Scent-marking and maintenance behaviour of captive red brocket males (*Mazama rufa*) kept eight hours in a new environment

Marcação de odor e comportamento de manutenção de machos veado-mateiros (*Mazama rufa*) mantidos oito horas em um novo ambiente

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ABSTRACT
Wild animals in captivity are often transferred to other facilities when cleaning or maintenance of the facilities where they live is carried out. The aim of this study was to assess the behavioural response of five red brocket males (Mazama rufa) after being moved to a new environment. The animals are kept in individual stalls, being eventually moved to an unfamiliar stall, previously occupied by other individuals. The latencies and frequencies of scent-marking and maintenance behaviours were recorded for eight hours after moving each animal to the new stall. Licking behaviour was the behaviour most frequently displayed (20 to 62 times), with latencies ranging from 0.02 to 12.17 min. It was followed by urinating/defecating with a frequency of 24 to 56 times (latency 2.13-46 min) and flehmen, with a frequency of 11 to 24 times (latency 0.1-21.08 min). Rubbing was observed in only two deer. The Pearson’s correlation coefficients showed a positive association between flehmen and licking latencies and urinating/defecating and sleeping latencies (P<0.05, for both). It also showed that flehmen and urinating/defecating frequencies are inversely related (P<0.05). The behaviour of M. rufa males after being moved to an unfamiliar stall were characterized by common exploratory and scent-marking behaviours.

Keywords: animal behavior, captive wild animal, Cervidae, neotropical deer, territory marking.

RESUMO
Os animais silvestres em cativeiro são frequentemente transferidos para outras instalações para limpeza ou manutenção das instalações onde vivem. O objetivo deste estudo foi avaliar a resposta comportamental de cinco machos de veado-mateiro (Mazama rufa) após serem transferidos para um novo ambiente. Os animais são mantidos em baías individuais, sendo eventualmente transferidos para uma baia desconhecida, previamente ocupada por outros indivíduos. As latências e frequências de marcação de cheiro e comportamentos de manutenção foram registrados por oito horas após a mudança de cada animal para a nova baia. O comportamento de lamber foi o comportamento mais frequente (20 a 62 vezes), com latências variando de 0,02 a 12,17 min. Seguido de urinar/defecar com frequência de 24 a 56 vezes (latência 2,13-46 min) e flehmen, com frequência de 11 a 24 vezes (latência 0,1-21,08 min). A fricção com chifres (rubbing) foi observada em apenas dois veados. Os coeficientes de correlação de Pearson mostraram associação positiva entre flehmen e latências de lamber e urinar/defecar e dormir (P<0,05, para ambos). Também mostrou que as frequências de flehmen e urinar/defecar estão inversamente relacionadas (P<0,05). O comportamento dos machos de M. rufa após serem transferidos para uma baia desconhecida foi caracterizado por comportamentos exploratórios e de marcação de cheiro comuns.

Palavras-chave: comportamento animal, animal silvestre em cativeiro, cervídeos, veado neotropical, marcação de território.

1 INTRODUCTION
The genus Mazama includes small deer species with simple antlers that inhabit Neotropical forests with dense vegetation (Eisenberg and Redford, 1999). Currently, the genus Mazama represents nine deer species occurring in Central and South America, with Mazama americana as the largest species and the most widely distributed throughout the neotropics (Merino and Rossi, 2010). The species, also known as red brocket, is recognized as cryptic species complex, in which M. rufa (Illiger, 1815) was recently validated as a separate species out of the M. americana complex (Peres et al., 2021).
Red brockets are very difficult to observe in nature, by its shy and wariness and slow and quiet walk through the forest (Varela et al., 2010). They are solitary and territorial deer, with some studies describing wild living in pairs during the mating season (Emmons and Feer, 1997) and showing a complex social interaction between adult females kept in captivity (MacNamara and Eldridge, 1987), suggesting that the species is territorial and polygenic, like most cervids (Varela et al., 2010). Hunting and other human activities directly affect the species, making it difficult to observe the red brocket individuals in open areas, limiting behavioural study of the species in nature (Varela et al., 2010). Therefore, captive conditions allow the proximity with the species facilitating the observation and better understanding of behaviours, such as marking, feeding, and reproduction (Azevedo, 2008). In addition, management of captive neotropical deer is an important tool for conservation, allowing to assess relevant behavioural information of the species that are difficult to access in the wild (Nogueira-Neto et al., 2021).

However, this is not an easy task, since these animals are highly susceptible to stress, which invariantly results in behavioural changes, characterized by loud snort, raising their tail and fleeing when threatened, or hitting the ground with their forelegs (Varela et al., 2012; Emmons and Feer, 1997).

In this context, understanding the behaviors related to space use and communication are essential to improving captivity conditions, as the scent-marking behaviors, which evolved to express territory occupancy, potential sexual mate and health status to other animals (Black-Décima, 2000; Walther, 1984). Neotropical deer use feces, urine and licking as chemical communication among individuals (Rivero et al., 2004). Depending on their age, sex, life history and captive environment, animals may behave differently and this variation could be an adaptative or some way to communicate their identity (Hertel et al., 2020). As there is a lack of information about scent-marking in *Mazama rufa*, we aim to assess the behavioral response of five red brocket males of the species, raised and managed in similar conditions of captivity, after being transferred to a new environment. Our hypothesis is that under such conditions, deer males will display a high frequency of exploratory and scent-marking behaviors. The results described here may provide a benchmark data on the red brocket behavior, which can offer insights into how to improve the management of red brocket in captivity.

2 MATERIALS AND METHODS

The study was approved by the Animal Ethics and Welfare Committee (Comitê de Ética e Bem-Estar Animal, CEUA, Protocol number: 005828/19) of the Faculty of Agrarian and Veterinary Sciences (Faculdade de Ciências Agrárias e Veterinárias, FCAV), UNESP, Jaboticabal, SP, Brazil.
We analyzed five red brocket males born and raised at Deer Research and Conservation Center, from São Paulo State University, in Jaboticabal-SP, Brazil. All males correspond to *Mazama rufa* (Illiger, 1815), identified as: male 1 (six years old and having velvet antlers), male 2 (three years old and having velvet antlers), male 3 (four years old and having hard antlers), male 4 (three years old and having hard antlers) and male 5 (eight years old and having hard antlers). The deer were kept indoors, in captivity stalls with 12m², walls 2 meters high and the floor covered with straw, without any contact with other animals, leaving these facilities only when need to be handled.

Water was provided ad libitum and 0.5 kg of concentrated feed for horses with 1 kg/animal day of ramie (*Boehmeria nivea*) and red mulberry leaves (*Morus alba*). The animals were driven from their home stalls to a physically identical but unfamiliar stall. The only difference between the home and unfamiliar stalls was that the unfamiliar had scents of other individuals of the same species, which previously occupied the stall.

All behaviours were recorded for eight consecutive hours using video cameras (Intelbras Multi HD 3000) fixed on the ceiling of the stall. All recordings were analyzed using VLC software version 3.0.8. We recorded four behavioural categories related to scent-marking (licking, urinating/defecating, flehmen and rubbing) and two related to maintenance (eating and sleeping, as indicators of ‘adaption’ to the new environment), as described in Table 1. The latencies and frequencies of these behavioural categories were recorded during eight hours of observation from the introduction of each deer in the new stall. We tested the normality of the data and estimated Pearson’s correlation coefficients to assess the dependency between pairwise behaviours. A cluster analysis was performed through the Ward criterion, also called Minimum Variance Clustering Method, which performed a hierarchical grouping, of the separated frequency and latency data to analyze similarity clustering patterns. In addition, we used the chi-square test to compare the frequencies of the observed behaviours of the five males. All statistical analyzes were carried out using the RStudio (2020) software package.
Table 1. Behavioural categories recorded of five captive red brocket deer males when kept in an unfamiliar environment (adapted from MacNamara and Eldridge, 1987 and Nogueira-Neto et al. 2021).

| Behavioural categories | Description |
|------------------------|-------------|
| Licking                | Individuals walked about their enclosures and paused at specific contact spots to move the tongue across the surface, usually of fence posts, trees, corners or edges of wooden doors or metal shelters. |
| Urinating/defecating   | Also called elimination postures. The animals assume an inconspicuous posture, hind legs slightly extended out and backward and the tail up. Then, the animals excreted urine or fecal pellets. |
| Flehmen                | The animal stands straight, extends its neck and lifts its head, opens its nostrils wide, with a small mouth opening with curling of the upper lip, often exposing the upper gum. Performed after smelling or licking an object, the ground, or their own or a conspecific animal’s urine or feces. |
| Rubbing                | The front of the head between the eyes and antlers was pressed and moved vertically up and down against objects. Also, vertical head movements while pressing the antlers against an object. |
| Eating                 | The animals mouthed and chewed fallen twigs and branches. |
| Sleeping/resting       | Act of lying down upright with legs folded under the body. The dorsoventral axis of the body is orientated in the vertical plane, and the animal then bears most of its weight on its hind feet, forefeet and rostrum. In the stretch position on the other hand the long axis of the body is more or less straight. The front feet are often tucked under the body, but sometimes may extend in front of the animal. |

3 RESULTS

Just after being moved from the familiar to the unfamiliar stall, the animals performed scent-marking behaviour. First, they licked the door and walls (latency 0.02-12.17 min) and then, showed some investigative behaviour as smelling, walking and ear movements. After smelling the door, floor or walls, all deer showed flehmen response (latency 0.1-21.08 min). Two males performed rubbing in doors and walls, associated with licking and forehead marking (latency 0.45-26.42 min). All males urinated/defecated the stall (latency 2.13-46 min), characterized by postures of elimination and brief and repeated expulsion of urine drops or fecal pellets. After the first performance of marking and investigative behaviours, all animals started eating in less than one hour (latency 9-48 min) after entering the new environment. They consumed branches, leaves and straw from the ground. Besides the short eating latency, all animals continued showing scent-marking behaviours. Finally, the males slept (latency 275-480.05 min), always in lying posture after exploring the unfamiliar environment (Table 2).
Table 2. Absolute frequencies and latencies (min.) of scent-marking and maintenance behaviours of five captive red brocket deer males kept during eight hours in an unfamiliar environment (SD: standard deviation; IQR: Interquartile range.

|                  | Frequencies |              |         |         |         |         |         |         |
|------------------|-------------|--------------|---------|---------|---------|---------|---------|---------|
|                  | Urinating/defecating | Licking | Rubbing | Flehmen |         |         |         |         |
| Male 1           | 24          | 20           | 0       | 23      |         |         |         |         |
| Male 2           | 24          | 50           | 0       | 24      |         |         |         |         |
| Male 3           | 56          | 53           | 20      | 11      |         |         |         |         |
| Male 4           | 53          | 62           | 10      | 13      |         |         |         |         |
| Male 5           | 35          | 55           | 0       | 14      |         |         |         |         |
| Mean             | 38,4        | 48           | 6       | 17      |         |         |         |         |
| SD               | 15,4        | 16,26        | 8,94    | 6,04    |         |         |         |         |
| IQR              | 30,5        | 23,5         | 15      | 11,5    |         |         |         |         |

|                  | Latencies (min) | Urinating/defecating | Licking | Rubbing | Flehmen | Eating | Sleeping |
|------------------|-----------------|-----------------------|---------|---------|---------|--------|----------|
| Male 1           | 2,31            | 5,24                  | 0       | 7,12    | 28      | 344    |          |
| Male 2           | 46              | 12,17                 | 0       | 21,08   | 48      | 470,05 |          |
| Male 3           | 7,33            | 2,16                  | 0,75    | 0,25    | 40      | 40     |          |
| Male 4           | 2,13            | 0,38                  | 26,42   | 4,15    | 24      | 275    |          |
| Male 5           | 32,28           | 0,03                  | 0       | 0,1     | 9       | 467    |          |
| Mean             | 18              | 3,99                  | 5,43    | 6,54    | 29,8    | 371,21 |          |
| SD               | 19,99           | 5,01                  | 11,73   | 7,72    | 15,03   | 92,21  |          |
| IQR              | 36,92           | 8,5                   | 13,58   | 13,92   | 27,5    | 181.02 |          |

In all observed males the licking behaviour was the more frequent scent-marking performed 20 to 62 times in eight hours. It was followed by urinating/defecating with a frequency of 24 to 56 times. Flehmen was performed with a frequency of 11 to 24 times in the study period. Rubbing was observed in only two deer with a frequency of 10 to 20 times in each male. We did not measure eating and sleeping frequencies, for these behaviours, we only analyzed their latencies, as described above.

The frequencies of licking, flehmen and urinating/defecating were very similar in male 1. Licking was the most frequent behaviour in males 2 and 5, with lower frequencies of occurrences of flehmen and urinating/defecating. These three animals did not rub. Males 3 and 4 had a similar pattern of behavioural frequencies with licking and urinating/defecating as its most common scent-marking behaviour, followed by a less frequent flehmen and rubbing (Figure 1).
When comparing behaviour frequencies, we observed an individual variation of all behaviour performed. Flehmen was observed in all animals, therefore it was more often shown by males 1 and 2. Licking frequencies were similar among males 3, 4 and 5, being more frequent in male 5 and less frequent in male 1 (outlier value). Urinating/defecating frequencies were also high in males 3, 4 and 5. Only two individuals presented rubbing behaviour, in this case, male 3 performed it more often than male 4 (Figure 1).

Two males (2 and 3) apparently showed pacing stereotypy, walking from one side of the stall to the other with circles of eight shape. In addition, one deer (male 3) rub-urate the tarsal gland, pawing the ground slightly forward-back and ventral-medial direction, in a way that brought the paws into proximity, urinating the tarsal glands while rubbing the glands with the ground. This last behaviour was performed by male 3 after the two-last urinating/defecating episodes and before sleeping.

The Pearson’s correlation showed a negative association between flehmen and urinating/defecating frequencies ($r = -0.916, P<0.05$), and a positive correlation between flehmen and licking latencies ($r = 0.954, P<0.05$) and urinating/defecating and sleeping latencies ($r = 0.929, P<0.05$). The cluster analysis recovered two groups based on similarities patterns of separated frequency and latency data (Figure 2). In the behavioural frequencies analysis, one group was formed by males 3 and 4, with low flehmen frequency and higher licking and urinating/defecating frequencies when compared with the other group, formed by males 1, 2 and 5. In this last group, male 2 and 5 showed more similar frequencies compared with male 1. In the behavioural latencies analysis, one group was also formed by male 3 and 4, and within this group, male 1 in a more distant position, characterizing the individuals with the lower latencies. The other group was constituted by
males 2 and 5 with higher behavioural latencies. Finally, the chi-square of the observed behavioural frequencies (licking, flehmen and urinating/defecating) was significant (P<0.05).

4 DISCUSSION

Territorial marking is a strategy of animal communication and is shown by most of the deer species (Bowyer et al., 1987, Black-Décima and Santana, 2011). After being moved to an unfamiliar stall all deer expressed exploratory behaviour, licking the door and walls, walking and smelling. These behaviours were shown combined with scent-marking, which also involves licking in association with rubbing, flehmen and urinating/defecating. Despite this descriptive study confirmed the hypothesis that the new environment promoted the marking and exploratory response of the five red brocket, it was observed that these behaviours are performed for a relatively short time, evidenced in the sleeping latency (less than 275 min). Licking was the most frequent behaviour just after being introduced in the unfamiliar stall, and it occurs with the shortest latency. This investigative behaviour is infrequent when animals remain in their home stall or familiar cage, increasing dramatically when they stay in an unfamiliar cage previously occupied and marked by conspecifics (MacNamara and Eldridge, 1987).

Urinating/defecating was also observed in all animals, but with a great latency. Thus, after performing the exploratory behaviour (walking, liking and smelling the stall surfaces) the deer started showing the elimination posture, expelling few drops of urine and/or fecal pellets. Once that started the urinating/defecating, they repeated constantly, reaching the second highest average frequency when compared with other analyzed scent-marking behaviours. This eliminative scent marking behaviour was similar to that shown by brown brocket deer after detecting feces from other
individuals in the latrine, by showing exploratory behaviour followed by urination and/or defecation (Black-Decima and Santana, 2011). After detecting a strange odor (of previous deer), the males urinated/defecated the stall, renewing marks, to slept with its scent throughout environment, showing a positive correlation between urinating/defecating and sleeping (P<0.05) and revealing that latrine served as centers of communication (Black-Decima and Santana, 2011). This behaviour it is also called counter-marking used in a variety of species to over-marking the urine and feces as a type of chemical communication that relates aged urine and glands secretions as two important agents that bucks used to scent-marking their territories (Dixon, 2018).

Flehmen was another behaviour performed by the five deer. Previous studies associated flehmen with conspecific odors in the stall, because individuals usually performed flehmen in responses to urine (Allen et al., 2016, MacNamara and Eldridge, 1987; Henderson et al., 1980). In our study, the variation in the flehmen response was characterized by two performance patterns, with one group of males with a low flehmen frequency and higher licking and urinating/defecating frequencies compared to the other group with a high frequency of flehmen and lower licking and urinating/defecating, as shown in the cluster analysis. The presence of pheromones in the stall probably influenced the flehmen frequency, as the males invested more time in sensing the animal marks which previously occupied the stall. In this sense, the more the animal invested in performing the flehmen, the less often it performed other behaviours and vice-versa.

The positive correlation latencies between licking and flehmen was possibly associated with the longer the animal takes to sense the pheromones, the longer it will take to respond by leaving its own scent. The negative correlation of flehmen and urinating/defecating (P<0,05) could be associated with different adaptive strategies that the animals chose when perceiving the stimulus in the environment, influencing the more scent-behaviour the animals performed to mark the same area. These intercorrelated behaviours are also called coping style, where the strategies to handle stressful situations are correlate with physiological mechanisms, such as stress response and pheromones levels (Koolhaas, et al., 1999; Cervantes and Delville, 2007).

Rubbing behaviour was object-oriented, scraping the forehead and antlers (in all stages of growth) against the door and walls, in alternate cycles, following the pattern previously described (Black-Décima, 2000; MacNamara and Eldridge, 1987). In our study, only two males performed rubbing, contrasting with the other three that did not rub in eight hours of observation. Specifically, the two male that rub, one had 20 rubbing cycles in eight hours with a short latency of less than one minute, compared with the other male that rubs in 10 cycles with a latency of 28 minutes. These behavioural variability among individuals could be associated with individual experiences or coping style, as has been discussed in many ungulates studies as an adaptive strategy to feeding, movement
and social communication (Dall et al., 2004; Hertel et al., 2020). It was also observed in the cluster analysis of behaviours latencies, the formation of two differenced groups of shorter or longer latencies, where males 2 and 5 started marking faster than the others, which could probably be associated with individual variation in temperament or habituation to handling (Hertel et al., 2020).

Another example of individual variation is the rub-urinating the tarsal glands performed by one male, pawing the ground for marking. This behaviour has been described in adult males of white-tailed deer associated with scraping activity, that involves smelling and licking the ground, branch and fences (Kinsell, 2010; Hirth, 1977). Thus, through tarsal glands males communicates social status, dominance and individual recognition, specifically during nights, since deer investigate the tarsal odor of other individuals more frequently during nocturnal periods (Muller-Schwarze, 1971; Sawyer et al., 1993).

In this study, male 1 and male 2, walked from one wall to another, without scent-marking or eating during pacing. This type of invariant and repetitive behaviour pattern had apparent no goal or function, being characterized as a stereotypic pacing (Mason, 1991). Pacing is common in captive wild animals, described it as an animal walking in a distinct, unchanging pattern within its cage, ranging speed from slow in the exact same pattern (Novak et al. 2016; Poirier and Bateson, 2017). Other authors reported individual variation in pacing behaviour, when studying individuals Rhesus monkeys housed in similar environments, with different proportions of animals that paced (Lutz et al., 2003). Need to walk, boredom, stress and brain damaged are some of the multiple causes designed for this abnormal behaviour (Poirier and Bateson, 2017). For this reason, a great number of people and institutions that keeps wild animals in captivity are looking for strategies to reduce the possible causes of pacing (Vidal et al., 2016; Rose et al., 2017).

The responses of five red brocket males during eight hours after being moved to an unfamiliar stall were characterized by common investigative and scent-marking behaviour as smelling, walking, licking, flehmen and urinating/defecating. These marking behaviours, in addition to approaching the understanding of red brocket behaviours in nature, could be used as a reference to monitoring adaptation to a new outdoor or indoor environment of the species in captivity.

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DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
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