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The effects of the addition of two environmental enrichments on the behavior and fecal cortisol levels of three small felids species (*Caracal caracal, Leptailurus serval, Leopardus pardalis*) in captivity

Avulse Radical[a,1], Simona Normando[b,1*], Patrizia Ponzio[a], Lucia Bono[c], Elisabetta Macchi[a]

[a] *Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Largo Paolo Braccini 2, 10095, Grugliasco (TO), Italy; ravulse@gmail.com; patrizia.ponzio@unito.it; elisabetta.macchi@unito.it*

[b] *Dipartimento di Biomedicina Comparata e Alimentazione, Università degli Studi di Padova, viale dell'Università 16, Agripolis, 35020 Legnaro (PD), Italy; simona.normando@unipd.it*

[c] *Parco Faunistico Cappeller, via Kimle, 36050 Cartigliano (VI), Italy; luciabono150@gmail.com.*

[1]= These two authors contributed equally to the present study

* correspondence: Simona Normando, e-mail: *simona.normando@unipd.it*

Highlights

- We evaluated the effects of two forms of enrichment on caracals, ocelots, servals
- Visibility and affiliative behaviour increased in all the species
- In ocelots, fecal cortisol was higher in the baseline than in any other condition

Abstract

Scientific evidences support the fact that environmental enrichment, when effective, can affect the behavior of animals in captivity in a way suggestive of an improvement in their quality of life. In the internationally published literature about enrichment in captive conditions, some species, such as primates, appear to have received much more attention than others, such as felids. Within the latter, some species, such as the caracal, appear not have been the focus of any enrichment study at all. Therefore, the aim of this study was to evaluate the effects of two suspended swinging rope-covered barrels and of a sloped platform on the behavior and fecal cortisol levels of three caracals (*Caracal caracal),* three servals (*Leptailurus serval*) and two ocelots (*Leopardus pardalis*) in captivity. Animals were observed using a continuous focal animal rule in four experimental conditions (before enrichment, addition of the barrels, addition also of the sloped platform, withdrawal of both putative enrichments), repeated for two cycles. Fecal samples were collected for cortisol levels analysis. Generalized Estimation Equations, with individual as subject, and experimental condition, cycle, sex, species, and the interaction species by experimental condition as independent variables were used. Pairwise comparisons for experimental condition by species were done to test for significance after Bonferroni correction for multiple comparisons. All the three species showed more social affiliative behavior only when both the barrels and the platform were in the enclosures ($p \leq 0.002$), In all species, animals were more visible (i.e., less out of sight) already after the introduction of the first enrichment (hanging barrels, $p \leq 0.005$). In the ocelots, fecal cortisol was higher in the baseline condition than in any other condition. Given the importance of affiliative social behavior in animal welfare, the addition of the barrels and the sloped platform appeared to be somewhat an effective environmental enrichment for the individuals under study.

Keywords: affiliative behavior, structural enrichment, visibility, zoo.

Introduction

Environmental enrichment is a simple and effective means of improving and enhancing animal welfare in any species - companion, farm, laboratory and zoo (Young, 2013). Add one or more factors to a relatively impoverished environment improves the physical/psychological welfare of the animals involved (Ellis, 2009). The provision of possibly enriching stimuli to animals kept in controlled environments (such as zoos, laboratories, and, to a somewhat lesser extent, in intensive breeding facilities and shelters), is a widespread and long-established practice. Its main aims are to improve the animals' quality of life and to increase the range or number of normal (i.e. wild) behavior patterns (Newberry, 1995; Young, 2013), within the whole context in which the animal is kept (Mellen and McPhee, 2001). The monitoring and evaluation of such environmental enrichment attempts is of the outmost importance. The provision of toys/objects/stimuli as forms of environmental enrichment is not granted to be effective in altering the behavior of the animals in the direction that the word "enrichment" implies (Newberry, 1995; Mellen and McPhee, 2001). For an environment intervention to be considered enriching, the changes it produces (whether behavioral, psychological, physical or physiological) must be linked to an improvement in the welfare of the animal (Ellis, 2009). The physiological changes can be assessed by the adaptative endocrine response (i.e. stress response) to an ambient stimulus (Kusuda et al., 2022). Stressful stimuli can induce ACTH release which, in turn, increases the synthesis and secretion of cortisol by the adrenal cortex (Schatz and Palme, 2001). Adrenal activity has been monitored by measuring serum glucocorticoids concentrations as one of the stress indicators (Sapolsky et al, 2000), but blood collection itself can elicit a stress response (Palme et al, 2005). Thus, a method for measuring the amount of fecal glucocorticoids, involving an easy sample collection, noninvasive procedures, regular collection intervals, and one that does not have to consider circadian variation, would be useful for monitoring the adrenal activity for stress evaluation (Kusuda et al., 2022). The utility of monitoring adrenal activity by measuring fecal cortisol and its metabolites has been validated in many species (Möstl and Palme, 2002) including Felids (Young et al, 2004; Rozhnov et al, 2009; Moreira et al, 2007; Narayan et al, 2013; Naidenko et al., 2019; Kusuda et al., 2022). Moreover, it is important to associate multiple parameters of different kind (e.g., behavioral and endocrine) when assessing the response of animals to changes in their environment, because the intrinsic limitations of each parameter, including cortisol (Otovic and Hutchinson, 2014), used by itself can lead to a higher risk of misinterpretation of the findings.

Publishing the results of such monitoring is also important, because it can be a starting point for other facilities when planning enrichment programs, individual differences notwithstanding (Alligood et al., 2017). In the internationally published literature about enrichment in captive conditions, some animals, such as primates, appear to have received much more attention than others, such as felids. A quick search on Scopus (www.scopus.com, accessed on 02/08/2022) using "environmental enrichment" and either "primates" or "felids", identifies 188 items for the former and 26 for the latter (of which nine refer to the domestic cat). There are almost forty species within the "*Felidae*" Family (Johnson et al., 2006) and some, such as the tiger (*Panthera tigris*) appear to have received more attention (e.g., Szokalski et al., 2012) than others in this regard. At the present, to our knowledge, the caracal (*Caracal caracal)* appears not have been the focus of any enrichment study at all to date. Therefore, the main aim of this study was to evaluate the effects of the addition of two forms of possible environmental enrichment on the behavior and on the fecal cortisol levels of three caracals (*Caracal caracal*), three servals (*Leptailurus serval*) and two ocelots (*Leopardus pardalis*) in captivity. The two forms of possible enrichment tested were two suspended swinging rope-covered barrels and a sloped platform.

Animals, Materials and Methods

Animals and their management

This research was carried out at the Zoo "Parco Faunistico Cappeller" in Northeastern Italy (45°42'50''04 N, 11°41'47''76 E). The study involved the three caracals, three servals, and two ocelots, whose details are given in table 1. All the eight animals involved had been born in captivity.

The animals were housed in three different enclosures (one for species), each consisting in an outdoor and an indoor area. Indoor area consisted of a wooden small house (9 square-meters (3x3m) for 2.5m height, approximately) for ocelots and servals and of two of such houses for caracals. Each house had an outdoor patio in addition to the indoor area. The floor of the indoor area was covered with hay. The outdoor area floor consisted of grass and gravel, and there were some trees and bushes providing shadow and hiding places. All the animals were individually fed once in the morning, six days a week. Water was provided *ad libitum*. Cleaning of the enclosures took place in the morning. During cleaning the animals were temporary confined in the outdoor part of the enclosure when the indoor part was cleaned and vice versa.

Design and Research Methods

The study took place between June and September 2012, after a period of preliminary observations. During preliminary observations, the animals were habituated to the presence of the experimenter, the working ethogram completed and the experimenter trained in individual and behavior recognition. The study consisted of four experimental conditions (EC) repeated in two identical cycles, following a “ABAB” design. The study thus included a total of eight one-week long phases as detailed in table 2. The experimental conditions were:

1. baseline condition, without the enrichments that were the focus of the study (NE),
2. introduction of the first putative enrichment (E1),
3. addition of the second putative enrichment, whereas the first one was left in place (E2),
4. withdrawal of the putative enrichments (Post).

The putative enrichments objects were added on Sunday morning just prior to the beginning of the behavioral observations. They were added in a part of the enclosure that was clearly visible from the observer’s position. The first putative enrichment to be added were two swinging light metal barrels, covered with woven hemp rope, suspended at approximately 50 cm from the ground. The second was a sloping wooden platform (160 cm long, 160 cm wide and at a distance of 70 cm from the ground at its higher side and 40 cm at its lower). The platform was designed so that the cats could both lie flat on the upper surface or stay sheltered between the platform and the ground. The decision on which putative enrichments objects to include in the study was done after consulting two people with <10 years' experience with felids in captivity. Feasibility and reducing the risk of harm to the animals were also considered. The order in which the objects were introduced in the enclosures (i.e., first the barrels then the platform) was due to availability of the materials. Figure 1 shows the barrels in the servals' enclosure during E2, the platform is also partially visible. Figure 2 shows the platform used in E2 in the Ocelots' enclosure, one of the barrels is also visible.

Behavioral data

During each phase, the behavior of the animals in each enclosure was recorded using a continuous sampling rule for 20 minutes for three times a day (between 10:00 and 11:40, between 13.00 and 14:40, and between 16:00 and 17:40) (Martin and Bateson, 1986). Four observations were scheduled for each phase (on Sunday, Monday, Wednesday and Friday). However, only three days of observations in the baseline phase of the second cycle were possible due to technical problems. For the same reason, it was impossible to perform behavioral observations on caracals for the last two days of the Post condition of the second cycle. The working ethogram used in the present study is detailed in table 3.

Behavioral data treatment

For statistical analyses, behaviors were divided into states and events. Events were behaviors lasting less than 5 seconds or consisting of a single action, such as swiping once with a paw at another animal. Duration and number of occurrences were recorded for states, number of occurrences for events. In order to standardize the analyses for the purpose of the present paper, only durations were analyzed, giving to events the standard duration time of one-second. Out of sight duration correspond to the time the animal was not visible in a day and was expressed as percentage of the total observation time of the same day. The three observations done in the same day were pooled together in order to equalize for possible circadian patterns of activity, as that found by Weller and Bennet (2001) for ocelots. All the other duration data were expressed as percentage of the time the animal was observed performing a behavior in a day on the total time that animal was visible (observed and not out of sight) in the same day, as suggested by Lehner (1996, p. 193) for subjects disappearing from view. When an animal was out of sight for 100% of the observation time in a day, all the duration data (except for out of sight) were considered missing data. This resulted in one missing observation for serval F1 and the male ocelot in the baseline of the first cycle, two for serval F2 in the baseline of the first cycle.

Statistical analyses of behavioral data

After exploratory analyses, Generalized Estimation Equations (GEEs) were run on the behavioral data using the software IBM® SPSS® (Armonk, New York, USA). Individual was included in the model as subject, and experimental condition, cycle, sex, species, and the interaction species by experimental condition as independent variables. Pairwise comparisons were made on EC by species when the factor resulted significant in the model. Regarding EC by species, the pairwise comparisons concerned only differences between experimental conditions within each species, so the Bonferroni corrected alpha was set as = 0.005/6 = 0.0083, because six comparisons were done.

Among the behaviors included in the working ethogram, scratching/kneading, ingestion, rolling, marking, rubbing, vocalizing, and yawning were seldom shown by the animals during the observations. Therefore, they were seldom recorded and excluded from the analyses. Also social interactions (agonistic) and solitary play were shown by the animals only occasionally during the observations and therefore were not included in the GEEs, but a descriptive analysis of the two behaviors was done. Social agonistic interactions are important for the risk of increased agonistic behavior due to competition over the enrichment (Wooster, 1997; Sha et al., 2012). Solitary play is relevant because it is deemed to be linked mainly to positive affective states (Ahloy-Dallaire et al., 2018). The category “Other” was not analyzed, as it consisted of miscellaneous behaviors.

Fecal cortisol levels determination

Sample collection

Fecal samples were collected in the morning between 9:00 and 11:00, before the animals were released, and arranged into sterile hermetic nylon bags, before being stored at -20°C until the analysis. In order to allow the identification of the animal the fecal samples belonged to, small meatballs composed by 30 grams of minced meat and six grams of pulverized non-toxic chalk of different colors (one for each individual in every enclosure) were prepared in advance and fed to each individual animal by the keeper in the morning before the meal. The keeper also controlled that the animal had ingested the ball completely.

Steroid Hormone Assay: Extraction and Determination of fecal cortisol concentration (FCC)

Ethanol extraction and determination of corticosteroids in the feces were carried out as previously reported (Prola et al. 2013; Cornale et al., 2015) using a multi-species cortisol enzyme immunoassay kit (K003; Arbor Assays, Ann Arbor, MI) validated for dried fecal extracts. Inter-assay and intra-assay coefficients of variation were less than 15% in all assays. The test’s sensitivity was determined by measuring the least amount of hormone standard consistently distinguishable from the concentration of standard zero and was calculated to be 17.3 pg/mL. Serial dilutions (1:4, 1:8, 1:16, and 1:32) of fecal samples from three caracals, two ocelots and three servals were assayed and all regression slopes were parallel to the standard curve (r2 = 0,987). The mean recovery rate of cortisol added to dried feces of caracal, ocelot and serval was 96%, 85% and 89% respectively (n=9). According to the manufacturer, the cortisol kit presents the following cross reactivity: 100% with cortisol, 18.8% with dexamethasone, 7.8% with prednisolone, 1.2% with corticosterone, and 1.2% with cortisone.

Fecal cortisol levels data treatment and statistical analysis

In order to allow for an intestinal transit time of about 24 hours (Young et al., 2004), for the analysis of fecal cortisol levels, the samples were assigned to a phase if they were collected from day 2 of that phase to day 1 of the following phase (e.g., samples collected in day 2, day 3, and day 4 of E2_ c1 and in day 1 of POST_c1 were all assigned to phase E2_ c1). A GEE, with individual as subject, and experimental condition (EC), cycle, sex, species, and the interaction EC by species as independent variables included into the model, was run on the fecal cortisol concentrations, and pairwise comparison made on EC by species (Bonferroni corrected for multiple comparisons). A prospect of the days in which it was possible to collect fecal samples from each individual is shown in table 4.3**.**

Results

All the considered dependent variables were affected by the interaction EC by species. The results of the performed inferential statistics, as regards to factors significantly affecting the behaviors and the cortisol levels are summarized in table 6, together with the results of the pair-wise comparisons for EC by species. Only two effects, i.e., affiliative behavior and visibility, appeared to be identical in all the species and linked to an enrichment condition. The three species showed more social affiliative behavior when both the barrels and the platform were in the enclosures in comparison to the previous two conditions (all $p \leq 0.002$). When examining individual raw mean data, a clear increase in the behavior was evident in the second cycle in all animals in E2 in comparison with both NE and E1. The most notable increase in the second cycle was that of Caracal F2, who showed the behavior for 6.9% in NE, 0.8% in E1, and 11.8% in E2. In the first cycle, the increase between NE and E2 was evident in five animals out of eight. Caracals and ocelots, but not servals, showed more affiliative behavior when both the barrels and the platform were in the enclosures also in comparison to when the enrichments were withdrawn ($p<0.001$). In all species, animals were more visible (i.e., less out of sight) when there were only the barrels than before the introduction of any putative enrichment (all $p \leq 0.005$). When examining individual raw mean data, the change was evident in all animals in cycle 1. The most notable change in cycle 1 was that of the female ocelot, who was not visible for 71.4% in NE and for 6.4% in E1.

Servals were less often recorded as “moving” when both the barrels and the platform were present than in any other condition (all $p \leq 0.006$). When the raw mean data referring to each individual were examined, such effect appeared to be due mainly to a big drop in the behavior in the two females during the first experimental cycle. F1 varied from 18.6% in NE to 1.6% in E2, with intermediate values in E1. F2 from 1.3% to 24.8%, with intermediate values in E1. In the second cycle, however, both females showed the behavior less in E1 than in any other phase. Caracals and ocelots showed less alertness behavior when both the barrels and the platform where present than in the two previous conditions (all $p \leq 0.004$). When examining individual caracals’ raw mean data, such effect appeared to be due mainly to a drop in the behavior in F2 and in the male in the first cycle (from 5.2% and 7.5% in NE to 4.7% and 3.3% in E2, respectively). In the second cycle, a drop (2.6% to 1.3%) was present only in F2. Both ocelots showed a decrease of the behavior during the first experimental cycle (from 6.4% and 1.7% in NE to 3.4% and 0.9% in E2, for the female and the male respectively, with intermediate values in E1), with a less clear pattern in the second cycle. Caracals showed less exploration when the enrichments were withdrawn than in any other condition (all $p \leq 0.004$). When individual raw mean data were examined, such effect appeared to be due mainly to a drop in the behavior in the two females during the first experimental cycle (from 0.8% and 0.5% in NE to 0.4% and 0.0% in Post, respectively), with no clear pattern in the second cycle.

Solitary play was recorded only occasionally (median duration 0.0 %, range 0.0% to 2.7% of observation time). The same was true for social agonistic behavior (median duration 0.0 %, range 0.0% to 6.5% of observation time, the longest duration being recorded in Caracal F1 on the first day of NE_Cycle1). The number of days the two behaviors were recorded for each individual for each EC by cycle are shown in table 6.

The most recorded behavior in all individuals was resting, which overall accounted for the 60.0% of the time the animals were visible. Ocelots’ fecal cortisol levels were higher before the introduction of any putative enrichment than in any other phase. The fecal cortisol levels patterns in the other two species were less clear as regards to the effect of the putative enrichments.

Discussion

The present study evaluated the effects of two suspended swinging rope-covered barrels and of a sloped wooden platform on the behavior and fecal cortisol levels of eight small wild felids in captivity. The animals involved were three caracals, two ocelots, and three servals. Direct focal animal continuous observations and an enzyme immunoassay were used. Apart from an increase of affiliative behaviors and visibility, most of the found behavioral effects differed among the three species included in the study. This agrees with what found in other studies that evaluated the responses to external stimuli in multiple felid species (Moreira et al., 2007; Suárez et al., 2017). For example, Suárez et al. (2017) found that Eurasian lynxes (*Lynx lynx*), bobcats (*Lynx rufus*), ocelots and Asiatic lions (*Panthera leo persica*) reacted to the presence of visitors in a way that was suggestive of a negative impact on their welfare (e.g., more resting and hiding and less playing), whereas jaguars (*Panthera onca*) showed an opposite pattern.

In the present study, a behavioral effect that was common in all three species was an increase of social affiliative interactions when both the forms of enrichment were in the enclosure, as compared to the previous conditions (i.e., no enrichment or the presence of hanging barrels only). Resende et al. (2009) reported that the behavior "social interaction" was only shown by the studied individuals (i.e., two Geoffroy's cats - *Leopardus geoffroyi* - one oncilla - *Leopardus tigrinus* - and two margays - *Leopardus wiedii*) during the enrichment phase (i.e., when a food based "surprise pack" was given), and not during non-enriched phases. Unfortunately, such social interactions are not further described in Resende et al (2009)'s paper. The only category in Resende et al (2009)'s working ethogram suggestive of a social behavior is "growling", defined in the paper as "Animal vocalizing" (Resende et al., 2009, p. 604). If indeed there was an increase in growling in cats of Resende et al.'s (2009) study, this finding disagrees with what found in the present study, because affiliative, and not agonistic, social behavior was increased in the present study. The difference could be due to many factors, including species, possible differences in weather conditions during the studies and type of enrichment. The increase in affiliative social behavior found in the present study can be interesting because of the link between affiliative behavior and positive emotional states, and thus improved welfare (Boissy et al., 2007; Rault 2012, 2019). It is also interesting because adults of the studied cat species, as most of the *Felidae*, are mainly solitary in the wild, but are often housed together in zoos (Mellen and Sheperdson, 1997), with beneficial effects of being housed in groups being reported for some (Antonenko et al., 2019, for Eurasian lynxes).

Although small wild cat species, such as the servals, are quite widespread in zoological parks at present, (Acaralp-Rehnberg 2020), there is not much literature on enrichment in these species. Most of the published studies on enrichment in these species tend to be old, to only describe the used enrichment programs, and/or to focus almost exclusively on the effects of food and odor enrichment (Mellen et al., 1981; Markowitz and LaForse, 1987; Carlstead et al., 1993; Sheperdson 1993; Sheperdson 1997; Powell, 1997; Wooster 1997; Mellen and Wells and Egli, 2004; Skibiel et al., 2007; Antonenko et al., 2019). For the abovementioned reason, comparison to the results of the present study is difficult. The only similarity in enrichment can be found with the study by Carlstead et al. (1993), insofar that Carlstead and colleagues provided leopard cats (i.e., *oncillas*, *Leopardus tigrinus*) with branches and hiding places and the sloped platform in the present study could be used as a partial hiding place if the cat positioned under it. By the way, the present study did not found an increase in exploratory behavior mirroring what found in the *oncillas*. Increasing exploration, and active behaviors in general, together with a decrease in pacing, and inactivity appears to be common findings also in studies using food and/or odor enrichment (Sheperdson 1993; Wooster 1997; Wells and Egli, 2004; Skibiel et al., 2007). In Carlstead's et al. (1993) study, exploratory behavior was decreased when the animals were moved in a barren environment, situation in which their cortisol concentrations remained chronically elevated. This suggests that reduced exploratory behavior could be an indicator of chronic exposure to aversive environmental conditions. It is interesting to note that the caracals in the present study significantly decreased their explorative behavior when the enrichments were withdrawn, suggesting that they may have found being denied the use of the already experienced enrichments an aversive situation.

It is important to note that factors related to the individual animal and/or to management could have contributed to the results of the present study as well as to those of most of the cited literature. Unluckily, although the present study was originally planned as to be a multi-center one, the repetitions in other zoos could not be carried out due to technical problems, except for one single cycle in a zoo housing two caracals and one serval. Interesting enough, also in this case, the serval showed less moving when both the barrels and the platform were present than in any other condition, and all three individuals were more visible when any of the putative enrichments was in place than before, although the female caracal tended to use the platform almost exclusively (unpublished data).

The present study has some limitations, most of which are common in studies done in zoos. For example, the limited sample size, the different species of the individuals, the non-independence of data gathered from individuals in the same enclosure. However, as noted by Allogood et al. (2017), when dealing with enrichment studies, it is important thing to focus on those specific individual animals, in that specific situation (including the fact that they can cohabit in the same enclosure) in order to be more effective in improving their quality of life. Moreover, given the paucity of studies on the behavior the involved three species in zoos, the present paper can be of use to both people deciding on possible enrichment forms for the animals in their care, and for researches wishing to deal more in depth in the topic.

Conclusions

In the present study the addition of two suspended swinging rope-covered barrels and of a sloped platform to the enclosures housing caracals, ocelots, servals, was linked to an increase of social affiliative behaviors and that of the barrels alone to an increase of visibility. Affiliative behaviors are deemed to be linked to positive welfare (Boissy et al., 2007) and visibility of the animals could be important for Zoo visitors. Although the role of individual differences should be considered, especially in experiments with only few animals, this is, to our knowledge, the first study addressing the effects of a form of environmental enrichment in the caracal, and one of the few evaluating non-food/non-olfactory enrichment for servals and ocelots. Therefore, the present study is likely to be a useful starting point both for institutions planning enrichment programs for these species, and for future, hopefully multi-centric, deeper and more complete investigations.

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Ethical note and conflict of interest declaration

The study is in accordance with the guidelines established by the Italian Animal Welfare Law (DL n. 26 - 4 March 2014, art. 2), and with all the relevant national and international regulations in force at the moment in which the study took place. The Institutional Ethical Review Board of the University of Turin reviewed and approved the research (CEBA protocol number 2722). None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper. Data are available upon request from the corresponding author.

Authorship statement

The idea for the paper was conceived by AR and SN. The experiments were designed by AR, SN, EM, PP, LB. The experiments were performed by AR, SN, EM, PP, LB. The data were analyzed by SN and AR. The paper was written by SN in collaboration with all the other authors.

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Table 1: Non-human animals involved in the study

Animal	Species	Sex	Age at study beginning	Source	Residence time at the Cappeller
CaF1	*Caracal caracal*	Female	10 years	Italy	8 years
CaF2	*Caracal caracal*	Female	10 years	Italy	8 years
CaM	*Caracal caracal*	Male	5 years	The Netherlands	4 years
OcF	*Leopardus pardalis*	Female	10 years	Germany	9 years
OcM	*Leopardus pardalis*	Male	11 years	Germany	9 years
SeF1	*Leptailurus serval*	Female	8 years	The Netherlands	7 years
SeF2	*Leptailurus serval*	Female	8 years	The Netherlands	7 years
SeM	*Leptailurus serval*	Male	12 years	The Netherlands	11 years

Table 2: Sequence of the experimental phases

Cycle	Phase	Name	Condition	Experimental phase	What is in the enclosure
1	Baseline	NE_c1	Baseline	First baseline	Standard enclosure
1	Swinging barrels	E1_c1	Introduction of the first enrichment	First enrichment introduced for the first time	Two suspended swinging barrels, covered in rope were added to each enclosure
1	Swinging barrels + sloped platform	E2_c1	Addition of the second enrichment	Second enrichment added for the first time	A sloping platform was added to each enclosure. The barrels were still in place
1	Post-enrichment baseline	POST_c1	Withdrawal of enrichments	First withdrawal of both the enrichments	Barrels and platforms were removed and the enclosure reverted to its standard situation
Two weeks pause					
2	Baseline	NE_c2	Baseline	Second baseline	Standard enclosure
2	Swinging barrels	E1_c2	Introduction of the first enrichment	First enrichment introduced for the second time	Two suspended swinging barrels, covered in rope were added to each enclosure
2	Swinging barrels + sloped platform	E2_c2	Addition of the second enrichment	Second enrichment added for the second time	A sloping platform was added to each enclosure. The barrels were still in place
2	Post-enrichment baseline	POST_c2	Withdrawal of enrichments	Second withdrawal of both the enrichments	Barrels and platforms were removed and the enclosure reverted to its standard situation

Table 3: The working ethogram used in the present study

Behavior	Description
Alertness	The animal remains generally inactive, but can move his/her head/eyes so that he/she can stare directly to a stimulus, and could flip his/her ears occasionally as he/she scans the surrounding environment (UKCBWG, 1995)
Exploring	The animal is gathering information from the environment, or at least appears to do so, mainly using vision (the animal looks intently at the object/structure/floor) or olfaction (the animal approaches the object/structure/floor with his/her nose and inhales and exhales rapidly and repeatedly).
Ingestion	The animal is introducing edible solid matter or liquid in his/her mouth, and swallowing it. In case of edible solid matter usually chewing movements are visible.
Marking (i.e., urine spraying)	While standing with tail raised vertically, the animal releases a jet of urine backwards against a vertical surface or object. The tail may quiver as urine is discharged (Stanton et al., 2015).
Moving	Locomotion (e.g., walking, trotting, galloping). During data gathering a "§"was added to the recording when the locomotion has the characteristic of a stereotypy (repetitive, invariant, no apparent purpose), but moving was analyzed all together in order not to introduce possible subjective bias.
Resting	The animal is lying in sternal or lateral recumbence, without being doing any of the other listed activities and appears to be inactive. Eyes can be either closed or open.
Rolling	Dropping from standing to sternal recumbence, if not already in sternal recumbence, and then rotating/turning one or more time from sternal to dorsal recumbence tucking the legs against the body (UKCBWG, 1995; McDonnell, 2003)
Rubbing	Cat puts any part or entire length of body in contact with something (e.g., an inanimate object, a structure or the floor) exercising pressure and friction against it (UKCBWG, 1995; Stanton et al., 2015).
Scratching and/or **Kneading**	Two behavioral patterns included: Scratching: cat repeatedly scrape its extended claws against a rough surface (UKCBWG, 1995) Kneading: the animal pushes forepaws pressing into the ground or other substrate in a rhythmic, "kneading" motion, alternating extension and flexion of the paws (Stanton et al., 2015).
Self-	The animal is licking the surface of his/her body, or rubbing his/her paw on another

Grooming	body part of his/hers (body care)	
Sitting	The animal shows flexed hind legs, rump in contact with the ground, forelegs extended, without being doing any of the other listed activities, and appears to be inactive.	
Social interaction (affiliative)	Any interaction among two or more individuals in which the behavior of one of the individuals directly influences the behavior of one or more of the other individuals. It includes (Stanton et al., 2015):	
	allo-grooming	The cat licks the fur of another cat's head or body.
	allo-rubbing	The cat puts any part (usually the head) or entire length of body in contact with a part of a conspecific's body exercising pressure and friction against it
	social play	The cat interacts with another cat in a "non-serious" manner (i.e. where there is no intention to harm), see "solitary play" for a more detailed definition of "play"
Social interaction (agonistic)	Any interaction among two or more individuals in which the behavior of one of the individuals directly influences the behavior of one or more of the other individuals in particular as regards the management of a (potential) conflict. It includes the following (Stanton et al., 2015):	
	Attack	The cat launches him/herself at (modifier) with extended forelegs and attempts to engage in physical combat
	Avoid	The cat moves, or changes direction while moving, in order to keep away from a conspecific who is displacing him/her
	Bare teeth	The cat opens its mouth slightly while pulling lips back to expose teeth
	Bite	The cat snaps teeth at and is successful in biting a conspecific. Biting is defined as the act of "opening and rapid closing of the jaws with the teeth grasping the skin of another animal", (McDonnel, 2003, p. 134)
	Chase	The cat runs rapidly in pursuit of another cat with apparent aggressive intents.
	Cuff	The cat strikes at another cat with forepaw and contact is made. Claws are usually extended.
	Displace	The cat provokes an avoidance behavior from another cat
	Ears flat	The cat flattens his/her ears to its head, so that they tend to lie flush with

		the top of the head (UKCBWG, 1995).
	Fight	The cat engages in physical combat with another cat.
	Flight	The cat runs away from a conspecific threatening him/her
	Growl	An aggressive/agonistic low-pitched, throaty, rumbling noise produced while the mouth is closed (Stanton et al., 2015).
	Hiss	The cat emits a drawn-out, low-intensity hissing sound produced by rapid expulsion of air from the cat's mouth, usually during exhalation.
	Rake	The cat makes kicking movements with one or both hind legs against another cat, with claws extended
	Strike at	The cat swipes his/her forepaw at another cat, as if to make him/her keep distances, but no contact is made.
	Threaten	The cat directs a display of aggressive behavior toward another cat without making any physical contact with him/her.
	Wrestle	The cat engages in physical contact with another cat, whereby the cat struggles with the other cat. Can include pulling the other cat toward oneself with his/her forelegs and perform raking movements with the hind legs.
Solitary Play	Play: activities appearing to have no immediate use or function, involving a sense of pleasure and elements of surprise. These activities are mostly modified versions of serious survival activities distinguished from the "serious" analogues by postures or expressions denoting less serious intent (McDonnell, 2003). Social play was excluded when more than one animal participated, however when one animal tried to solicit interaction from another, also by means of object play, but was ignored, this was included in the soliciting/solitary play category Individual play (locomotion play and play with objects) was included.	
Standing	The animal is standing on four legs (neither hind nor fore legs flexed), without being doing any of the other listed activities, and appears to be inactive.	
Vocalization	The animal emits a sounds via his/her mouth and/or nose using air conveyed via the respiratory system	
Yawning	Cat opens its mouth widely while inhaling, then closes mouth while exhaling deeply (Stanton et al., 2015)	
Other	Any other behavioral pattern not included in the list above.	
Not visible	The observer could not see the animal and identify/record his/her behavior	

Table 4: Fecal cortisol levels (ng/g). Ca = caracal, Se = serval, Oc = ocelot, F = female, M = male

		CaF1	**CaF2**	**CaM**	**SeF1**	**SeF2**	**SeM**	**OcF**	**OcM**
NE_c1	**NE_c1_d2**			17.30		37.40	43.90		
	NE_c1_d3	25.40	16.20	13.25		32.90	25.30	37.30	47.10
	NE_c1_d4		21.65			27.85	33.50		
E1_c1	**E1_c1_d2**	14.80		17.45	25.95	34.65	33.75	28.45	20.50
	E1_c1_d3	24.55		24.25	26.05	31.35	32.30	34.30	25.15
	E1_c1_d4	17.70	14.40					30.90	30.20
E2_c1	**E2_c1_d2**	22.85	23.45	25.00	30.35	33.10	27.10	33.75	24.40
	E2_c1_d3	16.30		20.75	32.05	31.05	32.70	36.15	33.00
	E2_c1_d4	13.85	20.25	19.25				30.25	36.15
P_c1	**P_c1_d2**	17.33	21.40	16.90	25.10	26.75	28.80	29.05	34.00
	P_c1_d3	23.69	19.98	22.70	22.15	23.15	24.90	34.50	27.85
	P_c1_d4	19.95	24.05	27.90				26.85	31.15
NE_c2	**NE_c2_d2**	26.80	13.10		36.20	29.50	27.20	26.05	
	NE_c2_d3	15.95	20.55	12.75	47.60	26.80	45.60	53.55	46.75
	E1_c2_d1	19.05	23.65						46.50
E1_c2	**E1_c2_d3**	17.33		24.85	24.60	34.00	34.85	42.30	
	E1_c2_d4	15.10	11.28	19.80	32.75	33.85	32.05	25.65	43.15
	E2_c2_d1							21.35	
E2_c2	**E2_c2_d2**	10.53	14.08	10.68	29.75	34.45	32.65	25.95	43.25
	E2_c2_d3	8.42	7.01	9.65	32.80	31.90	30.00	42.80	24.30
	E2_c2_d4			12.08				25.45	28.60
P_c2	**P_c2_d2**	5.03	5.50		22.15	26.20	27.50	24.55	28.15
	P-c2_d3	2.03	1.53	1.98	23.10	24.50	27.15	25.15	26.05
	P-c2_d4								24.95

Table 5: Results of the Generalized Estimation Equations. Ca = caracal, Oc = ocelot, Se = serval.

Factors affecting behaviors' durations and fecal cortisol levels								Pairwise comparisons		
Variable	Source	(Intercept)	Species	Experimental condition (EC)	Cycle	Sex	EC by species	Significant differences between ECs within each species (p)		
	df	1	2	3	1	1	6	Ca	Oc	Se
ALERTNESS	Wald chi-square (p)	120.15 (<0.001)	4.23 (0.121)	40.18 (<0.001)	30.20 (<0.001)	0.19 (0.661)	21588.00 (<0.001)	E2<E1 (0.001); E2<NE (0.001)	E2<E1 (0.004); E2<NE (0.006)	E2<E1 (<0.001); E2<Post (0.002); E1>NE (<0.001)
EXPLORATION	Wald chi-square (p)	238.52 (<0.001)	117.73 (<0.001)	9.25 (0.026)	10.00 (<0.001)	4.44 (0.035)	1519.20 (<0.001)	E2>Post (0.004); E1>Post (0.004); NE>Post (<0.001)	none	none

MOVING	Wald chi-square (p)	269.09 (<0.001)	18.07 (<0.001)	41.06 (<0.001)	1.63 (0.2)	3.28 (0.07)	137588.00 (<0.001)	E1<NE (<0.001); E1<Post (<0.001); NE<Post (<0.001)	E2>E1 (<0.001); E2>NE (<0.001)	E2<E1 (<0.001); E2<NE (0.004); E2<Post (<0.001); E1<Post (<0.001)
RESTING	Wald chi-square (p)	2811.93 (<0.001)	3.34 (0.188)	3.69 (0.296)	5.56 (0.02)	4.21 (0.04)	1467.00 (<0.001)	E1>NE (0.001)	none	E2>Post (0.003)
SELF GROOMING	Wald chi-square (p)	95.78 (<0.001)	15.11 (0.001)	10.34 (0.016)	2.14 (0.14)	0.95 (0.331)	1025.80 (<0.001)	none	E2>Post (<0.001)	E2>E1 (<0.001)
SITING	Wald chi-square (p)	73.28 (<0.001)	7.86 (0.02)	6.50 (0.09)	8.84 (0.003)	8.91 (0.003)	49.55 (<0.001)	none	E1>NE (0.004); E1>Post (<0.001); NE>Post (<0.001)	NE>Post (0.007)

SOCIAL INTERACTIONS (affiliative)	Wald chi-square (p)	14.19 (<0.001)	8.55 (0.014)	59.78 (<0.001)	2.41 (0.12)	4.33 (0.037)	318.47 (<0.001)	E2>E1 (0.001); E2>NE (<0.001); E2>Post (<0.001); E1<Post (0.008)	E2>E1 (<0.001); E2>NE (<0.001); E2>Post (<0.001); E1>NE (<0.001); NE<Post (<0.001)	E2>E1 (0.002); E2>NE (0.001)
STANDING	Wald chi-square (p)	126.01 (<0.001)	7.76 (0.021)	1.85 (0.605)	0.08 (0.77)	0.02 (0.896)	6148.00 (<0.001)	E1<NE (0.001); E1<Post (<0.001)	E1>Post (<0.001)	E2<Post (<0.001)
NOT VISIBLE	Wald chi-square (p)	83.03 (<0.001)	169.14 (<0.001)	59.41 (<0.001)	6.90 (0.01)	3.87 (0.049)	5718.20 (<0.001)	E1<NE (<0.001)	E1<NE (<0.001); E1<NE (<0.001); E1>Post (0.004); NE>Post (<0.001)	E1<NE (0.005); NE>Post (0.001)

CORTISOL	Wald chi-square (p)	75537.76 (<0.001)	6232.70 (<0.001)	365.18 (<0.001)	2.91 (0.09)	61.44 (<0.001)	518.44 (<0.001)	E1>Post (0.001)	E2>E1 (<0.001); E2<NE (<0.001); E1<NE (<0.001); NE>Post (<0.001)	E2>Post (<0.001); E1>Post (<0.001); NE>Post (<0.001)

Table 6: Number of days in which social agonistic behavior and solitary play were recorded.

Behavior	Animal	NE cycle1	E1 cycle1	E2 cycle 1	Post cycle 1	NE cycle 2	E1 cycle 2	E2 cycle 2	Post cycle 2
Social agonistic behavior	Caracal F1	2	0	2	0	1	0	0	0
	Caracal F2	2	0	1	0	1	0	0	0
	Caracal M	1	0	1	0	0	0	0	0
	Serval F1	0	0	0	0	0	0	1	0
	Serval F2	0	1	0	0	0	0	1	0
	Serval M	0	0	0	0	0	0	0	0
	Ocelot F	0	0	0	0	1	0	0	0
	Ocelot M	0	0	0	0	0	0	0	0
Solitary play	Caracal F1	0	0	0	0	0	0	1	0
	Caracal F2	0	1	0	0	0	1	2	0
	Caracal M	0	0	0	0	0	0	1	0
	Serval F1	0	1	1	0	1	1	0	0
	Serval F2	2	0	1	0	1	0	0	0
	Serval M	0	0	0	0	0	0	0	0
	Ocelot F	0	1	2	1	0	1	1	0
	Ocelot M	0	0	0	1	0	1	1	0

Figure captions

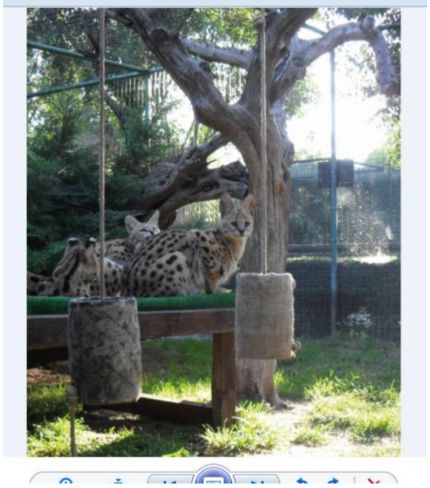

Figure 1: The rope covered barrels used as form of environmental enrichment in the present study in the servals' enclosure during E2, the platform is also partially visible.

Figure 2: The platform used in E2 in the present study in the Ocelots' enclosure, one of the barrels is also visible.

Ethical statement

The study is in accordance with the guidelines established by the Italian Animal Welfare Law (DL n. 26 - 4 March 2014, art. 2), and with all the relevant national and international regulations in force at the moment in which the study took place. The Institutional Ethical Review Board of the University of Turin reviewed and approved the research (CEBA protocol number 2722).