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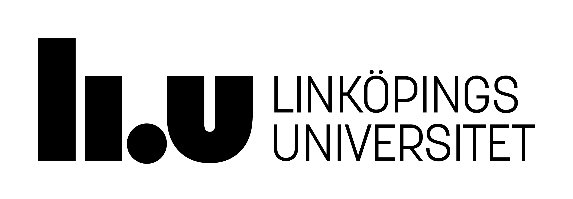
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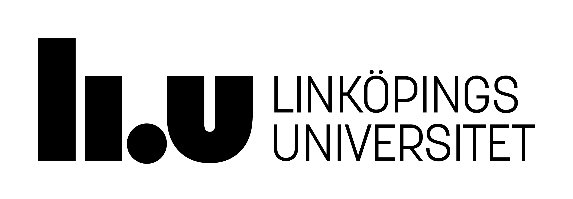
The effects of Cat Appeasing Pheromone on captive Iberian Lynx (*Lynx pardinus*)

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# **Abstract**

The most endangered felid species, the Iberian lynx (*Lynx pardinus*), is the reason why breeding centres were created around the Iberian Peninsula, hoping to reverse its population decline. This study was conducted in *Centro Nacional de Reprodução do Lince Ibérico*, in Portugal, and aimed at investigating the effects of a synthetic analogue of Cat Appeasing Pheromone (CAP) on the behaviour of 4 captive male lynx. The 2 months’ study was divided into Baseline and Treatment periods. During the latter, the control group was exposed to a placebo and the test group to CAP. The treatments were applied on a daily basis, and daily observations were conducted for two hours in the morning. The results showed a significant decrease of stereotypic behaviours in the test group. The periods prior to and after the experimental study were analysed to assess potential pre- and post-experimental study effects. Both groups showed a significant increase of stereotypic behaviours during the post-experimental period, which may be due to the relocation of the males after the experimental study, in order to pair them with females for breeding. Despite the small sample, the results confirm that CAP reduces stereotypies, and is a useful tool to enhance welfare in captive lynx. This study shows that pheromone therapy is a promising way of providing environmental enrichment for captive animals.

Keywords: CAP, Iberian lynx, pheromone therapy, stereotypy

# **Introduction**

The Iberian lynx (*Lynx pardinus* - Temminck, 1827) is one of the four extant species of the genus Lynx in the world, and it is, currently, the world’s most endangered feline. It has been Critically Endangered since 2002, but luckily in 2015 the IUCN has updated its status to Endangered (IUCN, 2015). It has a small natural range, since it is limited to Mediterranean scrubland of the Iberian Peninsula (Vargas *et al.*, 2009). In 1940, there were fifteen populations across the peninsula (Gil-Sanchéz & McCain, 2011), but the lynx’s habitat has gotten smaller over the past decades, and nowadays, there are only two populations in the South of Spain and one in the Southeast of Portugal. Anecdotal evidence pointed the cause of this species’ decline to be diseases of the European rabbit (*Oryctolagus cuniculus* - Linnaeus, 1758), the Iberian lynx’ basic prey. Gil-Sanchéz & McCain (2011) argue that rabbit diseases should not be considered the main reason for this decline, instead, the authors argue “human-caused mortality” to be the leading cause. In fact, the factors that contributed the most to such a population decline were habitat loss and fragmentation, direct persecution, and the population decline of the European rabbit (Vargas *et al.*, 2009). Taking all this into consideration, it became evident that something had to be done and there was enough evidence for Portugal and Spain to take action in the conservation of this species. As a consequence, breeding centres were created throughout the Iberian Peninsula with the aim of breeding suitable specimens for release into the wild, in an attempt to reverse the decline of the populations (Vargas *et al.*, 2009). Currently, there are four centres in Spain and one in Portugal, the *Centro Nacional de Reprodução do Lince* *Ibérico*, CNRLI (The Iberian Lynx National Breeding Centre). Like the rest of the centres of this Life+Iberlince breeding programme (Iberlince, 2011), the CNRLI faces some challenges. One of those is to create a captive environment that allows the animal to display most of its natural behavioural repertoire (Vargas *et al.*, 2009). This may be accomplished by providing enclosures that resemble their natural environment as much as possible and by promoting environmental enrichment (Vargas *et al.*, 2009) In addition, these centres try to reduce human-lynx interactions to a bare minimum, in order to avoid habituation to humans and potential domestication, which has long-term genetic and behavioural effects. Even though the programme strives to provide naturalistic enclosures, a captive environment always falls short on that task, especially considering the unavoidable size limitations. The home range of an adult lynx varies from 4 to 30 km2, depending on the sex of the animal and the availability of rabbit (Vargas *et al.*, 2009), and males tend to occupy larger home ranges than females (Vargas *et al.*, 2009; Jedrzejewski *et al.*, 2002). At the CNRLI, the total area of each of the 16 enclosures (Fig. 1) is 1000 m2, hence far less than the natural home range of the Iberian lynx.

Just like most breeding programmes, at the CNRLI there are adult lynxes of both sexes and cubs. The cubs are the ones meant for release and they undergo a training procedure before their release into the wild, which comprises hunting live prey, promoting human avoidance and short fasting periods. Not all adults breed every year, and their breeding status depends on the Programme’s management guidelines for each year (based on the animal’s genetic value and inbreeding coefficient). Some of the adult lynxes were born in the wild, but most are captive born. The wild-born animals were brought into captivity at a very young age, thus are well acclimatized to such living conditions.

Wild felids kept in captivity may exhibit behavioural changes that can be interpreted as a sign of poor welfare. Such behaviours can be simple abnormalities (such as apathy and abnormal aggressive behaviour) that seldom occur, or can be more frequent, being often referred to as stereotypies (Vargas *et al.*, 2009). These are repetitive and mostly functionless behaviours that result from attempts to adapt to an unfamiliar or unfulfilling environment (Macri & Patterson-Kane, 2011), and are common in many felids held in captivity (Vargas *et al.*, 2009). Swaisgood and Shepherdson (2005) and Clubb and Mason (2007) agree that space limitation is the most important stereotypy inducer for species with large home ranges, where they have to cover wide distances. Nevertheless, space is not the only factor to consider when trying to explain pacing or any other abnormal behaviours. Other potential factors may be psychological disturbances, tedium and social stress (Breton & Barrot, 2014), the presence of humans (Margulis *et al.*, 2003), the visibility towards other enclosures and the proximity of/to other animals. Swaisgood and Shepherdson (2005) gathered information from 41 studies on carnivores and concluded that there was a decrease of the observed abnormal behaviours once the enclosures were enriched. Environmental enrichment techniques may include the use of pheromones, and they are commonly used to prevent and correct these abnormal behaviours, but the occasional use of psychotropic drugs has also been tried (Vargas *et al.*, 2009). Affiliative pheromones have been found to prevent and decrease stereotypies, and felines seem less stressed and more relaxed when their environment was enriched with them (Martínez-Macipe *et al.*, 2015).

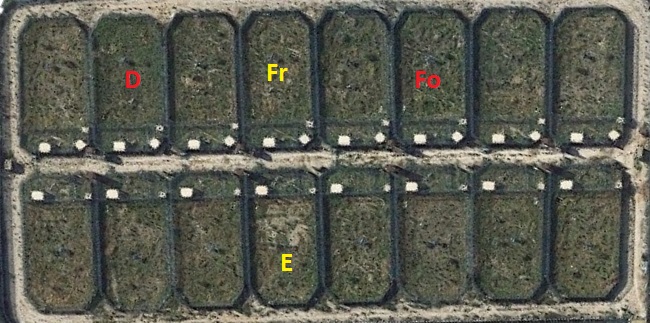
Karlson and Luscher (1959) were the first to identify pheromones, and they defined them as substances secreted by an organism to the outside, then causing a species-specific reaction on another conspecific. Pheromones do not necessarily have an odour and are not sensed in the same way as odours (Pageat & Gautier, 2003). Many animals, including felids, perform a behaviour that improves the perception of pheromones, called flehmen (Landsberg, 2006). During this behaviour, the animal curls back its upper lip, showing its front teeth, and moves its tongue while keeping its mouth half-open so that the pheromones can enter the vomeronasal organ (VNO) (Tirindelli *et al.*, 2009), which is part of the accessory olfactory system (Pageat & Gautier, 2003). The pheromones bind to pheromone binding proteins, activating certain receptors that stimulate the limbic system (Landsberg, 2006). This stimulation can have an effect on the emotional and/or the physiological state of the animal (Landsberg, 2006). Carnivores have glands that release pheromones, most of them are located in the skin and mucous membranes (Pageat & Gautier, 2003). These authors located six main areas from which pheromones are secreted in carnivores: the facial area, the pedal, perianal, genital and mammary complexes, and via urine and faeces. Most of the available literature focuses on pheromones secreted by glands in the facial area and the mammary complex. The synthetic analogues meant to be used in this study, Feliway® (an analogue of the facial pheromone F3) and Cat Appeasing Pheromone (CAP) belong to the feline facial pheromones and appeasing pheromones, respectively. The latter group is produced in the mammary complex, by the sebaceous glands (Martínez-Macipe *et al.*, 2015), and so far, appeasing pheromones have been isolated in some ungulates, dogs and cats (Pageat & Gautier, 2003). Appeasing pheromones have a calming effect on offspring and adults (Cozzi *et al.*, 2010), whereas the facial pheromone F3 promotes the emotional stability of the animal (Landsberg, 2006). Feliway® is commercially available, and is used by domestic cat owners who want to reduce their pets’ stress and anxiety levels, amongst other unwanted behaviours (Landsberg, 2006). At the time this thesis was written, CAP was not yet commercially available in Europe. Most studies (Spielman, 2000; Gaultier *et al.*, 2005; Macri & Patterson-Kane, 2011) conducted on wild felids held in captivity used Feliway® to test its effects on the reduction of stress, stereotypies and other abnormal behaviours. I was only able to find one study by Martínez-Macipe *et al.* (2015) using CAP, which successfully used it as enrichment. Ideally, this study would test the effects of both pheromones, but due to the available timeline, only one was tested. My CNRLI supervisor and I decided that a random draw would be the best way to impartially choose one of the two pheromones. CAP was the drawn one, thus the one to be tested.

Even though the centres involved in this breeding program strive to ensure the highest animal welfare standards, the applied environmental enrichment techniques are not always sufficient to prevent and/or reverse abnormal behaviours. Since this is the most endangered feline species, and it is imperative that the breeding efforts result in healthy and competent animals for release, the welfare in captivity is clearly important. This study aimed to assess the effects of synthetic feline pheromone on captive Iberian lynx males, and evaluate its potential role in reducing short and long-term stereotypies and other abnormal behaviours.

# **Materials and Methods**

## **Subjects**

Only males were used in this study, since at the time most females had cubs, and the study would compromise the cubs’ reintroduction training. The males were chosen considering their ability to endure two months of being restricted to the husbandry area (Fig. 2), and their location in the enclosure area (Fig. 1). Some males were sharing enclosures with females, thus making them unsuitable candidates. The experimental subjects were 4 Iberian Lynx males: Drago (8 years old), Enebro (7 years old), Foco and Fresco (both 6 years old). The males that displayed the highest frequency of stereotypies and/or other abnormal behaviours, Enebro and Fresco, were placed in the test group (and were exposed to the synthetic analogue of the pheromone) and the ones that displayed the least stereotypies, Drago and Foco, were placed in the control group (only exposed to the placebo). The location of their enclosures within CNRLI is represented in Figure 1, although the reader should bear in mind that the subjects only had access to the smaller section of the enclosure, the husbandry area (Fig. 2).

*Figure 1 - Enclosures at the CNRLI. Foco (Fo), Fresco (Fr), Drago (D) and Enebro (E) in enclosures 3, 5, 7 and 12, respectively*.

## **Experimental set-up**

The experiment was conducted at the CNRLI, in Silves, Portugal. There are a total of 16 enclosures (Figure 1), each of which is divided into two main sections: the large enclosure area and the husbandry area(Figure 2). The large enclosure area, measures 800 m2 and is meant to mimic the animal’s natural environment. The husbandry area is divided into two communicating areas, with a total area of 200 m2. The two communicating areas: the big husbandry area, where most platforms are located, and the small husbandry area, where the indoor quarter, is located.

The study period started on the 28th of September, 2015 and ended on the 30th of November, 2015. The experiment was conducted on a daily basis, from Monday to Saturday, during the males’ feeding time in the morning. The animals fast on Sundays, therefore the experiment was not performed on those days. To exclude potential novelty effects, all subjects were exposed to a control where water was sprayed on selected places within the enclosure during a period prior to the application of the CAP. This Baseline period lasted 4 weeks. After this, the test group was exposed to CAP for the remaining duration of the study (5 weeks), whereas the control group was exposed to the placebo.

This was a double-blind study, since at the time, the author, the observers and the keepers were unaware of which animals were part of which group, and which bottle contained CAP and the placebo. During the study, observers and keepers only knew that Enebro and Fresco received Treatment B, and Drago and Foco received Treatment C. Treatments C and B, respectively, the CAP and the placebo, and the spray bottles were provided by IRSEA - *Institut de Recherche en Sémiochimie et Ethologie Appliquée* (Research Institute in Semiochemistry and Applied Ethology). Treatment A (Feliway®) was also provided by IRSEA, but was not used, as mentioned in the Introduction section of this thesis.

## **Protocol**

The study protocol was designed in collaboration with the keepers and my supervisor at CNRLI, and was as follows:

1. The keepers sprayed 3 pumps (6 mL) of pheromone/placebo according to the order in Figure 2.
2. The keepers shut the animal inside MP, where it was offered food. When the animal had finished eating, the gate was opened and the animal was given access to the whole husbandry area.
3. The observers started the video recordings and this was the onset of data collection.

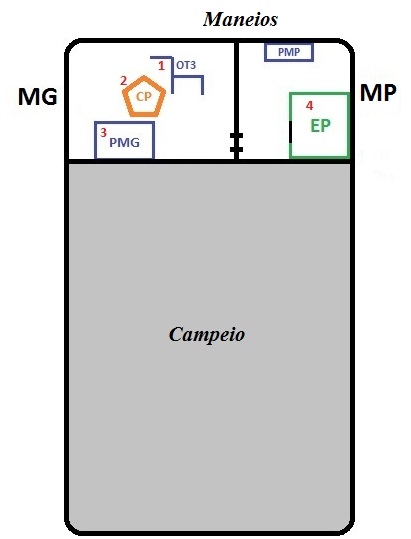


Figure 2 - Enclosure layout: grey section - main enclosure area; white sections: big and small husbandry areas; EP - indoor quarter; PMG/PMP - platforms; OT3 – look-out; CP – den. Numbers 1 to 4 mark places where the CAP/Placebo was sprayed.

## **Data collection**

In this study two types of recording methods were used, both done through a 24/7 video surveillance system.

First, the focal follow sampling method, with continuous recording of duration and frequencies of selected behaviours, and the location of the animals when performing these behaviours. Each session lasted 2 hours, starting as soon as the animals had finished eating. These recordings were only done on one group per day. The behaviours were recorded according to the ethogram shown in Table 1, using the observation sheet (Table 2).

Secondly, instantaneous scan sampling was performed over the 24 hours of each day during the three months prior to (July, August and September) and after (December, January and February) the study period, with instantaneous recording of selected behaviours as events, once every full hour for all subjects. These behaviours were recorded according to the CNRLI ethogram, which is similar, but more detailed than the one used in this study.

Table 1 – Ethogram (adapted from the CNRLI ethogram) with the behaviours deemed relevant for this study. Some of these behaviours were not observed.



Table 2 – Observations sheet used for recording data.



## **Data analysis**

Data were stored in Microsoft Excel® 2013 spreadsheets. Behaviours were grouped into categories, according to Table 3. Observed behaviours that were not part of this study’s ethogram (Table 1), were recorded as “o” (other) and grouped in the Inconclusive behaviours category. These behaviours were not statistically analysed, since they were considered not to be relevant for this study, given its aim. The percentage of time each animal performed each behaviour was calculated, and each animal’s activity budget was depicted in a histogram, for the data recorded with the focal sampling method. The percentage of events performing each behaviour within a category was calculated for the data recorded with the instantaneous scan sampling. The data collected with the first recording method did not meet the parametric criteria, as they were not normally distributed and showed unequal variances. For that reason, the unequal variance t-test (or Welch t’-test) was used. To analyse the data collected with the second recording method, Generalized Linear Models (GzLM) were used. All statistical analysis was carried out using the statistical package R 3.2.4 (R Core Team, 2014).

Table 3 – Observed behaviours from the general ethogram were grouped into categories to facilitate analysis.

|  |  |
| --- | --- |
| **Category** | **Behaviours** |
| Stereotypic | rc, rh, rsc, rsl |
| Relaxed | d, des, a |
| Territorial | mov, xp, xo |
| Exploratory | ex, s, tr, obs |
| Inconclusive | fv, o |

# **Results**

## **Pheromone Application**

The following tables show the percentage of time lynxes spent performing selected behaviours, and the statistical significance (p<0.05) of each comparison. Both tables show the same values, but they are arranged differently to facilitate the understanding of the results. The test group showed a significant decrease in the frequency of stereotypies when exposed to CAP (Table 3). The test group displayed significantly more stereotypies than the control group during the Baseline period (Table 4).

Table 3 - Percentage of time spent performing selected behaviours. Intra-group analysis. Red underlined values indicate significant (p<0.05) differences between Baseline and Treatment in the Test group. Green values indicate non-significant differences between Baseline and Treatment, within each group.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Control Group** | | | | **Test Group** | | | |
|  | **Baseline** | | **Treatment** | | **Baseline** | | **Treatment** | |
| Stereotypic | **0.54** | ± 1.12 | **1.79** | ± 4.33 | **11.24** | ± 13.55 | **3.68** | ± 3.95 |
| Relaxed | **41.19** | ± 26.67 | **30.21** | ± 19.53 | **5.15** | ± 10.30 | **5.28** | ± 7.06 |
| Territorial | **3.64** | ± 6.40 | **1.52** | ± 1.97 | **28.44** | ± 29.67 | **43.28** | ± 21.45 |
| Exploratory | **39.04** | ± 14.53 | **39.38** | ± 17.24 | **27.19** | ± 17.33 | **21.01** | ± 16.20 |
| Inconclusive | **15.59** | ± 21.02 | **27.1** | ± 26.02 | **27.98** | ± 26.46 | **26.75** | ± 27.66 |

Table 4 - Percentage of time spent performing selected behaviours. Inter-group analysis. Red underlined values within Baseline and Treatment indicate significant (p<0.05) differences between Control and Test groups. Green values indicate non-significant differences.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Baseline** | | | | **Treatment** | | | |
|  | **Control** | | **Test** | | **Control** | | **Test** | |
| Stereotypic | **0.54** | ± 1.12 | **11.24** | ± 13.55 | **1.79** | ± 4.33 | **3.68** | ± 3.95 |
| Relaxed | **41.19** | ± 26.67 | **5.15** | ± 10.30 | **30.21** | ± 19.53 | **5.28** | ± 7.06 |
| Territorial | **3.64** | ± 6.40 | **28.44** | ± 29.67 | **1.52** | ± 1.97 | **43.28** | ± 21.45 |
| Exploratory | **39.04** | ± 14.53 | **27.19** | ± 17.33 | **39.38** | ± 17.24 | **21.01** | ± 16.20 |
| Inconclusive | **15.59** | ± 21.02 | **27.98** | ± 26.46 | **27.1** | ± 26.02 | **26.75** | ± 27.66 |

The statistical significance of the results is also depicted in the boxplots shown further down. Note that the scales of the boxplots are not always the same. Furthermore, the line inside each box represents the mean, while the lower and upper edge mark the lower and upper quartile, respectively. The whiskers represent the standard deviation, and the open circles are the outliers.

Statistically significant (p<0.05) differences were found for

* Stereotypic behaviours between baseline and treatment period of the test group (t = 2.2919, df = 20.399, p-value = 0.03267; Figure 3);
* Stereotypic behaviours between the baseline periods of the test and control groups (t = 3.3374, df = 18.262, p-value = 0.003609; Figure 4);
* for Relaxed behaviours between baseline (t = 5.2169, df = 21.72, p-value = 3.245-5; Figure 5) periods of the test and control groups;
* for Relaxed behaviours between treatment (t = 5.9729, df = 31.914, p-value = 1.186-6; Figure 6) periods of the test and control groups;
* for Territorial behaviours between baseline (t = -3.4607, df = 19.767, p-value = 0.00250; Figure 7) periods of the test and control groups;
* for Territorial behaviours between treatment (t = 9.2988, df = 23.357, p-value = 2.566-9; Figure 8) periods of the test and control groups;
* for Exploratory behaviours between the treatment periods of the test and control groups (t = 3.8035, df = 47.978, p-value = 0.0004035; Figure 9).



Figure 4 - Percentage of time the control (C) and the test (T) group spent performing Stereotypic behaviours during the Baseline period. Asterisk indicates significant difference.

% of time performing behaviours

0 10 20 30 40 50

C T



Figure 3 - Percentage of time the test group spent performing Stereotypic behaviours during baseline (B) and treatment (T) periods. Asterisk indicates significant difference.

% of time performing behaviours

0 10 20 30 40 50

B T



Figure 5 - Percentage of time the control (C) and the test (T) group spent performing Relaxed behaviours during the Baseline period. Asterisk indicates significant difference.

% of time performing behaviours

0 20 40 60 80

C T



Figure 6 - Percentage of time the control (C) and the test (T) group spent performing Relaxed behaviours during the Treatment period. Asterisk indicates significant difference.

C T

% of time performing behaviours

0 10 20 30 40 50 60



Figure 8 - Percentage of time the control (C) and the test (T) group spent performing Territorial behaviours during the Treatment period. Asterisk indicates significant difference.

C T

% of time performing behaviours

0 20 40 60 80



Figure 7 - Percentage of time the control (C) and the test (T) group spent performing Territorial behaviours during the Baseline period. Asterisk indicates significant difference.

C T

% of time performing behaviours

0 20 40 60 80



Figure 9 - Percentage of time the control (C) and the test (T) group spent performing Exploratory behaviours during the Treatment period. Asterisk indicates significant difference.

C T

% of time performing behaviours

0 10 20 30 40 50 60

Statistically non-significant differences were found between the baseline and the treatment period in the control group (Table 3) for:

* Stereotypic behaviours (t = 1.3761, df = 29.758, p-value = 0.179) (Figure 10),
* Relaxed behaviours (t = 1.4531, df = 29.036, p-value = 0.1569) (Figure 11),
* Territorial behaviours (t = 1.3266, df = 19.206, p-value = 0.2002) (Figure 12) and
* Exploratory behaviours (t = 0.058, df = 33.413, p-value = 0.9541) (Figure 13).

For the test group, non-significant differences were found between the baseline and treatment periods (Table 3) for:

* Relaxed behaviours (t = 0.0445, df = 30.464, p-value = 0.9648) (Figure 14),
* Territorial behaviours (t = 1.7873, df = 31.6, p-value = 0.08348) (Figure 15),
* Exploratory behaviours (t = 1.1656, df = 37.363, p-value = 0.2512) (Figure 16).

Finally, non-significant differences were found between the control and test groups (Table 4):

* in the baseline periods of the Exploratory behaviours (t = 1.8932, df = 33.957, p-value = 0.06687; Figure 17) and
* in the treatment periods of the Stereotypic behaviours (t = 1.5797, df = 47.995, p-value = 0.1207) (Figure 18).



Figure 11 - Percentage of time the control group spent performing Relaxed behaviours during baseline (B) and treatment (T) periods.

% of time performing behaviours

0 20 40 60 80

B T

Figure 10 - Percentage of time the control group spent performing Stereotypic behaviours during baseline (B) and treatment (T) periods.



B T

% of time performing behaviours

0 5 10 15

Figure 13 - Percentage of time the control group spent performing Exploratory behaviours during baseline (B) and treatment (T) periods.



B T

% of time performing behaviours

0 20 40 60 80

Figure 12 - Percentage of time the control group spent performing Territorial behaviours during baseline (B) and treatment (T) periods.

B T

% of time performing behaviours

0 5 10 15 20 25



Figure 15 - Percentage of time the test group spent performing Territorial behaviours during baseline (B) and treatment (T) periods.

B T

% of time performing behaviours

0 20 40 60 80



Figure 14 - Percentage of time the test group spent performing Relaxed behaviours during baseline (B) and treatment (T) periods.

B T

% of time performing behaviours

0 10 20 30 40



Figure 17 - Percentage of time the control (C) and the test (T) group spent performing Exploratory behaviours during the Baseline period.

C T

% of time performing behaviours

0 20 40 60 80

Figure 16 - Percentage of time the test group spent performing Exploratory behaviours during baseline (B) and treatment (T) periods.

B T

% of time performing behaviours

0 10 20 30 40 50 60



Figure 18 - Percentage of time the control (C) and the test (T) group spent performing Stereotypic behaviours during the Treatment period.



C T

% of time performing behaviours

0 5 10 15

## **Pre- and Post-Experimental Study**

The following histograms depict the percentage of scans in which the lynxes were observed performing behaviours within the categories previously mentioned.

Figure 19 - Percentage of scans in which control group (blue bars) and test group (orange bars) performed each behavioural category (Ste – stereotypic; Rel – relaxed; Ter – territorial; Ex - exploratory; Inc - inconclusive behaviours), during the pre-experimental period.

Figure 20 - Percentage of scans in which control group (blue bars) and test group (orange bars) performed each behavioural category (Ste – stereotypic; Rel – relaxed; Ter – territorial; Ex - exploratory; Inc - inconclusive behaviours), during the post-experimental period.

The only statistically significant (p<0.05) difference between the two groups was found for the effect of the period (pre- or post- experimental study) on the frequency of Stereotypic behaviours (GzLM, χ2=5.7185, df=1,358, p-value=0.01679).

As for the remaining behaviours, no significant differences were found. The period had non-significant effects on the frequency of Relaxed behaviours (GzLM, χ2=1.2944, df=1,358, p-value=0.2552), Territorial behaviours (GzLM, χ2=0.57229, df=1,358, p-value=0.4494) and Exploratory behaviours (GzLM, χ2=2.9422, df=1,358, p-value=0.08629).

Both groups (control or treatment) also showed non-significant effects on the frequency of Stereotypic behaviours (GzLM, χ2=1.2092, df=1,357, p-value=0.2715), Relaxed behaviours (GzLM, χ2=0.058433, df=1,357, p-value=0.809), Territorial behaviours (GzLM, χ2=0.054945, df=1,357, p-value=0.8147) and Exploratory behaviours (GzLM, χ2=0.57428, df=1,357, p-value=0.4486).

Unfortunately, no comparison was done between data collected during the experimental period and the data collected during the pre- and post-experimental periods due to the differences in the collection methods. During the experimental period, observations were done using the focal follow sampling method, with continuous recording of duration and frequencies of selected behaviours. During the pre- and post-experimental periods, observations were done using instantaneous scan sampling, performed over the 24 hours of each day. Considering that the data was collected with these two different methods, it was not possible to perform such statistical comparisons.

# **Discussion**

The aim of this study was to assess the effects of CAP on captive Iberian lynx males, and evaluate its potential role in reducing short and long-term stereotypies and other abnormal behaviours. Regarding the latter, the test group showed a significant decrease in the time spent performing stereotypic behaviours between the baseline period (11.24%) and the CAP treatment period (3.68%) (Figure 3). These results concur with those of Spielman (2000) who found pheromones to reduce pacing in captive lions and tigers. Spielman also reported that after the completion of treatment, when the application of pheromones no longer took place, the pacing increased. Sajjad *et al*. (2011) also found that pacing increased for tigers held in a non-enriched environment. Macri & Patterson-Kane (2011) reported that pacing slightly decreased for solitary housed snow-leopards, although it increased for socially housed animals, when both groups were treated with synthetic analogues of cat pheromones.

The test group in the present study showed no significant differences between the time spent performing Relaxed behaviours during the baseline and the treatment periods, spending only about 5% of their time performing such behaviours. The control group spent more time performing these behaviours, although there was a non-significant decrease between the baseline’s 41% and the treatment’s 30% (Figure 11). This contrasts with the findings of Pitsko (2003) that found tigers to spend 76% of their time resting. The present study’s results also contrast with Macri & Patterson-Kane (2011) and Martínez-Macipe *et al.* (2015) who reported snow-leopards and lions to spend 53% and 92% of their time, respectively, performing relaxed behaviours. The differences seen between these studies and the present one might be due to the time of the day when sessions took place. Bashaw *et al.* (2007) showed that large felids are active mostly at dawn and dusk and spend most time resting around midday. Penadab *et al.* (2012) argued the same and also found that the captive Iberian lynxes in the *El Acebuche* centre were mostly active between 9:00 and 13:00, which coincided with the time when keepers were around the enclosures, doing the morning routines. This daily routine is similar to the one at the CNRLI (both centres are part of the same Breeding Program), and the observations of the present study were conducted in the morning, usually between 8:00 and 12:00. This might explain the low percentage of time spent performing Relaxed behaviours by the lynxes used in this study, compared to other captive wild felids.

Feliway® and CAP have been shown to reduce urine marking in domestic cats (Pageat&Tessier, 1997; Frank & Houpt, 1999; DePorter, 2013), however, the results of Macri & Patterson-Kane (2011) and of the present study contradict that. Urine marking was categorised as a Territorial behaviour, as some authors consider scent marking and scratching to be territorial marking behaviours (Pageat *et al.*, 2010). The test group performed these behaviours in 28% of the observed time in the baseline period, increasing to about 43% during the CAP treatment period (Figure 15).

Just like Gaultier *et al.* (2005) found in their study with tigers, lynxes also share behavioural traits with domestic cats, such as urine- and paw-marking, and grooming. In addition, both species show obvious individual personality differences. Some of the techniques used to prevent undesired behaviours in domestic cats can be applied to wild felids in captivity (Gaultier *et al.*, 2005), including pheromone therapy. To the best of my knowledge, there are no synthetic analogues of wild felids’ pheromones, for that reason a synthetic analogue of a domestic cat pheromone was used in the present study. Lynxes and domestic cats are part of the *Felidae* family and their semiochemicals have been shown to be effective on other species within this family, when the aim was to reduce abnormal behaviours and/or increase species-specific ones (Spielman, 2000; Gaultier *et al.*, 2005; Macri & Patterson-Kane, 2011; Martínez-Macipe *et al.*, 2015). Even though most of the mentioned studies used Feliway®, it has been shown that CAP also has positive effects. Cozzi *et al.*, (2010) found that CAP increased affiliative interactions between domestic cats and Martínez-Macipe *et al.* (2015) reported that it increased social and play activity in captive lions. No similar conclusions can be drawn from the present study, since the animals were not housed in groups. However, the used ethogram included play-like behaviours that, despite not being observed, would have been included in the Exploratory behaviours category. The control group spent more time performing such behaviours (39% in both periods) than the test group. The latter group showed a non-significant decrease in the time spent performing Exploratory behaviours between the baseline period (27%) and the CAP treatment period (21%) (Figure 16). These results are not in agreement with the previously mentioned findings by Cozzi *et al.*, (2010) and Martínez-Macipe *et al.* (2015).

The fact that the present study’s previously mentioned findings are not in agreement with others studies might be due to the number of animals used. Due to logistical constraints, it was not possible to use more than 4 animals in the present study. This sample size is much smaller than the eight animals used by Macri & Patterson-Kane (2011), the sixteen used by Cozzi *et al.*, (2010), the eighteen used by Spielman (2000) and Martínez-Macipe *et al.* (2015), the thirty-four used by Frank & Houpt (1999) and the sixty-eight used by Pageat & Tessier (1997).

Additionally, personalities’ differences have a larger effect when sample sizes are small, which is in line with Gaultier *et al.* (2005) findings for tigers. Even within the control and the test group of this study, obvious personality differences were found. Drago was the oldest male in this study and the most laid back. He spent most of the sessions laying down on the observational platform, and he did not seem too bothered by weather changes, other lynxes nearby or human presence. On the other hand, Foco was one of the youngest and he was more alert. Despite not seeming too bothered by the presence of other lynxes nearby, he seemed more disturbed by weather changes and human presence than Drago. He was the one that vocalised the most. The test animals, Enebro and Fresco, were more restless than the animals in the control group, but showed some differences between them as well. Enebro showed more avoidance towards humans and was less interested in other lynxes than Fresco. Fresco was more temperamental whenever the keepers were nearby, and he was the only one during this study to take part in a lynx-lynx interaction (through the fence) with his cub. These few personality differences may help to explain why the results of this study are not always in line with those of the other studies.

The only significant difference found in the Pre- and Post-Experimental Study analysis was for the effects of period on the Stereotypic behaviours. In fact, the control group performed these behaviours in 5.66% and 11.44% of the scans in the three months prior to and after the study, respectively, and the test group in 7.33% and 16.72% of the scans prior to and after the study, respectively. These findings are in accordance to the ones by Spielman (2000) that showed stereotypies to increase after the application of the pheromone had ceased. Despite this, Spielman states that such a change could be due to weather changes. The fact that, in the present study, the test group showed a significant increase in Stereotypic behaviours during the post-treatment period could lead one to believe that it might be caused by the pheromone. However, the fact that the control group also showed a significant increase, suggests that it might rather be due to external factors, *e.g.* the relocation of the males to different enclosures, and the fact that they were paired with females for the beginning of the mating season.

As for the remaining behavioural categories, the fact that no significant differences were found in their frequency before and after this study, and between both groups, might be due to several factors. The time of the year plays an important role in the lynxes’ behaviour. The pre-experimental period, July, August and September, is the hottest time in Portugal, and most of southern Europe. Penabad *et al.* (2012) stated that these months are the ones when lynxes are less active, mainly because of the high temperatures. In contrast, November, March and April are the months with the highest activity levels. Also, the lynxes’ placement within the enclosure area of the CNRLI changed between the pre- and the post-experimental period. This was a consequence of the new male-female pairings for the breeding season that, as usual, starts in December. In detail, during the pre-experimental period the males used in this study were placed in the same enclosures as during the experimental period, but had the whole area of the enclosures for themselves. During the post-experimental period, the males were placed in different enclosures and shared the enclosures with females. This sharing was done gradually, as first they were separated by fences. Males and females were only put together after there were signs that the females were receptive, so that courting and copulation could take place. It is inevitable and understandable that during this period the males’ behaviour was affected by the females, since oestrus peaks between December and January (Vargas *et al.*, 2009).

Even though several authors (Pageat & Gaultier, 2003; Lansdberg, 2006; Vargas *et al.*, 2009) agree that pheromone therapy is an advantageous treatment against abnormal behaviours in animals, it has its limitations. Hargrave (2014) believes that pheromones alone are not able to reverse long-term, learnt behaviours, and some stereotypies fall into this category. Pheromones represent a small part in the sensory system, seeing that animals resort to other senses when assessing their environment (Hargrave, 2014). Pageat & Gaultier (2003) agree and add that in a natural setting, when animals release pheromones, they usually adopt a specific pose and may expose body parts that are normally covered. In addition, animals may modify their surroundings, when scratching or spraying certain surfaces (Pageat & Gaultier, 2003). All these actions put together help the receiving animal to detect the pheromone, by opening the VNO (Pageat & Gaultier, 2003). These additional stimuli cannot be replicated in a study like the present one. To compensate for that fact, and to ensure that the receiving animal would sense the pheromone, a larger quantity of the synthetic analogue was used (6mL per location) than what would be naturally expelled by an animal (Pageat & Gaultier, 2003).

When working with animals, especially wild species, potential obstacles that may arise every now and then must be taken into consideration. For that reason, one has to be flexible and open to change. This study was no different, and suffered from some adjustments that were not initially planned.

Drago’s and Fresco’s indoor areas (EP in Figure 2) had no cameras and the video recording software did not allow video recordings of Enebro’s and Foco’s indoor areas’ cameras. For this reason, if Drago and/or Fresco were inside the building, there was no way of knowing what they were doing there, thus they were then recorded as “fv” (out of sight). If the other two animals, Enebro and Foco, were inside their indoor areas, the observers took real-time notes of their behaviours, despite not having the ability of making video recordings. The animals used their indoor areas only a few times, especially when the weather was rainy. It was evident that rain influenced the behaviour of the lynxes used in this study, which corroborates the findings of Schmidt (1999) and Penabad *et al.* (2012). These authors reported that lynxes display very low activity levels during rain, which goes in line with the behaviour of the males used in this study that spent most of the rainy days sheltered and/or hiding.

One of the two main purposes of CNRLI is the successful reintroduction of Iberian lynx specimens in the wild, in order to increase this animal’s wild population (Cunha Serra *et al.*, 2005). For that reason, the priority of the centre is the cubs and the successful completion of each one’s reintroduction training. This study was conducted at the same time as the reintroduction training was taking place, with the mother and the cubs in the main enclosure area. Thus, the males used in the study spent the entire time of this study in the husbandry area (Figure 2). Every other day, females and offspring would be moved between enclosures in the main enclosure area. On the days that females and cubs were not present in the males’ enclosure, keepers would place live prey (rabbits) in the main enclosure area, and the rabbits remained there for about a day, in plain sight of the males. This was done so that cubs could train their hunting skills when moved into that area, the following day. Schmidt (1999) found that the activity pattern of wild Eurasian lynx (*Lynx lynx* - Linnaeus, 1758) was mainly determined by the search and consumption of prey, and Penabad *et al.* (2012) found that captive Iberian lynxes’ activity pattern mirrors that of their wild conspecifics. Thus, one can extrapolate that the presence of rabbits might have had an effect on the results, as the males were “disturbed” every day, either by other lynxes or by live prey that they could not hunt.

The results of the present study are in accordance, but also contradict the ones of other studies, depending on the analysed behaviours. The small sample size, the dissimilar circumstances prior to, during and after this study, and the different personalities displayed by these animals make it difficult to draw definitive conclusions. Further studies are needed to corroborate these findings. It is my belief that a similar study done with a bigger sample, testing different synthetic analogues of feline pheromones, and in a time frame that does not overlap with the reintroduction training of the lynxes, will be more conclusive. With further research, it might even be possible to isolate a lynx-specific analogue pheromone that would be better targeted for such a study.

## **Conclusions**

The results showed that the test group displayed less stereotypies when treated with a synthetic analogue of Cat Appeasing Pheromone (CAP). This can be explained by the potential efficacy of the synthetic analogue of the pheromone, which is in line with the aim of this study. Also, this study showed that pheromone therapy is a promising way of providing environmental enrichment to captive wild felids.

## **Societal and Ethical Considerations**

The author declares she has no conflict of interest. This study obeyed the Portuguese, Swedish and European Union Animal Welfare laws and complied with the highest ethical standards. All the people that took part in the conduction of this study were freely involved in it. Feliway®, CAP and the placebo were gently provided by IRSEA - *Institut de Recherche en Sémiochimie et Ethologie Appliquée*, Quartier Salignan, France.

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