Revista Argentina de Ciencias del Comportamiento
ISSN: 1852-4206
debora.mola@unc.edu.ar
Universidad Nacional de Córdoba
Argentina

Figueira, Milene Paula; Rodrigues Silva, Fernanda Fatima; Ribeiro, Alice; Oliveira Silva, Ita; Boere, Vanner
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Revista Argentina de Ciencias del Comportamiento, vol. 15, núm. 2, 2023, pp. 26-34
Universidad Nacional de Córdoba
Córdoba, Argentina

Revista Argentina de Ciencias del Comportamiento

ISSN 1852-4206

Agosto 2023, Vol. 15, N°2, 26-34

revistas.unc.edu.ar/index.php/racc

Behavioral responses of captive maned wolves to olfactory enrichment: a preliminary study

Figueira, Milene Paula [a], Rodrigues Silva, Fernanda Fatima[a], Ribeiro, Alice[b], Oliveira Silva, Ita[c] and Boere, Vanner *[c,d]

Artículo Original

Abstract

Environmental enrichment is a technique that increases animal welfare. The behavior of maned wolves when exposed to four odors hidden outside the enclosure was investigated. Using the focal animal method and recording all occurrences, their behavior was analyzed for five minutes in each baseline phase (Basal), during exposure (Exp), and after withdrawal of the stimulus (Pos). Behavioral responses were categorized as positive (P+), negative (N-), and other (Ot). The average P+ increased significantly (p < .05) from the Basal to the Exp, but it was not statistically different (p = .6) between the Basal and the Pos. Neither N- nor Ot changed statistically in any of the phases (p > .5). The methodology we used with olfactory stimuli was efficient, seeming to increase the well-being of the maned wolves

Palabras clave:
animal welfare; behavior; canids; olfactory enrichment.

Resumen

Respuestas comportamentales de los lobos de crin en cautiverio al enriquecimiento olfativo: un estudio preliminar. El enriquecimiento ambiental es una técnica que aumenta el bienestar animal. Se investigó el comportamiento de los lobos de crin cuando se exponen a cuatro olores escondidos fuera del recinto. Usando el método del animal focal y registrando todas las ocurrencias, se analizó su comportamiento durante cinco minutos en cada fase de línea de base (Basal), durante la exposición (Exp) y después de retirar el estímulo (Pos). Las respuestas conductuales se clasificaron como positivas (P+), negativas (N-) y otras (Ot). El P+ promedio aumentó significativamente (p < .05) del Basal al Exp, pero no fue estadísticamente diferente (p = .6) entre el Basal y el Pos. Ni N- ni Ot cambiaron estadísticamente en ninguna de las fases (p > .5). La metodología que utilizamos con los estímulos olfativos fue eficiente, pareciendo aumentar el bienestar de los aguará guazú.

Keywords:
bienestar animal, comportamiento; cánidos, enriquecimiento olfativo.

Tabla de Contenido

Recibido el 3 de octubre 2021; Aceptado el 27 de diciembre 2021
Editaron este artículo: Mariana Bentosela, Paula Abate, Sebastián Miranda y Maylin Martínez Muñoz

Captivity can be tedious and severely limit the realization of species-typical behaviors that would be expressed in the wild (Meagher, 2019). Environmental enrichment (EE) can be defined as any technique or method to improve the biological functioning of a captive animal through modifications to its environment (Newberry,1995). EE is directly linked to animal welfare because it is a powerful way to improve the physical, psychological, and cognitive health of individuals (Newberry, 1995). EE is an ethical and legally mandatory procedure in zoos because it is recognized that animals are sentient beings (Pierce & Bekoff, 2018). Captivity is a controversial condition in which to maintain wild animals, but poor welfare can be considered a kind of cruelty, widely condemned by society (Pierce & Bekoff, 2018).

Implementing an EE program for zoo animals faces some theoretical and practical obstacles.

[a] Federal University of Viçosa, Postgraduate Program in Animal Biology, Viçosa, Minas Gerais, Brazil
[b] Federal University of Viçosa, Department of Animal Biology, Viçosa, Minas Gerais, Brazil
[c] Federal University of Southern Bahia, Institute of Humanities, Arts, and Sciences, Itabuna, Bahia, Brazil
[d] Federal University of Southern Bahia, Postgraduate Program in Environmental Technologies and Sciences, Porto Seguro, Bahia, Brazil
*Enviar correspondencia a: Boere, V. E-mail: vannerboere@uol.com.br

Citar este artículo como: Figueira, M. P.; Rodrigues Silva, F. F.; Ribeiro, A.; Oliveira Silva, I. & Boere, V. (2023). Behavioral responses of captive maned wolves to olfactory enrichment: a preliminary study. *Revista Argentina de Ciencias del Comportamiento, 15*(2), 26-34.

First, how animals respond to environmental changes must be understood and the way in which they express behaviors that are part of the species' natural repertoire (Meagher, 2019). The modified behavior should not increase the vulnerability of the animals to health problems or stress while in captivity. Second, the modifications must be inexpensive, appropriate to the conditions of each zoo, and safe for both animals and keepers (Boere, 2001). Therefore, preliminary studies must be carried out inexpensive, and low risk to avoid accidents for either the animals or the zoo staff.

The largest South American canid, the maned wolf (*Chrysocyon brachyurus*, Illiger, *1815, Canidae, Carnivora*), is relatively common in zoos (Vasconcellos et al., 2009). It is known that canids, such as maned wolves, belong to a mammalian family with high olfactory sensitivity (Langguth, 1975). Maned wolves are active animals that hunt small prey and consume fruits that are dispersed across their territory (Langguth, 1975). The daily journey of a maned wolf can be up to 16 km, mostly during the night, when the nostrils are important to distinguish a great diversity of food sources, prey, predators, and other wolves in the territory (Bandeira De Melo et al., 2007).

The maned wolf is distributed across some countries where scrubland, grassland, pampa, and high-altitude prairie occur (Rodden et al., 2004), but it is considered to be “near threatened” by the International Union of Conservation of Nature (Paula & DeMatteo, 2015). Some specimens have been captured in areas under threat by human activity or were found to have injuries from being run over, then being sent to wild animal care centers. Under these circumstances, few of these animals return to the natural environment, living their entire lives in captivity (Paula & DeMatteo, 2015). A part of this population that has been treated for health is cared for zoos, where they can eventually be studied in terms of physiology, morphology, behavior, and disease (Maia & Gouveia, 2002).

Only 3% of studies on EE address olfactory stimulation and increased well-being in canids (Clark & King, 2008). Accordingly, there are few studies on olfactory enrichment (OE) with captive maned wolves in peer-reviewed journals (Coelho et al., 2012; Cummings et al., 2007; Vasconcellos et al., 2009). In most cases, the wolves were subjected to tasks where they act as operative agents that make direct contact with stimuli within their own enclosure. Toys and hidden foods were introduced, causing the wolves to be active in seeking and manipulating objects (Coelho et al., 2012; Cummings et al., 2007; Vasconcellos et al., 2009). The tasks to the wolves required a general use of sensory, motor, and cognitive systems, but did not deal exclusively with olfactory stimuli.

This study aims to distinguish how odors can be part of an EE program, by investigating the behavioral effects of olfactory stimuli on maned wolves. The goal was to develop a method of EE that would increase well-being, measured against two targets: first, an increase in behavioral repertoire that is positive, that is, it results in health benefits, satisfies behavioral needs, and represents a state of “enjoyment” (Browning, 2020). The second objective is a decrease in the repertoire of negative behavior (Ferdowsian & Merskin, 2012) that may be associated with health problems, anxiety, fear, and prolonged stress in animals.

Methodology

The study was carried out with five healthy maned wolves housed in pairs (with the exception of one solitary animal). The individuals observed were: a male and a female at the São Carlos Ecological Park in São Carlos – São Paulo; a male and a female at the Parque Zoologico Municipal Quinzinho de Barros in Sorocaba – São Paulo; and a male at the Bosque Zoo Fábio Barreto in Ribeirão Preto – São Paulo. The individuals were adults between two and eight years old, and had been habituated to captivity since birth or had arrived very young at the institutions. All enclosures have a size of approximately 300m^2, with grass, trees, and a pond inside. Within each enclosure, there is a shelter where the wolf can hide. The wolves are fed a mix of fresh fruits, protein having animal origin, and industrialized dog food on a daily-basis during the morning. The study was conducted outside of the wolves’ mating season and none of them had any pups during the study.

The protocol used in this study is similar to that written in another article where it was observed the behavior of the Crab-eating foxes (*Cerdocyon thous*) exposed to olfactive enrichment (Figueira et al., 2021). The procedures are described below. After consulting the scientific literature on EE for canids (Coelho et al., 2012; Cummings et al.,

2007; Vasconcellos et al., 2009), four odorous stimuli (OS) were selected: 100 g of fresh minced beef, two chopped boiled eggs, 100 g of chopped parmesan cheese, and soiled sawdust of breeding box impregnated with mouse urine. Parmesan cheese is not recorded as being a regular food for captive maned wolves, but a food with a strong piquant odor, which the wolves would be highly responsive to was felt to be necessary. The olfactory stimuli were placed inside permeable cotton bags (10 cm x 10 cm x 5 cm), which allowed the animals to have access to only the content's odor, without being able to directly see it. The bags were each of the same color and the same size, and were not used for the same animal with the same odor.

In order to better analyze the effects of the OS, behaviors were categorized as positive (P+), negative (N-), or other (Ot; Table 1), based on the 'Five Domains' concept, according to the proposal of Mellor (2016). The behavioral categories (positive or negative) are associated physical/functional domains, and are linked to negative or positive affects assigned to the mental domain (Browning, 2020; Mellor, 2016). In addition to the need to express natural behavior, the affective experience is linked to the welfare condition of the animals (Browning, 2020). The behavioral categories are therefore also the result of evidence recorded in other studies on environmental enrichment for maned wolves (Coelho et al., 2012; Cummings et al., 2007; Vasconcellos et al., 2009).

Table 1.
An ethogram for the OE study on five maned wolves (Chrysocyon brachyurus) in captivity

Behavior	Behavior description	Behavioral category
Play	Individual interacts with the environment or with another animal in a playful way, relaxed.	P+
Non-agonistic social interaction	Individual shows friendly behavior towards another animal such as licking or grooming.	P+
Attempting to reach the OE	Individual tries to reach the OE with its paw through the cage.	P+
Self-maintenance	Individual bites or licks, slowly and calmly, parts of its own body.	P+
Sniffing	Individual moves its nostrils, pointing towards objects or regions of the enclosure.	P+
Sniff or point OE	Animal points its snout in the direction where the OE is or was placed.	P+
Agonistic behavior	Individual shows signs of aggression such as growls, baring of teeth, scratching or biting another animal.	N-
Biting the cage	Individual bites or pulls the cage with its teeth.	N-
Yawning	Self-defined behavior.	N-
Scratching itself	Individual rubs one leg or its mouth vigorously on its skin or hair.	N-
Stereotypy	Individual perform repeated movements, more than three times for no apparent reason.	N-
Climbing the railing or wall	Individual stands up and supports its front limbs on the railings or walls of the enclosure.	N-
Sneeze	Self-defined behavior.	N-
Others	This means any activity not listed in the behaviors described like P+ or N-	Ot
Out of sight	Focal animal is out of sight of the observer.	Ot

Note. P+, positive behavior; N-, negative behavior; and Ot, other behavior.

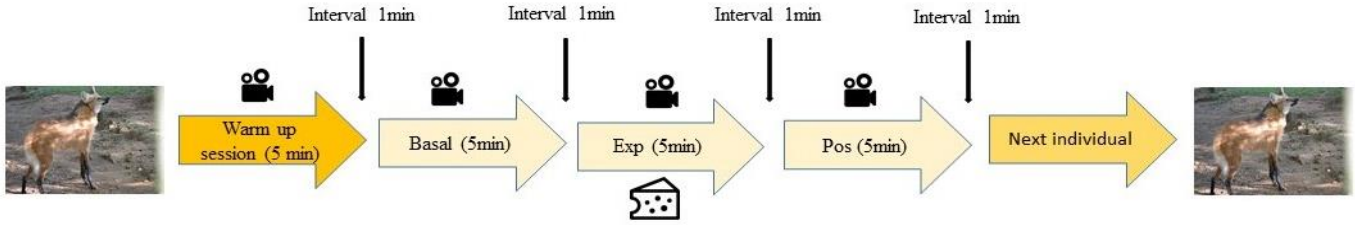
The P+ category brings together behaviors that increase animal welfare, while the N- category behaviors involved risks to health, suffering, and stress (Boissy et al., 2007; Ferdowsian & Merskin, 2012). Behavior considered ambiguous or indifferent to OS, which would have minimal influence on the well-being of maned wolves in captivity, were categorized as "other behaviors"

(Ot).

The behavior of the individual was recorded with a digital camera (Samsung® ST77, South Korea), which were fixed to a tripod in front of the cage of each enclosure at a height of 1.5 m. The filming took place in the morning between 8 am and 10 am, before the animals were fed. The order in which the OS were presented and focal wolves were previously defined by chance.

The film recording began with a “warmup” session, which consisted of repeating the same movements necessary for the assembly of the stimulus, but without actually exposing any OS (Figueira et al., 2021). This warmup session aimed to avoid a relationship between the researcher's movements and the stimulus itself (Goulart et al., 2009). Soon after, the OS were positioned in front and on the outside of each enclosure, starting the baseline session (Basal), a 5-minute session, where the wolf was filmed, but without presenting any OS. Following that time, with a one-minute interval, the exposure session (Exp) begins, when the researcher entered the corridor, placed the OS and left again. At the end of the Exp session, the researcher entered the external central area of the enclosure to remove the OS and left again, starting the post exposure (Pos) session, when the individual was filmed for another 5 minutes without the stimulus, which had been removed during the one-minute interval. The observations of the focal animal ended when the Pos session ended. Each wolf was exposed only once to the four stimuli, with no possibility of replicating the same stimulus. An experienced researcher collected and recorded the behavioral data. Each focal animal session was considered as an independent event, but the Basal, Exp, and Pos phases for each stimulus were assessed as dependent on each other, for each individual. The experimental schedule is depicted in Figure 1.

Figure 1.
Experimental schedule for exposing maned wolves (Chrysocyon *brachyurus) to olfactory stimuli*



The individuals' behavioral responses were analyzed using the focal animal method and with all behaviors recorded (Martin & Bateson, 1993). The total instances of each behavior were counted with a free behavioral analysis software (PROSTCOM; Conde et al., 1999). The average and standard errors for each behavioral category (P+, N- and Ot) were calculated for each phase of the session (Basal, Exp, Pos), resulting in a general mean for all stimuli. The average of each behavioral category was calculated from the mean for all wolves. To check for statistical differences, the Basal session was compared with the Exp session and the Pos session. Due to the small sample size, the distribution was non-normal, therefore non-parametric analyses were performed, applying the Wilcoxon test for paired samples with α ≤ 5% (Zar, 1999).

Ethical Aspects

The Ethical Committee of Animal Use (CEUA) of the Federal University of Viçosa approved the study, Protocol number no. 09/2013.

Results

The results from the statistical test are summarized in Table 2. The behaviors from the P+ category increased significantly ($p < .05$) from the Basal phase to the Exp phase, but were not statistically different ($p = .60$) in the Pos phase compared to the Basal phase (Table 2). There was a significant decrease ($p = .02$) in Ot category behaviors from the Basal phase to the Exp phase, but there was no difference between the Pos phase and the Basal phase ($p = .50$). The mean time for category N- behaviors did not differ significantly from the Basal phase to the Exp phase ($p = .66$) or from the Pos phase to the Basal phase ($p = .53$).

Table 2.
Behavioral response of maned wolves (Chrysocyon brachyurus)

Behavioral category	OS	Mean ± Standard error	*Z* value	*p*
P+	Basal	2.83 ± 2.31	- 1.99	.05*
	Exp	15.97 ± 8.68		
N-	Basal	1.38 ± .42	- .45	.66
	Exp	2.56 ± 1.29		
Ot	Basal	295.83 ± 2.59	- 2.27	.02*
	Exp	281.48 ± 9.50		
P+	Basal	2.83 ± 2.31	- .52	.60
	Pos	1.70 ± .89		
N-	Basal	1.38 ± .42	- .62	.53
	Pos	1.30 ± .62		
Ot	Basal	295.83 ± 2.59	- .68	.50
	Pos	297.03 ± 1.15		

Note. Experimental schedule of olfactory stimuli (OS), during the Basal (no stimuli), Exp (exposure to stimuli), and Pos (post stimuli) phases. Wilcoxon test (Z) and significance level (*P*) in comparison of the average time spent with behavioral responses P+ (positive behaviors), N- (negative behaviors), and Ot (other behaviors). *N* = 5.
* Significant difference

Discussion

The selected OS stimulated P+ and decreased Ot, but only during the time when the wolves were exposed to the stimuli. N- was unchanged during any phase. This result, taken as a whole, shows the relative success of the method, which was countered by the lack of reduction in negative behavior and the lack of a more lasting positive effect from exposure to the stimuli.

When responding to the presence of olfactory stimuli, increased behaviors indicative of well-being is part of the behavioral repertoire the wolves would carry out in a natural environment, being mimicked in captivity (sniffing or pointing at OS, attempting to catch the OS, and sniffing; Cummings et al., 2007). Taken together, these behaviors are related to a search for the source of the odor. The positive behaviors of non-agonistic social interaction, play, and self-maintenance are highly suggestive of a relaxed state and increased welfare (Boissy et al., 2007). It seems that the increase in positive behavior was focused on the OS, accompanied by a general relaxation (which supposes enjoyment).

The reduction of behaviors in the other category (Ot) suggests a trade-off with searching for the OS. The wolves seemed to change their motivation in order to perform the set of behaviors focused on the new stimulus (OS). Nevertheless, the interpretation of the reduction in Ot behaviors must be made cautiously, because it is an ambiguous behavioral category with regard to its influence on welfare. For example, resting is ambiguous as it could indicate apathy or effectively, economy of energy, such as is observed in nocturnal animals during the daytime in the wild (Cummings et al., 2007). Maned wolves are solitary and shy (Bandeira De Melo et al., 2007), thus the “out of view” behavior is part of a regular reaction of wolves when faced with an unknown stimulus, and it does not necessarily indicate a decrease in well-being, though it could be an elusive behavior, resulting from fear or anxiety (Mason, 2010).

It was defined operationally that enrichment would be assessed by attention directed towards the stimulus, accompanied by a state of relaxation and a decrease in negative behavior. The success of the method was compromised by the stability of N-, which neither increased nor decreased in any of the phases. A decrease in N- was expected, because it includes behavior that is harmful and undesirable for animals in captivity. This category contains stereotypical behaviors that suggest poor adaptation of the animal to captivity, although they may have positive aspects that alleviate some specific behavioral need (Browning, 2020). Scratching is another type of behavior that has been associated with anxiety in captive animals

that do not have skin disorders (Cannas et al., 2014). From the point of view of animal handling and safety, agonistic behavior can be risky as it may escalate into aggressions that result in severe injuries (Mason, 2010). These and other behaviors described as negative, taken together, can lead to health impairment and increased stress (Mason, 2010). For the staff zoo, negative behaviors are undesirable because they increase the risk of health problems and adaptation of captive animals. Therefore, a decrease in N- was not achieved with this enrichment method, partially frustrating the goal of increased the wolves well-being.

Comparison with other studies

In one study (Cummings et al., 2007), EE was carried out by exposing three maned wolves to toys, boomer balls, and hidden food, resulting in overall positive behavioral changes (i.e., increased activity and exploratory rates). This EE demanded some level of control from the wolves, with tactile exploration of the objects, some level of cognition, and use of the olfactory apparatus to find the stimuli. While toys and boomer balls took less time to explore, hidden food especially provoked an increase in positive behavior, suggesting that the introduction of objects is not an effective enrichment strategy (Cummings et al., 2007).

In another study, the authors introduced five foraging stimuli, as well as interspecific and intraspecific enrichment items, to three maned wolves (Coelho et al., 2012). Foraging EE from that study consisted of four different hidden or wrapped foods and an artificial bird's nest with two chicken eggs. The interspecific and intraspecific EE were a mix of visual items (stuffed animals), vocalization playbacks, and olfactory stimuli. The analysis and results from this study suggest that maned wolves responded positively to EE in a foraging context, but not to others (intraspecific and interspecific stimulus; Coelho et al., 2012).

The work carried out using enrichment through tasks is postulated to be more effective, because maned wolves appear to prefer to control the stimulus, thereby reducing their stress (Mason, 2010). The tasks used to obtain food, known as "contrafreeloading', are characterized by a strategy that challenges animals to obtain food in the presence of freely-available food (Vasconcellos et al., 2009). With this EE, individuals spend time and effort that results in a change of routine, expressing behaviors such as exploring and moving. The authors (Vasconcellos et al., 2009) observed eight maned wolves in a "contrafreeloading" experiment, exposing them to food concentrated on two trays or scattered about the enclosure. The authors found that the wolves preferred to search for the scattered food instead of the food concentrated on two trays, indicating that "contrafreeloading" is the natural disposition of maned wolves.

The present study has both convergences and divergences when compared to the experiments cited above. The tasks require some contact between the individual and the object, but this has some disadvantages, such as the cost related to the team's work in entering the enclosure to introduce, remove, and clean the objects. There are other disadvantages such as the accumulation of dirt and the accidental ingestion of debris from objects, which could require veterinary care. In relation to EE with objects, sensory miscellanea, and "contrafreeloading" (Coelho et al., 2012; Cummings et al., 2007; Vasconcellos et al., 2009), this method circumvents these problems by using significant stimuli placed in the external environment, preventing contamination of the enclosure and accidents due to ingestion of foreign bodies by the wolves. Furthermore, contrary to the olfactory stimulus of odors from other animals, this study's enrichment is convergent with a foraging context, which seems to be the most effective form of EE.

Some divergences arose in the comparison between this study and those of other researchers, because an increase in positive behavior was observed with the combination of olfactory exploration and greater relaxation. These differences may be related to dissimilar methods, or due to sample size, exposure time, or stimulus type. In the current study, five wolves were observed, while in other studies, the sample size ranged from three to eight (Coelho et al., 2012; Cummings et al., 2007; Vasconcellos et al., 2009). In this study, the exposure time was short, avoiding the observation of the prolonged effect of the stimulus. On the other hand, many stimuli used as EE (toys) seem to lose the interest of individuals in captivity (Kuczaj et al., 2002). We suggest that short exposure to various olfactory stimuli sequentially can maintain interest and P+ expression in wolves. The stimuli used were exclusively olfactory, unlike other studies that used

tasks, toys, and a mix of sensory stimuli. It is not clear whether one method has an advantage over the other; therefore, it is likely that OS enrichment can be complementary to EE programs that use different methods.

The method used in the present study was applied to 22 crab-eating foxes in zoos (Figueira et al., 2021). In that study, foxes increased P+ during and after exposure to the stimulus. N- increased from baseline to exposure, but decreased after stimulus withdrawal. For crab-eating foxes, the protocol used was successful as EE. The difference from that study with this one made with maned wolves, may be due to the small number of individuals, the characteristics of the enclosure and the behavioral disposition of the species *C. brachyurus* to be more elusive to novel stimulus.

A glimpse on neural and functional implications of OE

Task-based EE appears to be highly motivating and it allows the maned wolves to express behaviors that would be performed in the wild. Movement and performance of tasks stimulate areas of the brain related to cognition, as has been demonstrated in many experiments in laboratory animals and canids over time (Petrosini et al., 2009; Horowitz & Franks, 2020). Similarly, cumulative evidence has shown that stimulating odor-related areas of the brain in experimental animals triggers an increase in brain plasticity over a long time, resulting in brain and cognitive "resilience" to stress and aging (Horowitz & Franks, 2020). Brain and cognitive resilience are increased because EE can result in significant changes in brain biochemistry, synaptic connectivity, and neuronal function. The results from EE or OS are supposed to be augmented recovery capacity against brain injury, stress, and cognitive challenges. Considering the homology of behavioral results between laboratory animals when provided to EE, it seems that EE and OS tasks both lead to cognitive improvement. We speculate that for maned wolves in captivity, the EE could improve the adaptation to stressful events due the stimulation of cognitive and emotional neural substrates.

Limitations and importance of the study

Sensorial enrichment that uses harmless, non-stressful stimuli are likely to result in the greatest benefits to animal welfare (Wells, 2009). When introducing EE, there are pragmatic issues that must be taken into account, such as the risk of causing injury, the safety of the animals and handlers, and the cost of the method. Exposure to odors, as presented in this study, avoids contact between animals and objects, ensuring less contamination and fewer accidents involving ingestion of foreign bodies. The zookeepers are safer because they do not need to enter the enclosure, avoiding exposure and disturbance of the animals. The cotton cloth bag used to visually conceal the stimulus is cheap (approximately US $ 1.0 each) and can be reused for a long time. This method allows for enormous sensorial flexibility when alternating among several sources, making it safe, cheap, and flexible, with relative effectiveness in increasing the wolves' welfare.

The present study takes an approach that is not regular in other studies on EE in maned wolves. There was an assessment of positive behaviors, but also neutral and negative behaviors. Although it may seem arbitrary, the empirical and theoretical basis for defining these behavioral categories is well grounded in the present study. More importantly, this study not only evaluated positive behaviors, but also those behaviors that might be disadvantageous to the well-being of maned wolves, such as negative behaviors. This type of assessment in EE studies is not common, because most articles in the scientific literature show the effectiveness and efficiency of EE techniques. Therefore, the present study has the differential of showing the undesirable and potentially harmful consequences to the well-being of maned wolves with EE.

This study did have some weaknesses, such as its small sample size and short exposure time. Animals in zoos are not abundant and easily available for studies, because they are difficult to breed and often (in the case of canids) acquired due to the impossibility of introducing or reintroducing them to their original habitat. The management of animals in zoos is dependent of many protocols (health, safety, handling, ethical, and legal). Consequently, it is common in studies on enrichment to use few captive animals in zoos (e.g., Coelho et al., 2012; Vasconcellos et al., 2009). This means that one must be cautious in interpreting the results obtained, because biases such as personality and individual disposition may not allow for generalization (Coelho et al., 2012). In this and other canid studies carried out by the same team, the immediate aim is not to establish

an EE program, because the operating conditions at each zoo are different and long-term adaptations would be necessary. This study aimed to probe which techniques and methods could achieve effective sensory enrichment in maned wolves, and to make available the knowledge of researchers with the expertise of zoo staff. Consequently, the cautious five-minute three-phase protocol used in each observational session was sufficient to record the behavioral response of the wolves, without abruptly modifying routines pre-established by the zoo's technical personnel.

Conclusion

Olfactory stimulation, according to the methodology used, appeared to have been successful to increase positive behavior in maned wolves. However, the effect was not prolonged and negative behavior was unaltered. To achieve complete success with this method, further studies are required that allow the stimuli to remain for longer periods and that perform more tests using other odors. The present method is safe, flexible, low cost, and can be used complementarily with other EE methods for maned wolves.

Acknowledgments

Milene Paula Figueira was a CNPq (Brazil) fellow during her studies. The authors are immensely grateful to Dr Cecilia Pessuti and zoo staff for their collaboration with this study. We thanks to Mrs Liz de O. B. S. Souza for help us on schedule design. The authors received help to pay for the translation of the article from the Postgraduate Program in Environmental Technologies and Sciences, Federal University of Southern Bahia.

Data availability

The entire data set and analytical method details that supports the results of this study are available upon request to contact author, Vanner Boere (PO Box 51, Ilhéus, Bahia, Brazil, CEP 45653970; vannerboere@uol.com.br). The dataset is not publicly available because there has been no formal request to zoos for wide publicity of the data, with the exception of dissemination in scientific articles.

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