory enrichment aided to reducing the pacing in this species. A similar enrichment item, the frozen liver paste, was applied for three ocelots Sabiá's Park Zoo in Southern Brazil. This enrichment was considered the most relevant for the wild born male, which lived alone on the enclosure (Nahas *et al*., 2010). These results indicate that frozen blood and viscera are good enrichment items to ocelots.

During our enrichment session with a pumpkin with chicken meat inside, the animal exhibited a short interaction. In contrast, Wooster (1997) used the same enrichment for ocelots at Woodland Park Zoo in Seattle, USA and obtained a high interaction. Given that, we suggested that the smell of the pumpkin overpowered the smell of the meat. As a consequence, the animal lost interest due to vegetable smell. The same situation occurred during the enrichment "mice inside a hay ball" in which the individual demonstrated interest only while the hay was soaked with blood. The ocelot ignored the enrichment after it licked the blood that covered the ball and the animal did not eat the mice. We supposed that the hay smell was stronger than the mice smell.

The enrichment involving vegetables or grasses did not capture the ocelot´s interest. Durr & Smith (1997) concluded that the weak or intense stimuli can be uninteresting or reduce the exploratory behavior, respectively. In fact, these kinds of enrichments promoted neophobia in the animal and the increase of the stereotypic behavior figure eight pacing. The studies of Machado & Genaro (2010) showed that domestic cats living in restricted environments were very responsive to new situations and needed a long time to learn about a new object or environment. The most intense responses were recorded in individuals who lived in poorer environments, due to the fact they had nothing to direct their motivation to explore. However, these animals do not exhibit a rich behavioral diversity.

In the current study, the fish pool was one of the enrichments that stimulated more hunting, capture, and olfactory marking. These results corresponded with those observed by Shepherdson *et al*. (1993) in which the supply of live fish in a small pool increased fishing and decreased resting of the Fishing cat (*Prionailurus viverrinus* Bennett, 1833). Skibiel *et al*. (2007) compared the behavior of six species of captive felids to some enrichment procedures and they observed a pacing decrease for jaguars and ocelots when given "frozen fish". In contrast with our results, the experiments conducted by Bashaw *et al*. (2003) showed no trends toward pacing reduction after the fish enrichment.

The implementation of a visual barrier inside the ocelot's enclosure expanded the number of hiding places for the animal. This enrichment becomes a permanent part of the enclosure furniture. The efficiency of the barriers was measured with domestic cats (*Felis cattus* Linnaeus, 1758) in which these refuges were used to make some cats hide from others and to provide safe environments for cats (Rochlitz, 2007; Oliveira *et al*., 2015). This kind of enrichment reduced the

physiological stress as shown by Carlstead *et al*. (1993b). Authors recorded a low cortisol level in laboratory living domestic cats' urine, when there were hiding places in the enclosures. Herein, the individual used visual barrier as one of the main hiding places. This enrichment also helps in reducing the pacing. This behavior increased in leopard cats (*Felis bengalensis* Kerr, 1792) when they were kept in enclosures with no hiding places (Carlstead *et al*., 1993a).

Conclusion

This case study grants a comprehensive analysis of the captive ocelot during the Pre and Post-enrichment phases. Many zoos house small numbers of felids individuals, giving their solitary habits and broad home range. Therefore, to generalize our conclusions, more case studies of enrichment for captive ocelots should be encouraged.

Enrichment protocols tested were important for a temporary reduction of stereotypic behaviors on the animal. However, the enrichment should be applied periodically and whenever necessary modified to provide novelty for the animals. We concluded that our enrichment protocols aided to reducing self-grooming and pacing in this species. Moreover, sensory enrichments caused instantaneous reaction in the individual. Feeding enrichments like fish pool stimulated predatory behaviors, given that we emphasize the importance of live preys during felids enrichment programs. On the contrary enrichments involving vegetables or grasses did not promote interaction with the ocelot.

The pacing was the main abnormal behavior observed and the spatial restriction of the enclosure was the primary factor in the occurrence of this stereotypic behavior since wild cats need a wide area as in their natural habitats. We suggest that enclosures should be built to make visitors less visible to the animals, whereas the visual barriers were widely used by the ocelot not only to avoid visitors, but also during its resting periods.

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