DOI: 10.1590/1809-6891v24e-76165E

Ciência Animal Brasileira
Brazilian Animal Science

e-ISSN 1809-6891

Section: Veterinary medicine
Research article

Environmental enrichment techniques for black-eared-opossums (*Didelphis aurita* Wied-Neuwied, 1826) in captivity

Técnicas de enriquecimento ambiental para gambás-de-orelha-preta (*Didelphis aurita* Wied-Neuwied, 1826) em cativeiro

Carlos Eduardo de Noronha[1*], Rosana Suemi Tokumaru[1]

[1]Universidade Federal do Espírito Santo (UFES), Vitória, Espírito Santo, Brazil
[*]Corresponding author: cen.bfr@gmail.com

Abstract
This study aimed to analyze the effects of environmental dietary enrichment on the behavior of black-eared opossums that were in captivity at Centro de Triagem de Animais Silvestres (CETAS-ES), in the municipality of Serra-ES, from August to October 2022. Two food models were constructed for the study: the "Surprise Tubes" and the "Food Puzzle". A total of 24 juvenile black-eared opossums were selected, regardless of sex, and divided into eight groups, with three animals each. Then, four groups were exposed to one model and four to the other. Each group was submitted to two conditions: experimental, with the presence of the models, and control, without the presence of the models. Each condition lasted 24 hours and occurred on two consecutive days. They were filmed with a camera trap, resulting in 3,233 videos of 25 seconds. For the elaboration of the ethogram, 24 videos of each group were selected, recorded from 6:00 PM to 7:30 PM, when the animals were more active. The opossums interacted with both models, accessing, and eating the hidden food, preferring meat over fruit. No significant differences were found in the behavior of opossums in relation to the models and it was observed that the aggressive behavior significantly decreased when the models were present. It is concluded that the models can be used as an environmental enrichment for black-eared-opossums, bringing benefits to the reduction of aggressive behavior.
Keywords: *Didelphis aurita*; captivity; marsupials

Resumo
O objetivo deste trabalho foi analisar os efeitos do enriquecimento ambiental alimentar no comportamento dos gambás-de-orelha-preta que se encontravam em cativeiro no Centro de Triagem de Animais Silvestres (CETAS), no município Serra, no estado do Espírito Santo (ES), de agosto a outubro de 2022. Dois modelos alimentares foram construídos para o estudo: os "Tubos Surpresa" e o "Quebra-Cabeça Alimentar". Foram selecionados 24 gambás-de-orelha-preta, independente do sexo, juvenis, divididos em oito grupos, com três animais cada, sendo quatro grupos expostos a um modelo e quatro ao outro. Cada grupo foi submetido a duas condições: experimental, com a presença do modelo e controle, sem a presença do modelo. Cada condição durou 24 horas e ocorreram em dois dias consecutivos. Foram filmadas com câmera trap, resultando em 3233 vídeos, de 25 segundos cada. Para elaboração do etograma foram selecionados 24 vídeos de cada grupo, gravados entre as 18h00 e 19h30hs, período em que os animais se mostraram mais ativos. Os gambás interagiram com os dois modelos, acessando e comendo os alimentos escondidos, havendo preferência pela carne em comparação com a fruta. Não houve diferença significativa no comportamento dos gambás em relação aos modelos e observou-se que o comportamento agressivo diminuiu significativamente quando os modelos estavam presentes. Conclui-se que os modelos podem ser utilizados como enriquecimento ambiental para os gambás-de-orelha-preta, trazendo benefícios para a diminuição do comportamento agressivo.
Palavras-chave: *Didelphis aurita;* cativeiro; marsupial

1. Introduction

This study focuses on *Didelphis aurita* (Wied-Neuwied, 1826), known as the black-eared opossum, which belongs to the class *Mammalia*, family *Didelphidae*. It can be found in forested areas, from the east coast in Paraíba to Rio Grande do Sul, and can extend into the interior regions of Brazil, such as southern Mato Grosso do Sul, as well as eastern Paraguay [(1)]. Black-eared opossums are small to medium-sized marsupials with solitary, nocturnal, and nomadic habits. These animals are classified as omnivores for having a diversified diet, feeding on eggs, leaves, roots, invertebrates, and small vertebrates, such as snakes, thus helping in the control of venomous animals [(2)]. When

Received: May17, 2023. Accepted: July 31, 2023. Published: September 1, de 2023.

threatened, black-eared opossums do not usually attack, but exhibit aggressive behavior that includes opening their mouths to show their teeth and/or emit characteristic vocalizations(3-4). Furthermore, they feature death-feigning (thanatosis) and release a foul-smelling odor, which simulates unhealthy conditions for consumption, causing the predator to lose interest in preying on them (5).

Black-eared opossums are able to live both on land and high on trees, grasping and climbing branches with their long prehensile tail and short five-toed hands and feet, the first toe being clawless(6). Due to their ecological plasticity, they show great adaptive efficiency to the most varied habitats, easily adapting to the environment modified by humans, including rural and urban areas(7).

Despite the existence of laws to protect Brazilian fauna, such as laws no. 5197/1967 and 9605/1998, the abuse and mistreatment, hunting, or catching of wild animals are still widely practiced. Regarding black-eared opossums, these are often killed by attacks from domestic animals and humans or road kills(8-11).

Several factors compromise animal welfare in a captive setting. The welfare status of captive animals is an attribute that is linked to their quality of life and the way they interact with their surrounding environment. Animals face potential challenges in these interactions that can lead to frustration, scarcity, or overstimulation(12). In this sense, the incorporation of environmental enrichment techniques has emerged in an effort to improve the welfare of animals in captivity by providing appropriate environmental stimuli that mimic natural situations. It aims to create a more enriching and interactive environment(13), which allows the expression of characteristic behaviors of each species, positively influencing the physical and psychological development of the animal(14). Moreover, the use of entertainment strategies, based on the use of games or cognitive proposals of greater complexity, also favors the adequacy of the captivity environment, improving the welfare of animals(15).

Environmental enrichment, a field of animal behavior recognized by Yerkes and Hedinger in the first half of the 19th century, studies the importance of the physical and social environment for the welfare of captive animals by providing elements that allow them to express the natural behavior of the species(16-18). This technique consists of incorporating elements into captivity that can reproduce the natural habitat of the species, allowing the animals to exercise common activities of the wild, such as locomotion, foraging, and sheltering, among others. According to Mcphee et al.(19), there are five types of environmental enrichment:

Physical: It concerns the physical structure of the enclosure, the place where the animals are kept. For this type of enrichment, elements are introduced so that the enclosure resembles the animal's original habitat as much as possible. Examples include the use of branches, substrates, vegetation, and platforms.

Sensory: This type of enrichment is one of the most used and consists of stimulating the animals' five senses: visual, auditory, olfactory, tactile, and gustatory. Examples include aromatic herbs and sounds with vocalizations.

Cognitive: It is aimed at stimulating the intellectual capacities of animals, being done by means of toys or devices, with which animals are encouraged to interact to get a reward.

Social: It is linked to intraspecific or interspecific interaction that can happen within the enclosure. In this enrichment, animals have the opportunity to interact with other species that they would naturally live with in the wild or with individuals of the same species.

Dietary: It concerns the way the animals are fed in captivity. In general, attempts are made to offer a diet closer to that found in the wild, by providing carcasses, hidden food, and/or changes to routine feeding times.

Considering the scarce reports of environmental enrichment aimed at marsupials in captivity, especially for South American species, the objective of this work was to analyze the effects of dietary enrichment on the behavior of black-eared opossums, aiming to contribute to improvements in the treatment of this species kept in captivity.

2. Materials and methods

2.1 Study area

The study area was the Brazilian Wild Animal Screening Center (CETAS) owned by IBAMA- ES, the Brazilian Institute of the Environment and Renewable Natural Resources, located in the Environmental Protection Area (APA) of Jacuném Lagoon, in Barcelona, in the municipality of Serra-ES, Brazil. The research was authorized under order No. 12950928/2022 and case No. 02009.000902/2022-13, published in the IBAMA system.

CETAS-IBAMA-ES is a unit that aims to receive, identify, tag, screen, evaluate, recover, rehabilitate, and allocate healthy wild animals back to nature(20). In Espírito Santo, these animals are brought in from inspection seizures, rescues, or voluntary deliveries from individuals. They come from the Greater Vitória region, which comprises the municipalities of Vitória, Vila Velha, Cariacica, Viana, Guarapari, Serra, and Fundão.

Black-eared opossums are brought to CETAS-ES through various means including inspection seizure, rescue, and private individuals. These opossums are commonly victims of domestic animal attacks, cars collisions, or electrocutions. Thenat CETAS-ES, they are weighed, sexed, inspected, and evaluated by the caretakers and/or volunteers of the Marsupial Project. Subsequently, they are allocated and housed in enclosures (74 x 70 x 76 cm) until they are considered ready for release. The Marsupials Project is part of Últimos Refúgios Institute, a non-profit socio-environmental and cultural organization. Since 2017, the institute has been actively involved in the preservation of Brazilian marsupials, collaborating with CETAS-ES for their rescue and rehabilitation[21].

Since they are omnivores, black-eared opossums are fed fruits such as bananas, apples, oranges, papaya, and grapes daily. They are also fed meat, which can be quail or rat, three times weekly. The enclosures are cleaned every morning, at the same time as the water in the drinkers is changed and the leftover food in the feeders is removed. Young opossums are provided with special care and diet, including a blend of goat's milk and various fruits, supplied in 1-ml syringes. Moreover, if they are in litters, these are always kept together.

2.2 Experimental conditions

2.2.1 Animals

For the experiment, a total of 24 black-eared opossum juveniles were selected, including males and females, with weights ranging from 58 to 142g, under evaluation at CETAS-ES. The opossums were divided into eight groups, with each group consisting of three animals (Table 1), which were allocated to a cage with dimensions of 74 x 70 x 76 cm (Figure 3). The groups were formed according to the animals' arrival at CETAS-ES; therefore, some groups consisted of animals from the same litter. For identification purposes, each opossum in the group was individually tagged: the first animal with adhesive tape on the left paw, the second animal with adhesive tape on the right paw, and the last animal was not tagged.

2.2.2 Dietary enrichment

Dietary enrichment was achieved by the introduction of food hidden in containers constructed especially for the study, known as models, which were adapted to meet the specific needs of the black-eared opossums (*D. aurita*).

Model 1 - "Surprise Tubes"

Created based on the model developed by animal caregiver Aisa Coco[22] for *Procyon lotor* (raccoons), the "Surprise Tubes" model consists of a wooden rectangle measuring 20.5 x 25.5 cm, to which small PVC pipes of various shapes were attached (Figure 1). The food (animal feed, meat, and fruits) was concealed inside the pipes, encouraging the animal to reach for the food.

Figure 1. Photos of Model 1 - "Surprise tubes."

Model 2 - "Food Puzzle"

The "Food Puzzle" model was proposed by Banton-Jones[23] to be used with striped skunks (*Mephitis mephitis*). It consists of a wooden box with dimensions of 23.5 x 12 x 8.5 cm, which contains three smaller boxes measuring 5 x 5 cm, each with a lid and handle (Figure 2). The food items were stored separately in the smaller boxes, encouraging the opossum to attempt to open the boxes and obtain the food.

Figure 2. Photos of Model 2 - "Food Puzzle."

2.3 - Collection procedure

The eight groups of three opossums were randomly distributed among the enrichment models, with four groups subjected to Model 1 and the other four to

Model 2. Each group underwent two conditions: experimental and control, over two consecutive days. The order of the conditions was randomized among the groups (Table 1).

In the experimental condition, or dietary enrichment condition, the behavior of the animals was recorded when they were subjected to the model. The food was placed inside the experimental model, which was then introduced into the cage in the late afternoon, around 16h:30, remaining there for 24 consecutive hours. In the control condition, the behavior of the animals was recorded in the absence of the model, also for 24 consecutive hours. In this condition, the food was provided in standard CETAS-ES containers, which are rounded, with a diameter of 13 cm and an opening at the top.

The behavior of the animals in each condition was recorded using a KOVOSCJ camera trap, model H982, with 1080P full HD image quality and night vision. The camera was positioned outside the cage, supported by a tripod, facing towards the dietary enrichment model (Figure 3). Considering that opossums have crepuscular and nocturnal habits[24], the recordings were made from August 27 to October 22, 2022, starting at 16h:30 and ending at 04h. The camera was programmed to be triggered by animal movements and each recording lasted 25s.

Figure 3. Positioning of the trap camera for the experiment recording

Table 1. Distribution of environmental enrichment models and group composition

Group	Model	Order of presentation of the experimental condition**	Individuals	Sex	Weight at the beginning of the experiment
1*	Food Puzzle	2nd day	Alpha	Female	125g
			Beta	Female	114g
			Gama	Female	124g
2	Food Puzzle	2nd day	Ricky	Male	58g
			Speed	Male	64g
			Dolores	Female	65g
3*	Food Puzzle	1st day	Maria	Female	73g
			Suzana	Female	69g
			Rowena	Female	82g
4*	Food Puzzle	1st day	Eusébio	Male	127g
			Nilson	Male	109g
			Elizabeth	Female	73g
5	Surprise tubes	2nd day	Huguinho	Male	132g
			Zezinho	Male	142g
			Luisinho	Male	133g
6	Surprise tubes	2nd day	Honey	Female	65g
			Flowey	Female	67g
			Boris	Male	66g
7*	Surprise tubes	1st day	Afrodite	Female	76g
			Hércules	Male	74g
			Eros	Male	75g
8*	Surprise tubes	1st day	Golias	Male	79g
			Joel	Male	65g
			Thomas	Male	76g

Note: * The individuals selected to form these groups come from different litters. ** 1st experimental condition (presence of the model) before the control condition; 2nd control condition before the experimental condition (see text for detailed explanation).

2.4 Analysis procedure

For the analysis of images, the Boris software program (Behavioral Observation Research Interactive Software) was used to record the duration and frequency of occurrence of observed behaviors in the videos.

Comparisons between different enrichment models were conducted using the Mann-Whitney test, which is suitable for comparing independent non-parametric measures. This analysis allowed for determining if there were significant differences in behaviors concerning the different models used. All analyses were conducted using the JASP 0.16.1 software program, which provides statistical tools for data analysis. The choice of non-parametric tests was due to the absence of normal distribution in the observed data.

Thus, using only the videos recorded from 18h:00 to 19h:30, when the animals were most active, behaviors were identified and categorized to create an ethogram (Table 2). The observed behaviors were categorized as an event or a state. For events (short behaviors), the frequency of occurrence was recorded, and for states (prolonged behaviors), the duration of the behaviors was recorded.

Table 2. Behaviors identified in the recordings of *Didelphis aurita* and their respective definitions

Behavioral category	Behavior	Description
Exploratory	1- Explore environment (stationary)[1]	The opossum remains stationary in the enclosure, making regular head movements from side to side, as if sniffing the air.
	2- Explore environment (walking)[1]	The opossum moves around the enclosure, making regular head movements from side to side, as if sniffing the air.
	3- Explore the model[1]	The opossum moves over or around the model, looking, sniffing, biting, or manipulating the model with its hands.
Feed	4- Eat[1*]	The opossum brings the food to its mouth using its hands or takes the food with its mouth and ingests it.
	5- Drink[1]	The opossum drinks the bowl water.
Body maintenance	6- Clean[1]	The opossum licks, itches, or bites its own fur, paws, or fingers.
	7- Yawn[2]	The opossum performs involuntary and long inhales with its mouth open.
Movement	8- Climb[1]	The opossum climbs the branches present in the enclosure or climbs the bars.
	9- Manipulate cage bars[1]	The opossum scratches and bites the cage bars.
	10- Enter the burrow[2]	The opossum moves into the burrow.
	11- Leave the burrow[2]	The opossum moves out of the burrow.
	12- Carry food with tail[1]	The opossum uses its tail to carry food from one place to another.
	13- Carry object with tail[1]	The opossum uses its tail to carry the object from one place to another.
	14- Carry food with hand/mouth[1]	The opossum uses its hands or mouth to pick up food and carry it from one place to another.
	15- Carry object with hand/mouth[1]	The opossum uses its hands or mouth to carry an object from one place to another.
Social	16- Aggressive social interaction[1]	Biting, scratching, stealing food from another animal in the group.
	17- Peaceful social interaction[1]	Smell and look at another animal within the group.
	18- Look at another animal in the cage next door[1]	Direct eye contact with another animal from the adjacent enclosure.
Rest	19- Stay in the burrow[1]	The opossum remains stationary inside the burrow.

Note: Behaviors with the superscript number 1 represent a state, whereas those marked with the superscript number 2 represent an event. * Regarding the eating behavior, food was provided in bowls during the control condition and within the model during the experimental condition. In all conditions, meat, fruits, and animal feed were provided.

3. Results

3.1 Qualitative results

It was noted that among the four groups exposed to the “Food Puzzle” model, two of them managed to open all three boxes, and the opossums consumed all the provided food. In the remaining two groups, only one of the boxes was opened; one contained feed, the other contained meat, and the rest remained closed. Regarding the groups exposed to the “Surprise Tubes” model, it was observed that the opossums inserted their snouts into the holes of the pipes to reach the food, thus gaining easier access. No food was found in any of the groups at the time of the removal of the “Surprise Tubes” model.

Another behavior observed in the eight groups of black-eared opossums was the relationship between the size of the animals and the ease of feeding in the enrichment models presented. The *D. aurita* specimens with larger size and weight were able to open the boxes of the “Food Puzzle” model more easily, using mostly their mouths, whereas the smaller opossums presented to the same model, besides taking longer to open the boxes, managed to open them unintentionally by bumping into the boxes. In contrast, in the “Surprise Tubes” model, the smaller opossums had more agility and ease in reaching the food that was inside the tubes by introducing their snouts into the holes of the tube and entering the spaces between the tubes, whereas the larger opossums had more difficulty in reaching the food.

During the filming of the experimental condition of one of the groups subjected to the “Food Puzzle” model, two opossums died. They were found dead in the cage, near the entrance of the burrow, early in the morning, by the CETAS-ES caretaker who collected them, so it was not possible to see if there were apparent injuries, leaving the cause of death inconclusive. Therefore, it was necessary to start a new filming with new subjects. The data from this group were not used for the analysis. In the footage, it was also observed that the surviving opossum moved rapidly throughout the cage and did not interact with the model. No document on the cause of death of the two opossums was found in CETAS-ES.

Despite being present in the ethogram, since they were observed in the first analysis of all videos, the behaviors of carrying food/object with the tail were not observed in the videos selected for quantitative analysis and, therefore, are not included in Table 3. Moreover, the behavior of carrying objects with the mouth was witnessed, with the lids of the boxes of the “Food Puzzle” model, as well as twigs and leaves, being taken into the burrow.

3.2 Quantitative results

The frequency and duration of opossum behaviors in the presence (experimental condition) and absence (control condition) of the model were compared using Friedman’s tests (Table 3). A significant difference was observed between the experimental and control conditions in only two behavioral categories: social interaction and eating behavior.

Table 3. Frequency and duration of opossum behaviors in the presence (experimental condition) and absence of the model (control condition)

Category	Behavior	“Food Puzzle” model				“Surprise Tubes” model			
		Experimental		Control		Experimental		Control	
		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Exploratory	Explore the environment (stationary)	53.589	26.376	58.156	44.545	66.234	47.322	100.377	89.181
	Explore the environment (walking)	119.219	93.206	82.425	45.164	52.673	33.624	81.778	65.568
	Explore the model	21.433	17.586	-	-	74.673	79.108	-	-
Feed	Eat Feed	14.495	37.648	20.089	34.724	3.562	7.872	47.664	74.131
	Eat Meat	95.747	88.004	59.070	71.083	72.973	85.843	28.464	52.596
	Eat Fruits	30.106	62.699	39.232	42.629	10.748	17.919	55.529	46.878
	Drink	2.760	4.406	2.395	3.403	8.951	9.493	4.397	6.531
Body maintenance	Clean	13.353	17.991	25.411	31.024	20.769	22.309	10.041	22.887
	Yawn	0.083	0.289	0.250	0.622	0.250	0.452	0.333	0.651
Movement	Climb	80.082	57.633	69.420	65.381	31.087	43.842	52.110	53.191
	Handle cage bars	3.442	6.461	3.787	7.765	1.780	6.168	1.394	3.911
	Enter burrow	1.167	0.937	1.583	1.165	1.000	1.044	1.667	0.778
	Leave burrow	1.167	0.937	1.583	1.165	1.000	1.044	1.667	0.778
	Carry food with fand/mouth	1.358	2.027	2.583	5.210	2.331	4.624	2.325	7.359
	Carry object with hand/mouth	2.388	7.266	1.284	3.962	0	0	0	0
Social	Aggressive social interaction	16.386	14.164	42.244	143.540	5.876	6.060	1.754	4.470
	Peaceful social interaction	0.748	1.873	0.874	1.339	0.816	2.139	1.493	2.231

In the social interaction category, it was found that there were more aggressive than passive interactions (Table 3). Aggressive interactions were significantly more frequent than passive interactions both in the presence ($t = 4.32$, $p < .001$) and in the absence ($t = 3.24$, $p = .011$) of the model, shown by post-hoc comparisons with the Bonferroni correction. However, when comparing the experimental and control conditions, aggressive interactions were significantly more frequent in the absence of the model ($t = 3.51$ $p = .005$). Effect size was moderate (Kendall's $W = 0.3$).

Differences were seen in the duration of eating behavior, and the opossums ate more meat than fruit. (Table 3). The post hoc comparisons pointed to a significantly higher consumption of meat than fruit before the model ($t = 3.01$ $p = .02$). However, the effect size was small (Kendall's $W = 0.16$).

Statistically significant differences were not found when comparing the frequency and duration of behaviors in the presence of the Tubes model and the Puzzle model using Mann-Whitney tests. Table 3 shows that the standard deviation values were relatively high compared to the means, indicating individual variation in the frequency and duration of behaviors. The absence of significance in most of the performed comparisons (between experimental and control conditions and between models) may be related to this large behavioral variability observed between individuals.

4. Discussion

4.1 Discussion of qualitative results

Despite the fact that the size of *D. aurita* influenced the interaction with the presented enrichment models, the objectives of interaction with the model and access to food were achieved. These results were also achieved by Murray et al.[25] and Hogan et al.[26], in their research using food enrichment for *Petaurus australis* and *Lasiorhinus latifrons*, respectively. Murray et al.[25] had positive results in their experiment with *Petaurus australis*, an Australian arboreal marsupial with a predominant diet of eucalyptus nectar, sap, and pollen. The authors report that the experiment presented expected results since *P. australis* interacted with and investigated the gum tree, which was used for the food enrichment technique. Hogan et al.[26] studied the natural foraging behaviors of *Lasiorhinus latifrons*, a herbivorous Australian terrestrial marsupial, by exposing them to a circular lawn with roots, a eucalyptus branch, and buried food.

Interactions also occurred with parts of the "Food Puzzle" model outside the feeding situation. One of the animals was observed carrying the lid of a box into the burrow. The observation occurred in a video that was not selected for analysis since it was filmed outside the time range of greater activity of the animals. The observation of the behavior suggested, in a first analysis, the possibility of nest building[27]. However, since it was a behavior exhibited by only one opossum, the largest specimen of all participating animals, in a group with only juvenile females, excluding the possibility of pregnancy or nursing of joeys, it was later found that the animal had used the box for foraging in the burrow. A female was seen carrying the box into the burrow, in addition to the lid, twigs, and leaves, sleeping on the objects taken.

The death of the two opossums was considered an unexpected fact, as it is not common at CETAS-ES for juvenile specimens to die compared to neonates. The specimens that died had been at CETAS-ES for at least a week, were from different litters, and were healthy at the beginning of the recordings, before being exposed to the "Food Puzzle" experimental condition. According to the study by Baggio[28], agonistic and antisocial behavior, common in representatives of the genus *Didelphis*, is already present in the juvenile development stage. The author studied opossums in captivity and observed that young animals should be separated in different environments since disputes, fights, and even cannibalism can occur. The author reports that she observed some juvenile opossums escaping from the enclosure and entering another, with younger opossums, where a specimen was found feeding on one of the animals. In another study, by Kajin et al.[29], who examined a population of black-eared opossums in an Atlantic Forest area in Rio de Janeiro for nine years, a higher mortality rate was observed in lactating and young opossums, while the values found for adults were lower. The authors report that the high mortality of neonates may be linked to the reproductive strategy of marsupials, which show little investment in gestation and great investment in lactation, and the death of lactating opossums becomes common due to this stage of life being more critical for survival. In the case of weaned joeys, the authors explain that the high mortality rate may occur due to the vulnerability still present at this stage of life.

4.2 Discussion of quantitative results

We expected to obtain more significant differences between the conditions with the presence and absence of the models, especially in behaviors such as exploring the environment since they occurred frequently. Black-eared opossums are known to have intense scansorial activity, i.e., great climbing ability, as reported by Vieira e Camargo.[30]. The relatively high standard deviation values indicate that behavior varied widely around the mean, suggesting large individual variation and implying that studies with larger sample sizes could assess differences between models and between experimental and control conditions. The significant occurrence of more aggressive behavior in the absence of the model indicates that enrichment may have an effect on decreasing the aggressive behavior of opossums. This result provides support for the hypothesis that environmental enrichment can promote increased welfare in captive animals.

Black-eared opossums are considered solitary and antisocial animals and usually tend to avoid contact with other individuals of the same species by acting aggressively when in contact with another, except during breeding periods[31]. In this study, no sociability behavior was observed among black-eared opossums, even in groups with juveniles from the same litter, confirming the common solitary habit of the species. Some opossums reacted defensively when another opossum approached to catch food, threatening to bite, or advancing to push it away. Thus, the decrease in aggressive behavior in the presence of the models is indicative of an improvement in the quality of life of captive animals.

Furthermore, the results also indicated a significantly higher consumption of meat compared to fruit by black-eared opossums. According to Santori et al.[32], the preference of *D. aurita* for a certain type of food is not yet clearly established, as the available information on the feeding habits of these animals comes from analysis of fecal and stomach contents, as well as direct observation. Carvalho et al.[33] reported that although protein consumption from arthropod ingestion was one of the main diet items of the black-eared opossums they analyzed, there were no significant differences in relation to a diet composed of fruits and/or seeds when comparing animals from different climatic seasons, sexes, age classes, and habitat fragments. In turn, Hsu et al.[34] and Hume[35] found that the Virginia opossum (*Didelphis virginiana*), which is closely related to and has a similar diet as the black-eared opossum (*D. aurita*), is an opportunistic omnivore that, when kept in captivity, can survive on dog food with some natural supplements, such as fruits and crickets.

Since generalist food consumption by *D. aurita* is common, they easily adapt to different types of diets[36-37]. The feeding of black-eared opossums is directly related to food availability, so a hypothesis for meat preference, as observed in the present study, meets the four factors that influence an animal's food choice, according to Owen[38]: availability, palatability, accessibility, and the energy return obtained from the food.

5. Conclusion

The research demonstrated that black-eared opossums (*D. aurita*) successfully interacted with the "Surprise Tubes" and "Food Puzzle" models, adapted and built for the experiment, managing to access and eat the hidden food. Furthermore, it is important to note that significant effects were found when comparing the experimental and control conditions, such as the greater aggressive interaction in the absence of the enrichment models compared to when they were present, indicating that the models had an influence on decreasing this behavior. Another significant result was the higher consumption of meat than fruit in the presence of the models, which may be indicative of the dietary preference of these animals in captivity. We conclude that it is possible to apply environmental enrichment models for captive opossums using simple and low-cost materials. The results suggest that the use of enrichment can stimulate behaviors characteristic of the species, such as foraging and climbing, for example, and decrease aggressive behavior, providing an improvement in the quality of life for these animals in the captive environment. We highlight that this study used a small sample size, with four groups of three animals each, to test each enrichment model. Thus, we indicate that more studies with larger samples of captive black-eared opossums are needed to confirm the results found in this study.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: C. E. Noronha, R. S. Tokumaru. *Data curation:* C. E. Noronha, R. S. Tokumaru. *Methodology:* C. E. Noronha, R. S. Tokumaru. *Investigation:* C. E. Noronha. *Supervision:* R. S. Tokumaru. *Writing (original draft):* C. E. Noronha. *Writing (revision and editing):* C. E. Noronha, R. S. Tokumaru. *Formal analysis:* R. S. Tokumaru. *Visualization:* C. E. Noronha. *Validation:* R. S. Tokumaru. *Project administration:* C. E. Noronha, R. S. Tokumaru.

References

1 Gardner A. Mammals of South America, Volume 1: Marsupials, Xenarthrans, Shrews, and Bats. Chicago: University of Chicago Press; 2008. https://doi.org/10.7208/9780226282428 English

2 Perez Dictoro V. Que bicho é esse?. GUIA [Internet]. 14º de junho de 2021 [citado 2022 Dez 16];2(1):37-8. Disponível em: https://www.revistaguia.ufscar.br/index.php/guia/article/view/37 Portuguese

3 McManus JJ. Behavior of Captive Opossums, *Didelphis marsupialis virginiana*. American Midland Naturalist. 1970 Jul;84(1):144 English

4 Hunsaker II D, Shupe D. Behavior of New World Marsupials. In: Hunsaker II D, editor. The Biology of Marsupials. New York: Academic Press; 1977. p. 279–348. English

5 Delciellos AC, Loretto D, Antunes VZ. Marsupiais na mata atlântica. Ciência Hoje. 2006 Jan;66–69. Portuguese

6 Rossi RV, Bianconi GV, Pedro WA. Ordem *Didelphimorphia*. In: Reis NR, Peracchi AL, Pedro WA, Lima IP, editores. Mamíferos do Brasil. Londrina: UEL; 2006. p. 27–66. Portuguese

7 Faria MB, Lanes RO, Bonvicino CR. Marsupiais do Brasil: guia de identificação com base em caracteres morfológicos externos e cranianos. São Caetano do Sul: Amélie Press; 2019 Por-

tuguese

8 Milli MS, Passamani M. Impacto da Rodovia Josil Espíndula Agostini (ES-259) sobre a mortalidade de animais silvestres (*Vertebrata*) por atropelamento. Nat Online [Internet]. 2006 [citado 2023 Abr 21];4:40–6. Portuguese

9 Bueno PC. Sazonalidade de atropelamentos e os padrões de movimentos em mamíferos na BR-040 (Rio de Janeiro-Juiz de Fora). Revista brasileira de zoociências. 2010 Jan 1;12(3). Portuguese

10 Costa LS. Levantamento de mamíferos silvestres de pequeno e médio porte atropelados na BR 101, entre os municípios de Joinville e Piçarras, Santa Catarina. Biosci J [Internet]. 2011 Ago 30 [citado 2023 Abr 21];27(4):666–72. Disponível em: https://seer.ufu.br/index.php/biosciencejournal/article/view/7501. Portuguese

11 Rangel CH, Neiva CHMB. Predação de vertebrados por cães *Canis lupus F. Familiaris* (*Mammalia*: *Carnivora*) no Jardim Botânico do Rio de Janeiro. Biodiversidade Brasileira - BioBrasil [Internet]. 2013 [citado 2023 Abr 27];(2):261–9. Disponível em: https://revistaeletronica.icmbio.gov.br/BioBR/article/view/345. Portuguese

12 Broom DM. Welfare in relation to feelings, stress and health. Rev Electron Veterinária [Internet]. 2007 [citado 2023 Mar 17];8. Disponível em: https://www.researchgate.net/publication/26492349_Welfare_in_relation_to_feelings_stress_and_health English

13 Oliveira PKM, Carpi LC. Enriquecimento ambiental para ariranha (*Pteronura brasiliensis*) no zoológico de Brasília. Atas Saúde Ambient . 2016;4:30–46. Portuguese

14 Foppa L, Caldara FR, Machado SP, Moura R, Santos RKS, Nääs IA, Garcia G. Enriquecimento ambiental e comportamento de suínos: revisão. Revista Brasileira de Engenharia de Biossistemas. 2014 Dec 9;8(1):1–7. Portuguese

15 Dela Ricci G, Henrique Branco C, Teixeira Sousa R, Gonçalves Titto C. Efeito de diferentes técnicas de enriquecimento ambiental em cativeiro de onças suçuaranas (Puma concolor). Ciênc. anim. bras. [Internet]. 4º de julho de 2018 [citado 2º de março de 2023];19:1-10. Disponível em: https://revistas.ufg.br/vet/article/view/e-47693

16 Pizzutto CS, Sgai MGFG, Guimarães MABV. O enriquecimento ambiental como ferramenta para melhorar a reprodução e o bem-estar de animais cativos. Revista Brasileira de Reprodução Animal. 2009;Vol. 33:p. 129-138 Portuguese

17 Oliveira APG, Costa WM, Almeida RN de, Costa WM da, Dias NC da S, Vieira B de CR, et al. Uso de enriquecimentos ambientais como mitigadores de comportamentos anormais: uma revisão. Pubvet. 2014 Abr;8(7) Portuguese

18 Maia APDA, Sarubbi J, Medeiros BBL, Moura DJD. Enriquecimento ambiental como medida para o bem-estar de suínos. Revista Eletrônica em Gestão, Educação e Tecnologia Ambiental. 2013 Set 23;14(14) Portuguese

19 McPhee EM, Carlstead K. The Importance of Maintaining natural Behaviors in Captive Mammals. In: Kleiman DG, Thompson KV, Baer CK, editores. Wild Mammals in Captivity - Principles and Techniques for Zoo Management [Internet]. Chicago: The University of Chicago Press; 2010. p. 303–13 English

20 Ministério do Meio Ambiente (BR). Centros de Triagem de Animais Silvestres (Cetas) [Internet]. IBAMA; 2016 Nov 18 [citado 2022 Jul 27]. Disponível em: http://www.ibama.gov.br/fauna-silvestre/cetas/o-que-sao-os-cetas#:~:text=Contatos%20dos%20Cetas-,Sobre%20os%20Cetas,ou%20entrega%20volunt%C3%A1ria%20de%20particulares. Portuguese

21 Projeto Marsupiais [Internet]. Últimos Refúgios. [citado 2022 Dez 04]. Disponível em: https://www.ultimosrefugios.org.br/projeto-marsupiais Portuguese

22 Coco A. Animal enrichment and how you avoid zoo animals to get bored [Internet]. Aisa Coco. 2018 [citado 2022 Ago 19]. Disponível em: https://www.aisacoco.com/animal-enrichment/ English

23 Banton-Jones K. Block Puzzle Feeder [Internet]. Wild Enrichment. 2019 [citado 2022 Ago 19]. Disponível em: https://wildenrichment.com/small-mammals/block-puzzle-feeder/ English

24 Hokoç JN, De Araújo Lima SM, Moraes AMM, Ahnelt P. A Visão em Marsupiais: Características e Evolução. In: Cáceres NC, editor. Os Marsupiais do Brasil: Biologia, Ecologia e Conservação. Campo Grande: Editora UFMS; 2012. p.159-171 Portuguese

25 Murray AJ, Waran NK, Young RJ. Environmental Enrichment for Australian Mammals. Animal Welfare. 1998 Nov;7(4):415–25 English

26 Hogan LA, Johnston SD, Lisle A, Horsup AB, Janssen T, Phillips CJC. Stereotypies and environmental enrichment in captive southern hairy-nosed wombats, *Lasiorhinus latifrons*. Applied Animal Behaviour Science. 2010 Aug;126(1-2):85–95. English

27 Monticelli PF, Gasco A. Nesting behavior of *Didelphis aurita*: twenty days of continuous recording of a female in a coati nest. Biota Neotropica. 2018 Ago 2;18(3) English

28 Baggio F. Cuidados com filhotes de *Didelphis sp.* (Gambás). Curitiba: Universidade Positivo; 2021 p. 1–41. Disponível em: https://repositorio.up.edu.br/jspui/bitstream/123456789/3361/1/FABIANA%20BAGGIO.pdf. Portuguese

29 Kajin M, Cerqueira R, Vieira MV, Gentile R. Nine-year demography of the black-eared opossum *Didelphis aurita* (*Didelphimorphia*: *Didelphidae*) using life tables. Revista Brasileira de Zoologia. 2008 Jun;25(2):206–13. English

30 Vieira EM, Camargo NF. Uso do Espaço Vertical por Marsupiais Brasileiros. In: Cáceres NC, editor. Os Marsupiais do Brasil: Biologia, Ecologia e Conservação. Campo Grande: Editora UFMS; 2012. p.347-364 Portuguese

31 Tardieu L, Adogwa AO, Garcia GW. *Didelphis* species, neotropical animals with the potential for intensive production: Part 1 Review of taxonomy, natural history, general biology, animal behaviour, and nutrition. Tropical Agriculture (St Augistine). 2017; Vol. 94:p. 157-174 English

32 Santori RT, Lessa LG, Astúa D. Alimentação, nutrição e adaptações alimentares de marsupiais brasileiros. In: Cáceres NC, editor. Os marsupiais do Brasil: biologia, ecologia e conservação. Campo Grande: Ed. UFMS; 2012. p. 385–406 Portuguese

33 Carvalho FMV, Fernandez FAS, Nessimian JL. Food habits of sympatric opossums coexisting in small Atlantic Forest fragments in Brazil. Mammalian Biology. 2005 Nov;70(6):366–75 English

34 Hsu M, Harder JD, Lustick SI. Seasonal energetics of opossums (*Didelphis virginiana*) in Ohio. Comparative Biochemistry and Physiology Part A: Physiology. 1988 Jan;90(3):441–3. English

35 Hume ID. Nutrition of marsupials in captivity. International Zoo Yearbook. 2005 Jan;39(1):117–32. English

36 Santori RT, Cerqueira, R, Kleske CC. Digestive anatomy and efficiency of *Philander opossum* and *Didelphis aurita* (*Didel-*

phimorphia, *Didelphidae*) in relation to the feeding habits. Revista Brasileira Biologia. 1995; 55:323-329 English

37 Ceotto P, Finotti R, Santori R,Cerqueira R. Diet variation of the marsupials *Didelphis aurita* and *Philander frenatus* (*Didelphimorphia*, *Didelphidae*) in a rural area of Rio de Janeiro state, Brazil. Mastozoología Neotropical. 2009;16(1):49-58. Disponível em: https://www.redalyc.org/articulo.oa?id=45712055005 English

39 Owen J. Feeding strategy. Chicago: University Of Chicago Press; 1982 English