ENVIRONMENTAL ENRICHMENT FOR CAPTIVE CAPUCHIN MONKEYS (*Sapajus* spp.) USING NATURAL MATERIAL

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Abstract. Captive environments are relatively less complex as compared to wild, and consequently contain less stimuli for any animal within them, which may be stressful. One approach to mitigate stress in captive animals is the application of environmental enrichment techniques. Capuchin monkeys are among the most common primates in captivity, therefore, evaluating the efficacy of environmental enrichment techniques for these species is important. In this study, we evaluated the effects of natural material as environmental enrichment for *Sapajus* spp. We performed a comparative study of the behavior of seven individuals when their enclosure was non-enriched and enriched with bamboo, leaf-litter and bromeliads. Our results suggested that activities such as body care and affiliative interactions were less frequent when environmentally enriching objects were placed in their enclosure. They also spent more time in object manipulation, and monitoring behavior significantly reduced during these periods. Of the three materials used, we consider bamboo the most efficient enrichment material, since it remained in the enclosure longer and so attracted capuchin attention for a more extended period, and significantly modified their behavior.

Key-words: welfare, captivity, *Sapajus apella, Sapajus libidinosus, Sapajus nigritus.*

Resumo. Enriquecimento ambiental para macacos-prego (*Sapajus spp.)* usando materiais naturais. Ambientes de cativeiro podem ser estressantes aos animais uma vez que são relativamente menos complexos que ambientes naturais e contém menos estímulos. Uma das abordagens para reduzir o estresse de animais em cativeiro é a aplicação de técnicas de enriquecimento ambiental. Macacos-prego estão entre os primatas mais comumente encontrados em cativeiro. Portanto, é importante avaliar a efetividade de técnicas de enriquecimento ambiental para essas espécies. Neste estudo tivemos como objetivo estimar a eficiência de materiais naturais como enriquecimento ambiental para espécies do gênero *Sapajus*. Para tanto, comparamos o comportamento de sete indivíduos quando submetidos à adição de bambu, folhiço e bromélias em seus recintos. Diante dos enriquecimentos ambientais, os macacos-prego reduziram a frequência de atividades de cuidado corporal e interações afiliativas. Eles também aumentaram a frequência de manipulação de objetos e reduziram a frequência de monitoramento do ambiente durante os períodos com enriquecimento. Concluímos que o bambu foi a ferramenta mais eficaz de enriquecimento, pois permaneceu mais tempo intacto no recinto e com isso atraiu a atenção dos macacos-prego por mais tempo, modificando de forma significativa seu comportamento.

Palavras-chave: bem-estar, cativeiro, *Sapajus apella, Sapajus libidinosus, Sapajus nigritus.*

Introduction

Animals may be kept in captivity for a variety of reasons, which includes medical researches (Mittermeier *et al.*, 1994; Bailey, 2005), entertainment, education (Hyson, 2004), rescue (Tribe & Brown, 2000), and conservation (Kleiman *et al*., 1986). The maintenance of wild animals in captive environments as "sanctuaries", breeding colonies and zoos is an important tool for both species conservation and human health (Bailey, 2005). However, it can compromise the welfare of the captive individuals, since the enclosures usually differ markedly from native habitat in structure and composition. Captive environments are always smaller than the areas animals would naturally occupy, and consequently contain fewer stimuli, and this may be stressful for animals (Ross *et al.,* 2009). Many captive environments also contain other sources of stress such as noise or husbandry routines (e.g. handling, nursing, breeding) (Morgan & Tromborg, 2007; Dickens & Benley, 2014; Quadros *et al.*, 2014). Stress induced by these factors can compel the species to modify their behavior and activity budgets to adapt into the environment (Young, 2003; De Azevedo, 2007; Novak *et al.,* 2017; De Almeida *et al.*, 2018). Among behavioral changes, stereotypies are key for eavesdropping animal welfare. Stereotypies are expressed as repetitive motor behaviors without an apparent purpose and are commonly considered to be indicators of stress (Mason & Rushen, 2008).

Institutions that keep captive animals are responsible for their well-being (Bloomsmith *et al.*, 1991; Waza, 2005). As an effort to reduce negative impacts, a variety of forms of environmental enrichment have been attempted (Young, 2003). Environmental enrichment improves the environments of captive animals, with the aim of promote their mental and physical well-being. Such initiatives generally fall into such categories as "food-based", "structural", "sensory", and "cognitive" enrichment (Newberry, 1995; Clark, 2017). These activities aim to increase the possibilities that captive animals express their motor, exploratory and cognitive abilities in ways that most closely resemble their natural forms (Young, 2003). Such enrichment has great potential to promote physiological and mental well-being in captive animals as it can substantially reduce stress levels (Mellen & Macphee, 2001; De Almeida *et al.*, 2018). Even though the application of environmental enrichment is now widely used, systematic testing of its effectiveness is rarely conducted (Mellen & Macphee, 2001).

Capuchin monkeys, primates from the genus *Cebus* and *Sapajus*, are commonly kept in captivity (Lynch-Alfaro *et al.*, 2012, 2014). They are known by their high behavioral flexibility, intelligence, and memory (Fragaszy *et al.*, 2004). They are very active and spend half of their days foraging and feeding (Ross, 1988; Fragaszy *et al.*, 2004). Their social groups can be as large as 35 individuals (Ludwig, 2005), so they usually use large areas that may reach 800 ha (Spironello, 2001). Within this, they may travel as much as ~3 km daily (Edwards *et al*., 2017). Therefore, when such primates are kept in captivity, it is highly desirable to provide activities that simulate those they commonly perform in the wild (Fragaszy, 2005). One method is to introduce into the captive enclosure natural materials with which species would normally have contact in the wild. However, any such materials must be nontoxic, innocuous, and preferably low-cost (Bloomsmith *et al.*, 1991; Boinski *et al.*, 1999; Costa *et al.*, 2018).

Bearing this in mind, the objective of the current study was to estimate the efficiency of three kinds of environmental enrichment 1) leaf-litter; 2) bromeliads; 3) bamboo, with the addition of earthworms in all treatments. We choose this material because capuchins are known to eat bromeliads and bamboo in the wild (Fragaszy *et al.,* 2004), and descend to the ground to forage for animal resources (Torralvo *et al.*, 2017). We tested if the provided material has induced any behavioral changes in capuchins by observing the animals before and after enrichment introduction. We predicted that during environmental enrichment the individuals would: 1) Increase foraging and feeding activities; 2) Reduce resting time; 3) Decrease in agonistic and increase in affiliative interactions; 4) Increase the behaviors "Monitoring" and "Object manipulation"; and 5) Reduce stereotyped behavior. By testing in this manner, we also aimed to generate basal guidelines for the choice of environmental enrichments suitable for captive capuchin monkeys that are natural, generate low waste of products and are cost-effective.

Material And Methods

Study site

The study was carried out in the São Bráz Conservationist Sanctuary (IBAMA: 2/43/96/0001-0) located in the Santa Maria municipality of Rio Grande do Sul state, Brazil (29°41'50.89"S; 53°55'13.57"W). The sanctuary is approximately 5 ha in area and consists of 75 enclosures which inhabit some 400 exotic and native animals. Visitation is prohibited to the general public but schools and college groups are allowed.

Subjects and housing

We studied seven adult individuals: one male and one female *Sapajus libidinosus* Spix 1823, two female *S. apella* Linnaeus 1758, two males and one female *S. nigritus* Goldfuss 1809. They were housed in the same enclosure (4.30 m x 6.20 x 3.5 m). Animals were fed every morning (09:00-10:00) with fruits and eggs, with drinking water provided *ad libitum.* The enclosure, placed in an open field, contained trunks, ropes, a shelter, and small bushes around it with which the capuchins could have physical contact.

Data collection and analysis

We recorded capuchins behavior for twenty five consecutive days, for a total of ~206 hours of observation between December of 2010 and February of 2011. Of these, some 55.5 hours constituted the control phase, before any environmental enrichment had been placed inside the enclosure. The remaining period was spent observing the capuchins' behavior following environmental enrichment. We used as material (1) leaf-litter, (2) bromeliads (Bromeliaceae), (3) bamboo (Poaceae) pieces with holes. Within each enrichment, some 25 earthworms (*Eisenia andrei*) were hidden daily. All of the materials were natural and were available in the sanctuary gardens, except the bromeliads that were purchased commercially (price ~US$ 3.63/unit), which resembled with those found in the animals' native forest habitat. Enrichment materials were inserted in the enclosure early in the morning before the beginning of behavioral observations, and were replaced daily, if necessary. Remnants were removed at the end of sampling, and replacement material subsequently introduced.

Data collection occurred daily between 8:30-12:30 and 14:00-18:00 hours. During the recordings, the observer remained quiet besides the enclosure and avoided eye contact with the animals. We recorded the behavior using instantaneous scan sampling every 5 minutes (4 minutes of sampling, with 1 minute interval) (Altmann, 1974), using the following categories: 1) Foraging; 2) Feeding; 3) Resting; 4) Body care; 5) Affiliative interactions; 6) Agonistic interactions; 7) Alert; 8) Object manipulation; 9) Monitoring; 10) Stereotyped behavior; 11) Out of sight ; 12) Others (Adapted from Santos & Reis, 2009) (Tab.

Table 1. Ethogram of capuchin monkeys used for data collection (adapted from Santos & Reis, 2009).

Behavior category	Description
Feeding	Individuals introduce food items into their mouth and ingested them.
Foraging	Individuals moved on the trunks, branches, leaves, structures in the enclosure and ground in an apparent search for food sources (such as insects).
Locomotion	Individuals moved either on the ground or on aerial structure (trunks and fences) by jumping, running, climbing up or down.
Resting	Individuals remained still seated, lying or sleeping.
Body care	An activity where animals clean their own pelage (autogrooming) or other body part (nose, ears, anus).
Affiliative interactions	Social interaction directed at other individuals with "friendly" intent, such as allogrooming, playing, copulating.
Agonistic interactions	Social interaction directed to other individuals with an aggressive approach such as piloerection, teeth threat display, chasing or attacking.
Alert	Individuals interrupted its activity and remains motionless with an erect posture and eyes fixed at a point for a few seconds.
Object manipulation	Individuals hold or put in the mouth objects without apparent intent of feeding.
Monitoring	Individuals stare at a given item in the environment for a few seconds.

Table 1. Continuation.

Stereotyped behavior	Repetitive movement without apparent function (e.g. moving in circles, head and body twirl).
Out of sight	Animals were in the shelter and the observer could not record the behavior during the scan.
Others	Activities that did not fit any of the other categories. This included such activities as defecating and scratching.

1). In order to compare capuchin behavior with different kinds of environmental enrichment and control period, we calculated the frequency of each behavioral category. Each category was analyzed separately. The duration of observation periods for each environmental enrichment category were not identical (control: 55.5 hours; leaf-litter: 51.4 hours; bromeliads: 51.3 hours; and bamboo: 48.3 hours), so we parameterized the data by dividing the frequency of each behavior by the total number of scans for each period of observation (morning or afternoon). As our data was non-parametric, we used a Kruskal-Wallis to compare all treatments and Dunn *post hoc* test compare pairs of categories. All analysis were run on the R 3.4.0 program (R Core Team, 2016). The study was non-invasive and complied with appropriate Brazilian laws.

Results

Overall, capuchin monkeys manipulated all of the inserted environmental enrichment, thus the treatments were efficient in stimulating the animals. The frequency of most behavioral categories did not differ between pre- and post-enrichment, and this was true for all enrichment types (Tab. 2). We also did not corroborate most of our hypotheses. However, we find that body care decreased in the presence of leaf-litter (Dunn test: H=-2.1379; p=0.0163), bromeliads (Dunn test: H=-2.5864; p=0.0048) and bamboo (Dunn test: H= 4.020; p<0.001) compared to control period. The frequency of affiliative interactions during bamboo and bromeliad enrichments were lower than control (bamboo: H= 2.0455; p=0.0204; bromeliads: -2.5431, p=0.0055). Leaf-litter enrichment presented higher frequency of affiliative interactions if compared to bromeliads (Dunn test: H= -2.2430; p =0.0124). Monitor behavior decreased significantly during bamboo enrichment compared to control period (Dunn test: H= 2.2609; p<0.0119). Monitoring was also smaller during bamboo enrichment compared to bromeliads (Dunn test: H=2.3666; p=0.0090). Following the provision of all three enrichment types object manipulation increased (leaf-litter: H=3.8403; p=0.0001; bromeliads: H=2.7962; p=0.0026; and bamboo: H= -4.9231; p<0.0001) (Fig. 1). Surprisingly, the category “others” was lower in control (Dunn test: H=2.28766; p=0.0111) and bamboo (Dunn test: H=3.1894; p=0.0007) when compared to its frequency with leaf-litter.

Table 2. Results from Kruskall-Wallis analysis for each behavioral category.

Behavior category	H	p-value
Feeding	6.9839	0.0724
Foraging	0.8766	0.8310
Locomotion	3.8431	0.2789
Resting	2.1789	0.5361
Body care	16.726	0.0008*
Affiliative interactions	9.5303	0.0230*
Agonistic interactions	2.5472	0.4668
Alert	4.7743	0.1891
Object manipulation	27.067	0.001*
Monitoring	9.0281	0.0289*
Stereotyped behavior	1.344	0.7187
Out of sight	0.5527	0.9072
Others	13.365	0.0039*

* significant p-values

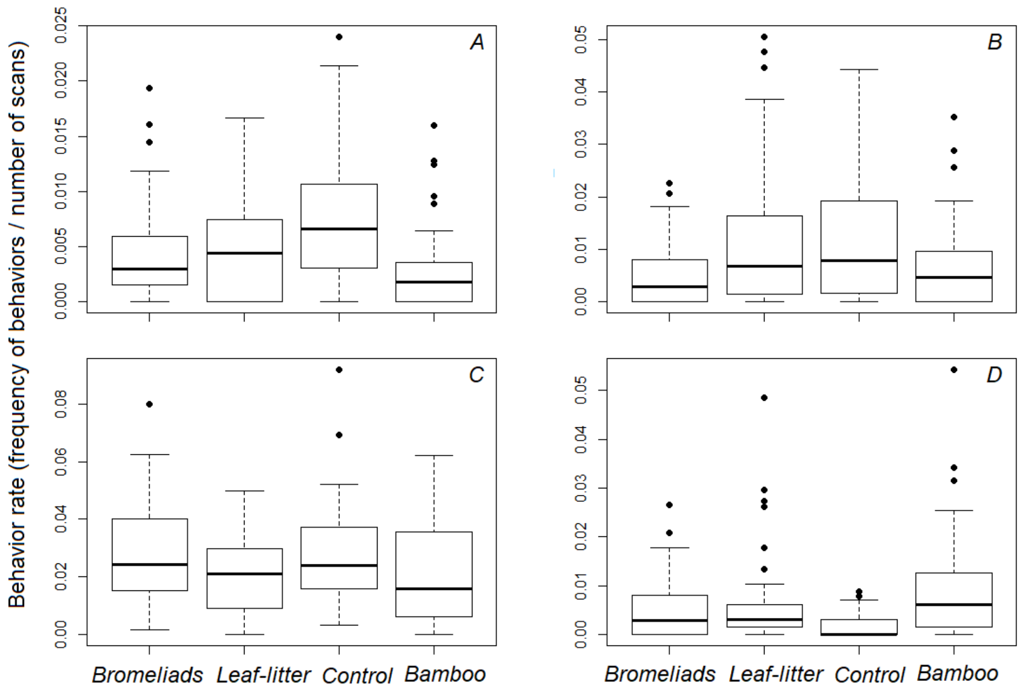

Figure 1. Behavior rate (frequency of behaviors/number of scans) of A) Body care; B) Affiliative interaction; C) Monitoring and; D) Object manipulation by seven captive *Sapajus* spp. individuals, when provided with different kinds of environmental enrichment: *Bromeliads, Leaf-Litter, Bamboo* and *Control* (this final category indicating an absence of environmental enrichment).

Discussion

The capuchin monkeys were attracted to all the materials used for environmental enrichment, since they are very curious and explorative species. Though, most of our hypotheses were not corroborated. The capuchin monkeys did not spend much time in foraging and feeding activities, as we expected because of the addition of earthworms (animal protein) in the enrichment. There are two possible reasons for this: 1) the number of added earthworms was too low (25/day) or 2) capuchins did not consider earthworms as relevant food sources. We recorded multiple times that capuchins manipulated the earthworms but did not eat them. This result is not surprising, as capuchins have rarely been recorded eating earthworms in the wild (but see Barnett *et al*., 2002). Also, whenever in contact with novel food capuchins usually explore it but remain cautious about ingesting it (Sabbatini *et al.,* 2007).

We expected that resting would decrease during enrichment due to the insertion of elements inside the enclosure which elicited attention and object manipulation. However, this hypothesis was not corroborated. They spent less time resting during the control period as well, which may be related to the typically active behavior of capuchins (Ross, 1988; Zhang, 1995). Even if the enclosure size may affect locomotion rates (Webb *et al.,*2018), we believe that this result is mostly related to the fact that wild capuchins have high foraging and traveling rates, as well as low rates of resting and social interactions (Rímoli *et al.,* 2008). Even in captivity capuchins can spend much of their energy in vertical (~45 m/hr) and horizontal (541 m/hr) travel throughout the day (Edwards *et al*., 2017). We also expected a decrease in agonistic and an increase in affiliative interactions. Although we saw the opposite trend for affiliative interactions, and no difference was found in the frequency of agonistic interaction. Besides that, we believe it is unlikely that it would produce any remarkable change in the already established social system of the group. Such findings would also be positive, once the enrichment stimulate the animals without disrupting their established social system (Westergaard & Fragaszy, 1985).

Capuchins possess social learning and show object manipulation both in nature and in captivity (Ottoni & Izar, 2004). Indeed, as expected, object manipulation increased during enrichments and the animals often manipulated the introduced material. Our results coincide with those found by Westergaard & Fragaszy (1985) who reported an increase in object manipulation by capuchins when their enclosure was enriched. Such activities can be helpful in buffering stress related hormones like corticosterone (Boinski, 1999). Boinski *et al.* (1999) found that *Sapajus apella* decrease body care activities during enrichment. Likewise, macaws are known to reduce body care (preening) during environmental enrichment (De Almeida *et al*., 2018).

Studies show that capuchin monkeys in captivity can invest 22 % of their time budget in monitoring the environment (Ferreira, 2017).

Accordingly, we expected that frequency monitoring behavior would increase, once new items were inserted into the enclosure, and capuchins tend to be curious with novelty in its environment. However, we found a decrease in this activity, particularly during bamboo enrichment. This is probably linked to the fact that, during bamboo enrichment, the animals were more likely to be engaged in object manipulation (an activity that increased during this phase).

Stereotyped behavior occurred at very low frequency (1.946 %), even during the control phase (without enrichment). This is probably why we detected no changes in the general pattern of stereotyped behavior over the course of the study. There is good and widespread evidence that behaviors potentially indicator of stress occur in captive animals in general (Mason & Rushen, 2008). Such behaviors are often related to individual personality in capuchins (Ferreira *et al*., 2016). In our study only one animal, a female *S. libidinosus,* showed any form of stereotyped behavior.

Of the three enrichments deployed, leaf-litter, bromeliads and bamboo, the third appeared to be the most effective, since it induced changes in all of the four behavioral categories mentioned above. Additionally, such material remained in the enclosure for much longer than bromeliads and leaf-litter, materials that were both quickly destroyed or discarded by capuchin monkeys. Bamboo also had positive effects for enriching enclosures for a number of Old World primates, including gibbons, mona monkeys and brown lemurs (Costa *et al.*, 2018). Our findings reinforce such results by showing that bamboo is also an effective enrichment for Neotropical primates. However, we caution that bamboo may be a dangerous material if the levels of aggressive behavior in the group is high, in this case leaf-litter and bromeliads enrichments would be more appropriate.

In general, dry leaves, bamboo and bromeliads with the addition of small invertebrates provide effective environment enrichment for *Sapajus* monkeys. The introduced material induced motor activities of captive animals that, in the wild, are extremely active. Thus, we strongly recommend the use of such items as environmental enrichment for captive capuchin monkeys. We also found that bamboo was the most efficient enrichment tool since it remained intact for longer in the enclosure and induced significant positive changes in the capuchins behavior.

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