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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

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# Keywords:

Affiliative behavior, Structural enrichment, Visibility, Zoo

### **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 inter- nationally 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 2 suspended swinging rope-covered barrels and of a sloped platform on the behavior and fecal cortisol levels of 3 caracals (Caracal caracal), three servals (Leptail-urus serval) and 2 ocelots (Leopardus pardalis) in captivity. Animals were observed using a continuous focal animal rule in 4 experimental conditions (before enrichment, addition of the barrels, addition also of the sloped platform, withdrawal of both putative enrichments), repeated for 2 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 ( $P \le 0.002$ ), In all species, animals were more visible (i.e., less out of sight) already after the introduction of the first enrichment (hanging barrels,  $P \le 0.005$ ). In

the ocelots, fecal cortisol was higher in the base-line 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.

#### 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 keptin 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 kept (Mellen and McPhee, 2001). The monitoring and evaluation of such environmental enrichment attempts is of the outmost

**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                        |

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 offecal 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 (Möstland Palme, 2002) including Felids (Young et al, 2004; Rozhnovet 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 it-self 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 9 refer to the domestic cat). There are almost 40 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 studyat 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 3 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 3 caracals, 3 servals, and 2 ocelots, whose de-tails are given in Table 1. All the 8 animals involved had been bornin 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  $(3 \times 3 \text{ m})$  for 2.5 m 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, 6 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

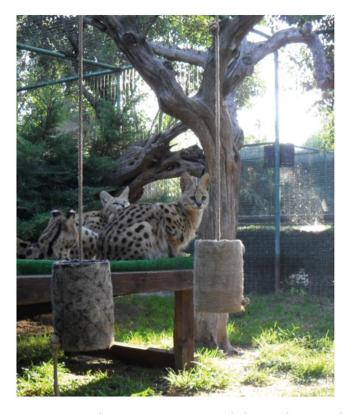
The study took place between June and September 2012, after a period of preliminary observations. During preliminary observations, the animals were habituated to the presence of the experimenter, the working ethogram completed and the experimenter trained in individual and behavior recognition. The study consisted of 4 experimental conditions (EC) repeated in 2 identical cycles, following a "ABAB" design. The study thus included a total of 8 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.

**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<br>to each enclosure. The barrels<br>were still in place            |
| 1        | Post-enrichment baseline POST_c1   |         | Withdrawal of enrichments            | First withdrawal of both the enrichments        | Barrels and platforms were<br>removed and the enclosure<br>reverted to its standard<br>situation |
| Two weel | ks 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<br>to each enclosure. The barrels<br>were still in place            |
| 2        | Post-enrichment baseline           | POST_c2 | Withdrawal of enrichments            | Second withdrawal of both the enrichments       | Barrels and platforms were<br>removed and the enclosure<br>reverted to its standard<br>situation |



**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.

### 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 Sun-day, 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 2 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 an- other 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 percent-age 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 ani- mal 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, 2 for serval F2 in the baseline of the first cycle.



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

# 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). 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 6 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 be-tween 9:00 and 11:00, before the animals were released, and ar- ranged into sterile hermetic nylon bags, before being stored at  $-20^{\circ}$ 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 multispecies 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 3 caracals, 2 ocelots, and 3 servals were assayed and all regression slopes were parallel to the standard curve ( $r^2 = 0.987$ ). The mean recovery rate of cortisol added to dried feces of caracal, ocelot and serval was 96%, 85%, and 89% respectively ( $r^2 = 0.987$ ). 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.

# 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 5, together with the results of the pair-wise comparisons for EC by species. Only 2 effects, that is, affiliative behavior and visibility, appeared to be identical in all the species and linked to an enrichment condition. The

3 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 ev- ident 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  $P \le 0.006$ ). 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 inter- mediate 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 plat-form 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%-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 pat- tern 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.

**Table 3**The working ethogram used in the present study.

| Behavior                  | Description                           |  |
|---------------------------|---------------------------------------|--|
| Alertness                 | The animal rema                       | ains generally inactive, but can move his/her head/eyes so that he/she can stare directly to a stimulus, and could flip his/her  |
|                           | -                                     | y as he/she scans the surrounding environment (UKCBWG, 1995).  |
| Exploring                 | =                                     | othering information from the environment, or at least appears to do so, mainly using vision (the animal looks intently at the floor) or olfaction (the animal approaches the object/structure/floor with his/her nose and inhales and exhales rapidly and                   |
| Ingestion                 |                                       | troducing edible solid matter or liquid in his/her mouth, and swallowing it. In case of edible solid matter usually chewing visible.   |
| Marking (i.e.,            | While standing v                      | with tail raised vertically, the animal releases a jet of urine backwards against a vertical surface or object. The tail may quiver  |
| urine spraying)<br>Moving | Locomotion (e.g                       | arged (Stanton et al., 2015).  , walking, trotting, galloping). During data gathering a "\$"was added to the recording when the locomotion has the a stereotypy (repetitive, invariant, no apparent purpose), but moving was analyzed all together in order not to introduce |
|                           | possible subjecti                     |  |
| Resting                   | The animal is lyi<br>be either closed | ng in sternal or lateral recumbence, without being doing any of the other listed activities and appears to be inactive. Eyes can or open.  |
| Rolling                   |                                       | tanding to sternal recumbence, if not already in sternal recumbence, and then rotating/turning one or more time from sternal   |
| Rubbing                   |                                       | bence tucking the legs against the body (UKCBWG, 1995; McDonnell, 2003).  rt or entire length of body in contact with something (e.g., an inanimate object, a structure or the floor) exercising pressure  |
|                           |                                       | nst it (UKCBWG, 1995; Stanton et al., 2015).   |
| Scratching                |                                       | patterns included:   |
| and/or<br>Kneading        | _                                     | epeatedly scrape its extended claws against a rough surface (UKCBWG, 1995).  nimal pushes forepaws pressing into the ground or other substrate in a rhythmic, "kneading" motion, alternating extension   |
| Kilcuding                 |                                       | ne paws (Stanton et al., 2015).  |
| Self- Grooming<br>Sitting |                                       | king the surface of his/her body, or rubbing his/her paw on another body part of his/hers (body care).  vs flexed hind legs, rump in contact with the ground, forelegs extended, without being doing any of the other listed activities,                                     |
| Social<br>interaction     | Any interaction                       | among two or more individuals in which the behavior of one of the individuals directly influences the behavior of one or er individuals. It includes (Stanton et al., 2015):   |
| (affiliative)             | allo grooming                         | The cat licks the fur of another cat's head or body.   |
|                           | allo-grooming<br>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  |
|                           | sacial play                           | pressure and friction against it.  The set interests with earther set in a "pan estima" manner (i.e. where there is no intention to harm), see "calitary play"   |
|                           | 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                    | Any interaction                       | among two or more individuals in which the behavior of one of the individuals directly influences the behavior of one or   |
| interaction               |                                       | er individuals in particular as regards the management of a (potential) conflict. It includes the following (Stanton et al., 2015):  |
| (agonistic)               | Attack<br>Avoid                       | The cat launches him/herself at (modifier) with extended forelegs and attempts to engage in physical combat.  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  |
|                           | Chase                                 | the jaws with the teeth grasping the skin of another animal" (McDonnell, 2003, p. 134)  The sat runs rapidly in pursuit of another sat with apparent aggressive intents  |
|                           | Cuff                                  | The cat runs rapidly in pursuit of another cat with apparent aggressive intents.  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<br>Growl                       | The cat runs away from a conspecific threatening him/her.  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  |
|                           | Daka                                  | during exhalation.   |
|                           | Rake<br>Strike at                     | The cat makes kicking movements with one or both hind legs against another cat, with claws extended.  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 a                    | ppearing to have no immediate use or function, involving a sense of pleasure and elements of surprise. These activities are  |
|                           |                                       | versions of serious survival activities distinguished from the "serious" analogues by postures or expressions denoting less  |
|                           |                                       | IcDonnell, 2003). Social play was excluded when more than one animal participated, however when one animal tried to n from another, also by means of object play, but was ignored, this was included in the soliciting/solitary play category                                |
|                           |                                       | locomotion play and play with objects) was included.   |
| Standing                  |                                       | anding on four legs (neither hind nor fore legs flexed), without being doing any of the other listed activities, and appears to  |
| Vocalization              |                                       | s a sounds via his/her mouth and/or nose using air conveyed via the respiratory system.  |
| Yawning                   |                                       | outh widely while inhaling, then closes mouth while exhaling deeply (Stanton et al., 2015).  |
| Other                     | · ·                                   | vioral pattern not included in the list above.   |
| Not visible               | The observer co                       | uld 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   | ОсМ   |
|-------|----------|-------|-------|-------|-------|-------|-------|-------|-------|
| 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 |

Solitary play was recorded only occasionally (median duration 0.0%, range 0.0%-2.7% of observation time). The same was true for social agonistic behavior (median duration 0.0%, range 0.0%-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 8 small wild felids in captivity. The animals involved were 3 caracals, 2 ocelots, and 3 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 3 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 (*Pantheraleo 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., 2 Geoffroy's cats-Leopardus geoffroyi-one oncilla-Leopardus tigrinusand 2 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).

**Table 5**Results of the generalized estimation equations.

| Variable                                | Source                    | (Intercept)          | Species             | Experimental condition (EC) | Cycle             | Sex               | EC by species         | Significant diffe species (p)   | rences between E  | Cs within each  |
|---|---------------------------|----------------------|---------------------|-----------------------------|-------------------|-------------------|-----------------------|---|---|---|
|   | df                        | 1                    | 2                   | 3                           | 1                 | 1                 | 6                     | Ca  | Oc  | Se  |
| ALERTNESS                               | Wald<br>chi-square<br>(p) | 120.15<br>(<0.001)   | 4.23<br>(0.121)     | 40.18 (<0.001)              | 30.20<br>(<0.001) | 0.19<br>(0.661)   | 21588.00<br>(<0.001)  | E2 <e1 (0.001);<br="">E2<ne (0.001)<="" td=""><td>E2 &lt; E1 (0.004);<br/>E2 &lt; NE (0.006)</td><td>E2 &lt; E1<br/>(&lt;0.001);<br/>E2 &lt; Post<br/>(0.002); E1 &gt; N<br/>(&lt;0.001)</td></ne></e1> | E2 < E1 (0.004);<br>E2 < NE (0.006)   | E2 < E1<br>(<0.001);<br>E2 < Post<br>(0.002); E1 > N<br>(<0.001)                            |
| EXPLORATION                             | Wald<br>chi-square<br>(p) | 238.52<br>(<0.001)   | 117.73<br>(<0.001)  | 9.25 (0.026)                | 10.00 (<0.001)    | 4.44<br>(0.035)   | 1519.20<br>(<0.001)   | E2>Post<br>(0.004);<br>E1>Post<br>(0.004);<br>NE>Post<br>(<0.001)   | none  | none  |
| MOVING                                  | Wald<br>chi-square<br>(p) | 269.09<br>(<0.001)   | 18.07<br>(<0.001)   | 41.06 (<0.001)              | 1.63 (0.2)        | 3.28 (0.07)       | 137588.00<br>(<0.001) | E1 < NE<br>( < 0.001);<br>E1 < Post<br>( < 0.001);<br>NE < Post<br>( < 0.001)   | E2>E1<br>(<0.001);<br>E2>NE<br>(<0.001)   | E2 < E1<br>(<0.001);<br>E2 < NE (0.004);<br>E2 < Post<br>(<0.001);<br>E1 < Post<br>(<0.001) |
| RESTING                                 | Wald<br>chi-square<br>(p) | 2811.93<br>(<0.001)  | 3.34<br>(0.188)     | 3.69 (0.296)                | 5.56 (0.02)       | 4.21 (0.04)       | 1467.00<br>(<0.001)   | E1>NE (0.001)   | none  | E2>Post<br>(0.003)  |
| SELF GROOMING                           | Wald<br>chi-square<br>(p) | 95.78<br>(<0.001)    | 15.11<br>(0.001)    | 10.34 (0.016)               | 2.14 (0.14)       | 0.95<br>(0.331)   | 1025.80<br>(<0.001)   | none  | E2>Post<br>(<0.001)   | E2>E1<br>(<0.001)   |
| SITING                                  | Wald<br>chi-square<br>(p) | 73.28<br>(<0.001)    | 7.86 (0.02)         | 6.50 (0.09)                 | 8.84<br>(0.003)   | 8.91<br>(0.003)   | 49.55<br>(<0.001)     | none  | E1>NE (0.004);<br>E1>Post<br>(<0.001);<br>NE>Post<br>(<0.001)   | NE>Post<br>(0.007)  |
| SOCIAL<br>INTERACTIONS<br>(affiliative) | Wald<br>chi-square<br>(p) | 14.19<br>(<0.001)    | 8.55<br>(0.014)     | 59.78 (<0.001)              | 2.41 (0.12)       | 4.33<br>(0.037)   | 318.47<br>(<0.001)    | E2>E1 (0.001);<br>E2>NE<br>(<0.001);<br>E2>Post<br>(<0.001);<br>E1 <post<br>(0.008)</post<br>   | E2>E1<br>(<0.001);<br>E2>NE<br>(<0.001);<br>E2>Post<br>(<0.001);<br>E1>NE<br>(<0.001);<br>NE <post<br>(&lt;0.001)</post<br> | E2>E1 (0.002<br>E2>NE (0.001  |
| STANDING                                | Wald<br>chi-square<br>(p) | 126.01<br>(<0.001)   | 7.76<br>(0.021)     | 1.85 (0.605)                | 0.08 (0.77)       | 0.02<br>(0.896)   | 6148.00<br>(<0.001)   | E1 <ne (0.001);<br="">E1<post<br>(&lt;0.001)</post<br></ne>   | E1>Post<br>(<0.001)   | E2 < Post<br>( < 0.001)   |
| NOT VISIBLE                             | Wald<br>chi-square<br>(p) | 83.03<br>(<0.001)    | 169.14<br>(<0.001)  | 59.41 (<0.001)              | 6.90 (0.01)       | 3.87<br>(0.049)   | 5718.20<br>(<0.001)   | E1 <ne<br>(&lt;0.001)</ne<br>   | E1 < NE<br>(<0.001);<br>E1 < NE<br>(<0.001);<br>E1 > Post<br>(0.004);<br>NE > Post<br>(<0.001)                              | E1 < NE (0.005<br>NE > Post<br>(0.001)  |
| CORTISOL                                | Wald<br>chi-square<br>(p) | 75537.76<br>(<0.001) | 6232.70<br>(<0.001) | 365.18<br>(<0.001)          | 2.91 (0.09)       | 61.44<br>(<0.001) | 518.44<br>(<0.001)    | E1>Post<br>(0.001)  | E2>E1<br>(<0.001);<br>E2 <ne<br>(&lt;0.001);<br/>E1<ne<br>(&lt;0.001);<br/>NE&gt;Post<br/>(&lt;0.001)</ne<br></ne<br>       | E2 > Post<br>(<0.001);<br>E1 > Post<br>(<0.001);<br>NE > Post<br>(<0.001)                   |

 $\label{eq:capacity} \mathsf{Ca} = \mathsf{caracal}, \ \mathsf{Oc} = \mathsf{ocelot}, \ \mathsf{Se} = \mathsf{serval}.$ 

**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 | Caracal F1 | 2         | 0         | 2          | 0            | 1          | 0          | 0          | 0            |
| behavior         | 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            |

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; Shepherdson et al., 1993; Mellen and Shep- erdson, 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 (Shepherdson et al., 1993; Wooster 1997; Wells and Egli, 2004; Skibiel et al., 2007). In Carlstead et al.,'s (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 ani- mal 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 multicenter 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 Alligood 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 3 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 multicentric, deeper and more complete investigations.

### **Conflict of interest**

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the pa- per. Data are available upon request from the corresponding author.

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## **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 Institu- tional Ethical Review Board of the University of Turin reviewed and approved the research (CEBA protocol number 2722).

# **Authorship statement**

The idea for the paper was conceived by AR and SN. The ex-periments 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 author

#### References

- Acaralp-Rehnberg, L.K., Coleman, G.J., Magrath, M.J.L., Melfi, V., Fanson, K.V., Bland, I.M., 2020. The effect of behind-the-scenes encounters and interactive presentations on the welfare of captive servals (*Leptailurus serval*). Animals 10 (4), 743.
- Ahloy-Dallaire, J., Espinosa, J., Mason, G., 2018. Play and optimal welfare: Does play indicate the presence of positive affective states? Behav. Process. 156, 3–15.
- Alligood, C.A., Dorey, N.R., Mehrkam, L.R., Leighty, K.A., 2017. Applying behavior-analytic methodology to the science and practice of environmental enrichment in zoos and aquariums. Zoo Biol. 36, 175–185.
- Antonenko, T.V., Ulitina, O.M., Pysarev, S.V., Matsyura, A.V., 2019. Different enriched environments for Eurasian lynx in the Barnaul Zoo. Ukr. J. Ecol. 9 (4), 671–675.
- Boissy, A., Manteuffel, G., Jensen, M.B., Moe, R.O., Spruijt, B., Keeling, L.J., Winckler, C., Forkman, B., Dimitrov, I., Langbein, J., Bakken, M., Veissier, I., Aubert, A., 2007. Assessment of positive emotions in animals to improve their welfare. Physiol. Behav. 92 (3), 375–397.
- Carlstead, K., Brown, J.L., Seidensticker, J., 1993. Behavioral and adrenocortical responses to environmental changes in leopard cats (*Felis bengalensis*). Zoo Biol. 12 (4), 321–331.
- Cornale, P., Macchi, E., Miretti, S., Renna, M., Lussiana, C., Perona, G., Mimosi, A., 2015. Effects of stocking density and environmental enrichment on behaviorand fecal corticosteroid levels of pigs under commercial farm conditions. J. Vet. Behav. 10, 569–576.
- Ellis, S.L., 2009. Environmental enrichment: practical strategies for improving feline welfare. J. Feline Med. Surg. 11 (11), 901–912.
- Johnson, W.E., Eizirik, E., Pecon-Slattery, J., Murphy, W.J., Antunes, A., Teeling, E., O'Brien, S.J., 2006. The late Miocene radiation of modern Felidae: a genetic assessment. Science 311, 73–77.
- Kusuda, S., Funahashi, T., Adachi, I., Yamamoto, H., Nagao, E., Matsui, K., Akiba, Y., 2022. Fecal Glucocorticoid Metabolites as a Noninvasive Indicator of Stress in the Tsushima Leopard Cats (*Prionailurus bengalensis euptilurus*): Application to Health Care. Animals 12, 1072.
- Lehner, P.N., 1996. Handbook of Ethological Methods. Cambridge University Press, Cambridge, UK II ed. UK..
- Markowitz, H., LaForse, S., 1987. Artificial prey as behavioral enrichment devices for felines. Appl. Anim. Behav. Sci. 18 (1), 31–43.
- McDonnell, S., 2003. A Practical Field Guide to Horse Behavior the Equid Ethogram. Eclipse Press, The Blood Horse Inc, Hong Kong, China.
- Martin, P., Bateson, P., 1986. Measuring behaviour: an introductory guide. Cambridge University Press, Cambridge, UK.
- Mellen, J., McPhee, M.S., 2001. Philosophy of environmental enrichment: past, present and future. Zoo Biol. 20, 211–226.
- Mellen, J.D., Sheperdson, D.J., 1997. Environmental enrichment for felids: an integrated approach. Int. Zoo Yearb. 35, 191–197.
- Mellen, J.D., Stevens, V.J., Markowitz, H., 1981. Environmental enrichment for ser- vals, Indian elephants and Canadian otters at Washington Park Zoo. Portland.Int. Zoo Yearb. 21 (1), 196–201.
- Moreira, N., Brown, J.L., Moraes, W., Swanson, W.F., Monteiro-Filho, E.L.A., 2007. Effect of housing and environmental enrichment on adrenocortical activity, behavior and reproductive cyclicity in the female tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). Zoo Biol. 26 (6), 441–460.

- Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74.
- Moreira, N., Brown, J.L., Moraes, W., Swanson, W.F., Monteiro-Filho, E.L.A., 2007. Effect of housing and environmental enrichment on adrenocortical activity, behavior and reproductive cyclicity in the female tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). Zoo Biol. 26, 441–460.
- Naidenko, S.V., Berezhnoi, M.A., Kumar, V., Umapathy, G., 2019. Comparison of tigers' fecal glucocorticoids level in two extreme habitats. PLoS One 14 (4), e0214447. doi:10.1371/journal.pone.0214447.
- Narayan, E.J., Parnell, T., Clark, G., Martin-Vegue, P., Mucci, A., Hero, J.M., 2013. Faecal cortisol metabolites in Bengal (*Panthera tigris tigris*) and Sumatran tigers (*Panthera tigris sumatrae*). Gen. Comp. Endocrinol. 194, 318–325.
- Newberry, R.C., 1995. Environmental enrichment increasing the biological rele-vance of captive environments. Appl. Anim. Behav. Sci. 44, 229–243.
- Otovic, P., Hutchinson, E., 2014. Limits to using HPA axis activity as an indication of animal welfare. ALTEX 32, 41–50.
- Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E., 2005. Stress hor-mones in mammals and birds: Comparative aspects regarding metabolism, ex- cretion, and noninvasive measurement in fecal samples. Ann. N. Y. Acad. Sci. 1040, 162– 171.
- Powell, K.E., 1997. Environmental enrichment programme for Ocelots Leopardus pardalis at North Carolina Zoological Park. Asheboro. Int. Zoo Yearb. 35 (1), 217– 224.
- Prola, L., Cornale, P., Renna, M., Macchi, E., Perona, G., Mimosi, A., 2013. Effect of breed, cage type, and reproductive phase on fecal corticosterone levels in doe rabbits. J. Appl. Anim. Welf. Sci. 16, 140–149.
- Rault, J.-L., 2012. Friends with benefits: Social support and its relevance for farm animal welfare. Appl. Anim. Behav. Sci. 136 (1), 1–14.
- Rault, J.-L., 2019. Be kind to others: prosocial behaviours and their implications for animal welfare. Appl. Anim. Behav. Sci. 210, 113–123.
- Resende, L.S., Remy, G.L., de Almeida Ramos Jr, V., Andriolo, A., 2009. The influence of feeding enrichment on the behavior of small felids (Carnivora: Felidae) in captivity. Zoologia 26 (4), 601–605.
- Rozhnov, V.V., Lukarevskiy, V.S., Hemandez, H.A., Sorokin, P.A., Litvinov, M.N., Kotl-yar, A.K., Udin, V.G., Naydenko, S.V., 2009. Noninvasive approach to the assessment of activity of the hypothalamic-pituitary-adrenal system of the Amur tigers. Dokl. Biol. Sci. 430, 57–59.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.
- Sha, J., Han, S., Marlena, D, Kee, J., 2012. Effects of single-use and group-use enrichment on stereotypy and intragroup aggressive and affiliative behaviors of a social group of squirrel monkeys (Saimiri sciureus) at the Singapore zoo. J. Appl. Anim. Welf. Sci. 15 (4), 358–371.
- Schatz, S., Palme, R., 2001. Measurement of faecal cortisol metabolites in cats and dogs: a non-invasive method for evaluating adrenocortical function. Vet. Res. Commun. 25 (4), 271–287.
- Shepherdson, D.J., Carlstead, K., Mellen, J.D., Seidensticker, J., 1993. The influence of food presentation on the behavior of small cats in confined environments. Zoo Biol. 12 (2), 203–216.
- Skibiel, A.L., Trevino, H.S., Naugher, K., 2007. Comparison of several types of enrichment for captive felids. Zoo Biol. 26 (5), 371–381.
- Stanton, L.A., Sullivan, M.S., Fazio, J.M., 2015. A standardized ethogram for the *feli-dae*: A tool for behavioral researchers. Appl. Anim. Behav. Sci. 173, 3–16.
- Suárez, P., Recuerda, P., Arias-De-Reyna, L., 2017. Behaviour and welfare: the visitor effect in captive felids. Anim. Welf. 26 (1), 25–34.
- Szokalski, M.S., Litchfield, C.A., Foster, W.K., 2012. Enrichment for captive tigers (*Panthera tigris*): current knowledge and future directions. Appl. Anim. Behav. Sci. 139 (1-2), 1–9.
- UKCBWG (UK Cat Behaviour Working Group), 1995. An Ethogram for Behavioural Studies of the Domestic Cat (*Felis silvestris catus*). UFAW, Wheathampstead, UK.
- Weller, S.H., Bennett, C.L., 2001. Twenty-four-hour activity budgets and patterns of behavior in captive ocelots (*Leopardus pardalis*). Appl. Anim. Behav. Sci. 71 (1), 67–79.
- Wells, D.L., Egli, J.M., 2004. The influence of olfactory enrichment on the behaviour of captive black-footed cats, *Felis nigripes*. Appl. Anim. Behav. Sci. 85 (1-2), 107– 119.
- Wooster, D.S., 1997. Enrichment techniques for small felids at Woodland Park Zoo. Seattle. Int. Zoo Yearb. 35 (1), 208–212.
- Young, R.J., 2013. Environmental enrichment for captive animals. John Wiley & Sons.
  The Universities Federation for Animal Welfare, UK.
- Young, K.M., Walker, S.L., Lanthier, C., Waddell, W.T., Monfort, S.L., Brown, J.L., 2004.Noninvasive monitoring of adrenocortical activity in carnivores by fecal gluco-corticoid analyses. Gen. Comp. Endocrinol. 137, 148–165.