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Close and frightening: predator presence affects cortisol levels in captive felines

Ksenia A. Volobueva* & Sergey V. Naidenko

ABSTRACT. Exhibitions, where animals of various species are kept close to each other, are increasingly used in zoos and live collections. Such co-housing may not be suitable for some species: the presence of a large predator may cause stress to smaller heterospecifics (for example, potential prey) and even when the enclosures have no direct intersections and are merely close together. We evaluated the effects of a potentially dangerous predator on the welfare of typical potential prey/competitors kept in close proximity to this predator. Amur wildcats and caracals were kept one at a time in enclosures next to lynx. Stress level was assessed by analysing the dynamics of glucocorticoids (cortisol's metabolites) using non-invasive methods. The results showed that the presence of a predator in a neighbouring enclosure with an Amur wildcat did not change their cortisol concentrations significantly. However, hormone levels in animals kept at some distance from the lynx were twice as high as when they were in close proximity to the predator. Unlike Amur wildcats, caracals were more sensitive to the presence of a heterospecific. For Amur wildcats the presence of shelters in enclosures and the absence of direct lynxes' pursuit may have been sufficient to reduce the negative impact of a large predator.

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KEY WORDS: Amir wildcat, caracal, Eurasian lynx, cortisol, stress, interspecific interactions.

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Близко и страшно: присутствие хищника влияет на уровень кортизола у кошачьих в условиях неволи

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РЕЗЮМЕ. Экспозиции животных разных видов в непосредственной близости друг от друга все чаще используются в зоопарках и живых коллекциях. Такое совместное проживание может быть не подходящим для некоторых видов: присутствие крупного хищника может вызвать стресс у более мелких гетероспецификов (например, потенциальной добычи), даже если вольеры не имеют прямых пересечений и просто находятся рядом друг с другом. Мы оценили влияние потенциально опасного хищника на благополучие типичной потенциальной добычи/конкурентов, содержащихся в непосредственной близости от этого хищника. Дальневосточные лесные коты и каракалы содержались по очереди в вольерах рядом с рысью. Уровень стресса оценивали, анализируя динамику глюкокортикоидов (метаболитов кортизола) с использованием неинвазивных методов. Результаты показали, что присутствие хищника в соседней вольере с дальневосточным лесным котом не приводило к значительному изменению их концентрации кортизола. Однако уровень гормона у животных, содержавшихся на некотором расстоянии от рыси, был в два раза выше, чем в случае, когда они находились в непосредственной близости от хищника. В отличие от дальневосточных лесных котов, каракалы оказались более чувствительными к присутствию гетероспецифика. Для дальневосточных лесных котов наличие укрытий в вольерах и отсутствие прямого воздействия со стороны рыси, возможно, было достаточным для снижения негативного воздействия крупного хищника.

КЛЮЧЕВЫЕ СЛОВА: дальневосточный лесной кот, каракал, евразийская рысь, кортизол, стресс, межвидовые взаимодействия.

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Introduction

High levels of chronic stress in captive animals are quite a serious problem for zoos, reintroduction centres and living collections. Typically, one of the main purposes of such institutions (in addition to educational purposes) is animal breeding. At the same time, high levels of stress have a negative impact on reproduction and offspring rearing in mammals (Husak & Moore, 2008; Alekseeva *et al*., 2020).

In captivity, a large number of factors can negatively impact the behaviour and physiology of animals. Inappropriate enclosure size can limit locomotor activity and affect growth rates (Pearce & Patterson, 1993). Changes in ambient temperature are a common source of discomfort for caged animals, for example, low temperatures lead to elevated glucocorticoid levels in Amur tigers and pigs (Hillman *et al*., 2004; Ivanov *et al*., 2017). Meanwhile, rabbits demonstrate abnormal maternal behaviour and increased frequency of sexual behaviour at excessively high temperatures (Marai & Rashwan, 2004). Even daily routines such as feeding can elicit physiological reactions associated with stress (Malinow *et al*., 1974). Moreover, one of the most common sources of stress in zoos can be conspecific and heterospecific individuals (including humans, keepers, and visitors), causing a significant increase in aggression, a grooming decrease and a reduction in the frequency of affiliative behaviour (Morgan & Tromborg, 2007).

Interactions between individuals, including those of different species, can also have a negative impact on the animals (Wielebnowski *et al*., 2002). Direct physical contact between animals is not necessary for this. Auditory, chemical (olfactory) or visual signals are sufficient to influence the activity of hypothalamic-pituitary-adrenal (HPA) axis and trigger the release of glucocorticoids (Carlstead *et al*., 1993; Blanchard *et al*., 1998; Slos & Stoks, 2008). In zoos, where animals are kept in the presence of representatives of both their own and other species, negative effects of such influences are quite common. However, it is not easy to identify the consequences visually, for example, through the animals' behaviour. The absence of pronounced external indicators of disorders makes it very difficult to assess the condition of individuals which determines reproductive success and longevity. Nevertheless, the question of what elicits a stronger negative reaction in animals remains open: signals from conspecifics or heterospecific individuals, the presence of larger neighbours, predators or herbivores.

One of the common ways to assess the condition of animals is to estimate glucocorticoid levels (Bayazit, 2009). These hormones secreted by the adrenal cortex are involved in a wide range of responses of the organism to external environmental factors (Romero & Butler, 2007). However, under conditions of prolonged (chronic) stress, the effects caused by glucocorticoids become maladaptive and lead to physiological and behavioural disorders (Liu *et al*., 2006), depressed reproductive system activity and reduced immunity (Sheriff *et al*., 2011). Various methods exist for monitoring of glucocorticoid levels, both in captivity and in the wild, with non-invasive methods being frequently used in recent years (Naidenko *et al*., 2011; Palme, 2019). These methods often allow working without direct contact with the animal. Additionally, the advantage of using hair and various excreta (such as urine, faeces) to analyse hormone levels is the lack of the contact with the animal during the sampling procedure, which allows for more reliable results (Hulsman *et al*., 2011; Crossey *et al*., 2020; Larm *et al*., 2021). The faecal glucocorticoid metabolites (FGMs) measurement is a valuable tool to assess the influence of different factors on adrenocortical activity in individuals of different species kept in captivity (Palme, 2019; Keay *et al*., 2006) and living in the wild (Gerlinskaya *et al*., 1993; Millspaugh & Washburn, 2004).

The family Felidae is one of the large carnivores' families, comprised of 42 species, including the domestic cat (Brown & Comizzoli, 2018). The Eurasian lynx (*Lynx lynx*) is the third-largest predator in Europe, after the brown bear (*Ursus arctos*) and wolf (*Canis lupus*). It is the largest representative of the subfamily of small cats (Felinae) in Eurasia, with average body mass ranging from 15–25 kg (Naidenko, 1997) exceeding the size of the European wildcat (*Felis silvestris*, 3–8 kg) by 3–4 times (Koordinierte, 2004). The caracal (*Caracal caracal*) is a medium-sized cat (8–19 kg) of the same subfamily inhabiting Africa, the Middle East, Central Asia, and arid regions of Pakistan and north-western India. The habitats of the Eurasian lynx and caracal overlap slightly in the wild, primarily in the southwest of Turkey, where animals live in the same habitats (İlemin *et al*., 2020). There are also a few observations indicating coexistence of the lynx and caracal in the eastern Alborz Mountains region in Iran (Moqanaki *et al*., 2016; Mousavi *et al*., 2016). The similar diet of both species (main prey being small ungulates, lagomorphs, and birds (Jansen *et al*., 2019; Khorozyan & Heurich, 2023) in these territories may lead to competition between them. Based on the size of the animals, it can be assumed that the caracal will avoid encounters with a larger competitor (İlemin *et al*., 2020).

The Amur wildcat (*Prionailurus bengalensis euptilurus*) is one of the least studied species of the Felidae family in Russia (Pavlova & Naidenko, 2012; Yudin, 2015) reaching a body weight of 9 kg by winter. This subspecies of the leopard cat is distributed in the Russian Far East, Manchuria, Taiwan and Tsushima islands (Ghimirey *et al*., 2022). The limited available data on the biology of the Amur wildcat makes it an attractive subject for research, and it is frequently housed in zoos and living collections. In the wild the ranges of the lynx and the Amur wildcat overlap in the Russian Far East, creating a scenario where the lynx can potentially prey on the smaller predator (Sunde *et al*., 1999; Nájera *et al*., 2019).

In zoos, animals of the same taxonomic group, including felids, are often displayed in close proximity to each other. The aim of our study was to evaluate the impact of a potentially dangerous predator on the well-being of smaller felines, kept in close proximity to each other, specifically in adjacent enclosures. We hypothesized that the impact would be more pronounced when placing a potential prey (the Amur wildcat) next to a familiar potential predator (the Eurasian lynx) compared to pairing of competitor species (the caracal and lynx).

Materials and Methods

Study site

The study was conducted at the Joint Usage Centre “Live Collection of Wild Mammals” of the Severtsov Institute of Ecology and Evolution RAS (Biological Station “Tchernogolovka”), located 50 km northeast of Moscow, Russia (56.000º N, 38.220º E). The experiment was conducted from 13.09.2021 to 10.10.2021. The temperature range during the experiment varied from –3°C (7.10.2021) to +24°C (13.09.2021), with an average temperature of +7°C.

Objects

The objects of the study were six adult individuals of the Amur wildcat (3 males and 3 females), four adult males of caracal and three adult males of Eurasian lynx.

Husbandry conditions

During the experiment, the animals were housed outdoors individually under natural light and temperature conditions in three open-air enclosure complexes. The caracals were kept in enclosures ranging from 8 to 12 m², while the Amur wildcats were housed in enclosures of 16 m². All enclosures had a height of 2–2.2 m and were covered with a metal mesh at the top. Inside each enclosure, there was an artificial shelter: a wooden box (shelter) measuring 1.8 × 1 × 0.8 m for caracals and 0.9 × 0.6 × 0.5 m for the Amur wildcat, covered with sheet iron on top. Additionally, each enclosure for the Amur wildcat was equipped with an elevated hiding spot — a shelf — measuring 70 × 70 cm, 1.5 m in height.

The animals were fed once a day, 6 days a week, and were fasted 1 day a week. The daily diet consisted of whole chicken: about 0.3–0.5 kg minced meat per Amur wildcat, and about 1 kg per caracal and lynx. Animals were provided with water ad libitum.

Samples collection

The Amur wildcats were housed in two identical complexes, each with five enclosures, arranged in the shape of “U” (see Fig. 1), far from other enclosures with animals. The cats were kept in four enclosures (in enclosures 1–2 and 4–5) in the first complex and in

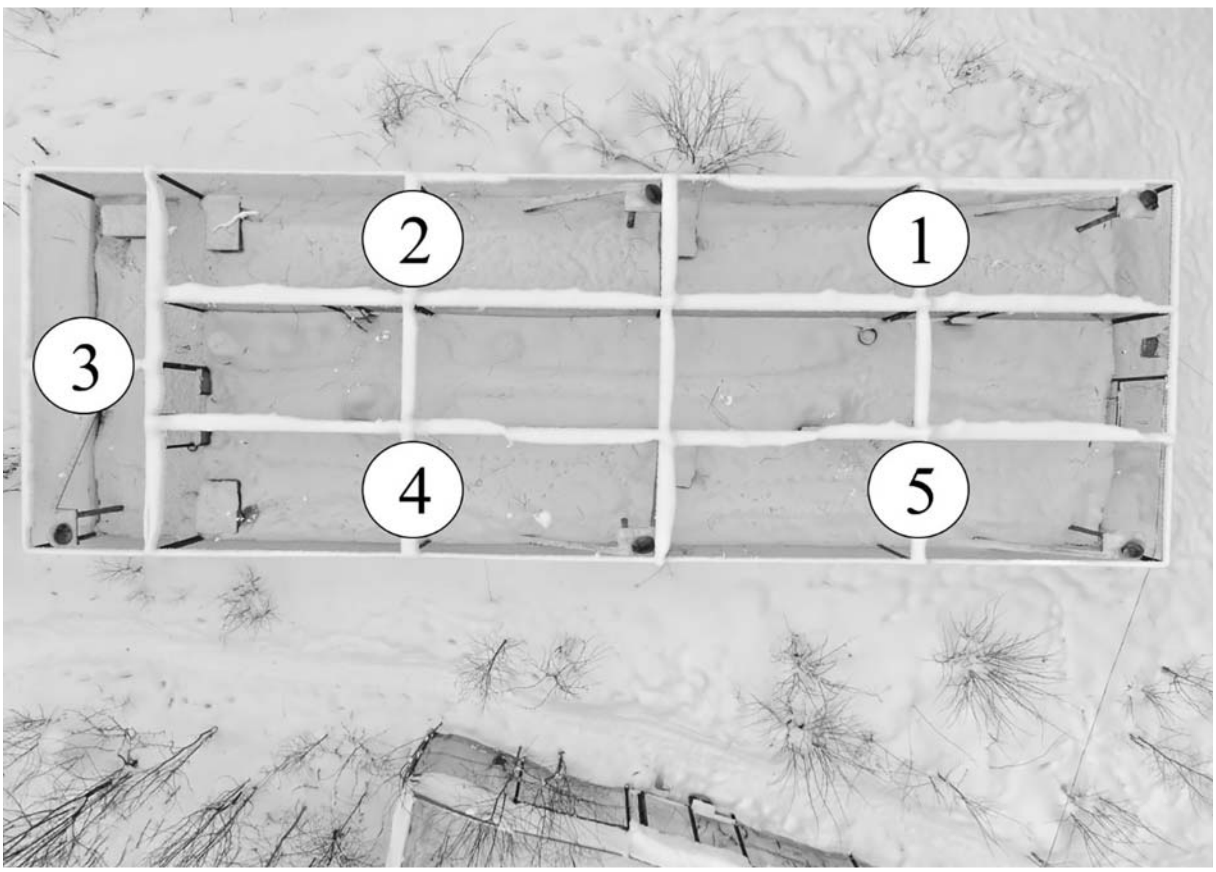

Fig. 1. Photograph of the enclosure complex for Amur wildcats used in the experiment.

two enclosures (enclosures 2, 4) in the second complex. Their faeces were collected over a week to determine the baseline cortisol levels in the animals. Subsequently, a male Eurasian lynx was placed in enclosure number three, and samples were again collected from the Amur wildcats over the course of a week. Two Amur wildcats were in close proximity to the predator (group “close”: enclosures 2 and 4, enclosure fences were modified (reduced to 25 mm mesh size) to eliminate the risk of injury from the lynx), while two other cats were located at a distance of 8 m from the lynx in direct view of the predator (group “far”: enclosures 1 and 5).

To assess FGMs levels in the caracal, a male lynx was placed in an enclosure adjacent to the caracal’s cage. Similar to the Amur wildcat, faeces were collected from the caracals during the first week of the experiment in the absence of lynx to determine basal glucocorticoid levels. Subsequently, lynx was transferred to an adjacent enclosure and faeces samples were collected for further analysis for over another 7 days. The walls of the enclosure were modified with fine metal mesh with a cell size of 25 mm, as in the case of the Amur wildcat, to prevent injuries to the animals.

The faeces were collected twice a day, in the morning and in the evening. Each sample aliquot was packed in a separate plastic bag, labelled (animal name, sex, date, and time of collection). Prior to extraction, the samples were frozen and stored at a temperature of –18°C.

Steroid extraction and analysis

We used the previously described method of steroid extraction with 90% methanol (Jewgenow *et al.,* 2006; Pavlova & Naidenko, 2008). Briefly, 0.1 g of wet faeces was weighed in an Eppendorf tube on Ohaus Pioneer scale (Ohaus Europe Gmbh, Nanikon, Switzerland), and 0.9 ml of 90% methanol was added. The tubes were shaken for 30 minutes using an “Ecros” shaker (ECROSKHIM, Saint-Petersburg, Russia). Subsequently, the tubes were centrifuged for 10 minutes at 4000 rpm using the Eppendorf MiniSpin centrifuge (Eppendorf, Gamburg, Germany). Afterwards, 400 μl of the supernatant was transferred to a clean Eppendorf tube and 400 μl of distilled water was added. The extracts were stored at –18°C until measurements were taken. The results were recalculated based on the dry weight of the faeces by drying the sample aliquot in a desiccator cabinet at 80°C to a constant weight after the whole procedure, and then calculating the percentage moisture content. This extraction method provided the most complete extraction of FGMs from feline faeces (Naidenko *et al*., 2019).

FGMs levels in the Amur wildcat were determined using “ImmunoFA_Cortisol” kits (Immunotech, Moscow, Russia). Preliminary validation of the antibodies used to measure FGMs levels in the Amur wildcat by non-invasive methods was conducted earlier (Pavlova & Naidenko, 2008). The sensitivity of the kit was 36 ng/g. The cross-reactivity of the used antibodies to cortisol was 6.0% for prednisolone, 0.9% for 11-deoxycortisol, 0.6% for corticosterone, and < 0.08% for other steroids.

To analyze the FGMs dynamics in the caracal “Cortisol-IFA” kits (“CHEMA”, Moscow, Russia) were used. The test sensitivity was 44 ng/g. The cross-reactivity of the antibodies used for cortisol was as follows: prednisolone — 5.6%, 11-deoxycortisol — 0.9%, corticosterone — 0.6%, for other steroids — < 0.1%. The possibility of assessing the HPA axis activity of the caracal by EIA using antibodies against cortisol (“Cortisol-IFA” kit (“CHEMA”, Moscow, Russia)) was performed by determining the changes in FGMs levels of caracals after the immobilization. The study was conducted in July 2020. This experiment was performed with two adult caracals (one female and one male) weighting 14.25 kg and 17.18 kg respectively, at the Joint Usage Centre “Live Collection of Wild Mammals” of the Severtsov Institute of Ecology and Evolution RAS (biological station “Tchernogolovka”). Animals were immobilized with Zoletil (about 1.8 mg/kg body weight; Virbac, France). After injection caracals were placed in their enclosures, where they were kept until the end of the experiment. Faeces were collected twice a day, in the morning and in the evening, a week before and after the immobilization. Prior to extraction, the samples were frozen and stored at a temperature of –18°C. Steroid hormones were extracted by the standard procedure as was previously described (Jewgenow *et al*., 2006; Pavlova & Naidenko, 2008). A significant increase in the level of FGMs in both caracals was detected 47 hours after their immobilization (the first faeces sample). The result of the experiment showed that the change in the levels of FGMs reflect the changes in the HPA axis activity in the caracal. An increase was 1.83 times in male and 3.45 times in females and the antibodies to cortisol (“Cortisol-IFA” kit (“CHEMA”, Moscow, Russia)) were used for assessment of stress severity of caracals by non-invasive methods.

The FGMs concentrations were determined using a heterogeneous enzyme-linked immunosorbent assay (ELISA) method with the Multiskan FC Microplate Photometer (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). The optical density in the plate wells was measured at a wavelength of 450 nm, and compared with standard values. Measurements were taken in duplicates, and the coefficient of variation (CV) was determined. If the CV was more than 5%, the measurements were repeated; if the CV was less than 5%, the mean value was accepted for further analysis. The intra-assay CV was 2.3 ± 0.2% (n = 304), and the inter-assay CV for the control sample with a concentration of 100 ng/ml measured on different plates was 1.8 ± 0.05% (n = 9).

Statistics

Statistical analysis was performed using the program Statistica 12 (StatSoft Inc., Tulsa, OK, USA). Factorial ANOVA was used to evaluate factors affecting FGMs concentrations. The identity and the presence of the predator (before/after lynx transfer) were considered as factors. The baseline FGMs levels (in the absence of the predator) were evaluated for six Amur wildcats; “close” to the predator — for the same six animals; and "far" from the lynx — for four individuals. FGMs levels before and after placing the Eurasian lynx into the enclosure far from Amur wildcats were compared (baseline and "far", $n = 4$); the difference in FGMs concentrations before and after the transfer of the lynx into the enclosure adjacent to the cat was assessed (baseline and "close", $n = 6$); additionally, the influence of the distance factor between the enclosures of the Amur wildcats and the lynx was evaluated ("far"–"close", $n = 4$). Individual significant peaks in FGMs concentrations were defined as values exceeding the means by two or more standard deviations (Ivanov *et al*., 2014). The Mann-Whitney test was used to analyze changes in FGMs concentrations in an individual animal. Our preliminary analysis showed that the air temperature did not affect the FGMs level of any individuals ($p > 0.05$), so we neglected the effect of this factor in the further analysis.

Ethical approval

Data collection protocol No.21 dated 24.04.2018 was approved by the Commission for the Regulation of Experimental Research (Bioethics Commission) of the Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences. The performed procedures had no obvious adverse effects on the animals.

Results

Amur wildcat

Comparison of FGMs levels in the absence of a predator and its "close" proximity in the adjacent enclosure showed that the "presence of a predator" factor did not have a significant effect on FGMs concentrations ($F = 0.01$; df = 1; $p = 0.91$) (Fig. 2), whereas the individual had a reliable effect ($F = 8.45$; df = 5; $p = 0.000001$). The combined effect of these factors was not significant ($F = 0.12$; df = 5; $p = 0.99$). Among animals kept in proximity to lynx, 2 of 6 Amur wildcats showed significant peaks in FGMs concentrations (higher than mean ± 2SD after 12 and 48 hours). Significant differences between the baseline FGMs levels and their concentrations in the presence of the predator were observed in only one animal (Mann-Whitney U Test: $Z = -2.22$, $p = 0.027$) out of six.Comparing FGMs levels in the absence of the predator and "far" distance from the predator, the individual factor had a significant effect on FGMs concentrations as in the first group ($F = 10.66$; df = 3; $p = 0.000005$), whereas the "predator presence" factor did not significantly affect this parameter ($F = 3.09$; df = 1, $p = 0.08$) (Fig. 3). When examining individual FGMs profiles, significant FGMs peaks after placing the lynx into the complex were observed in 3 out of 4 animals kept far from the predator (at 48, 84, 96, 120 hours; one individual showed two peaks with a 24-hour difference). The combined effect of these factors had a significant impact ($F = 3.5$; df = 3; $p = 0.02$).

Comparing FGMs levels in Amur wildcats in the presence of a predator ("far" or "close") no significant influence of the distance to the predator was found ($F = 2.47$; df = 1; $p = 0.12$) (Fig. 4). Overall, the FGMs level during the period spent in the close enclosure (4372 ± 1147 ng/g) was almost half that during the stay in the far enclosure (8650 ± 3182 ng/g). Individual characteristics of the animals had a significant impact on FGMs levels ($F = 8.01$; df = 3; $p = 0.0001$). The combined effect of the factors "distance from predator" and individual was not significant ($F = 2.2$; df = 3; $p = 0.08$).

No significant differences were found between morning and evening samples ($p > 0.05$).

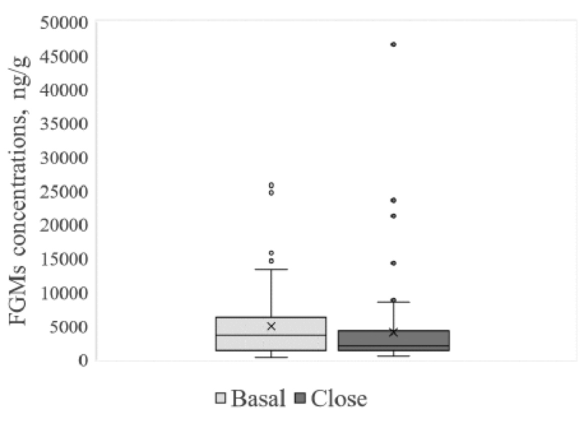

Fig. 2. FGMs levels during the absence of a predator and its "close" proximity of the Amur wildcat: Basal — basal FGMs concentrations, Close — FGMs concentrations of individuals kept at closer enclosure. Plotted are the median (horizontal line in the box), mean values (cross), lower and upper quartiles (horizontal box boundaries), and minimum and maximum values (whiskers); dots indicate outliers.

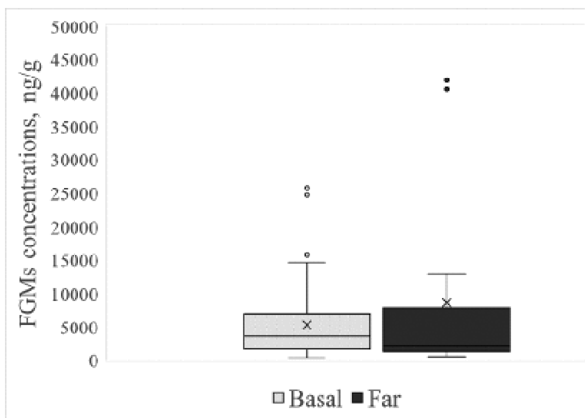

Fig. 3. FGMs levels during the absence of a predator and "far" distance from the predator of the Amur wildcat: Basal — basal FGMs concentrations, Far — FGMs concentrations of individuals kept at far enclosure. Plotted are the median (horizontal line in the box), mean values (cross), lower and upper quartiles (horizontal box boundaries), and minimum and maximum values (whiskers); dots indicate outliers.

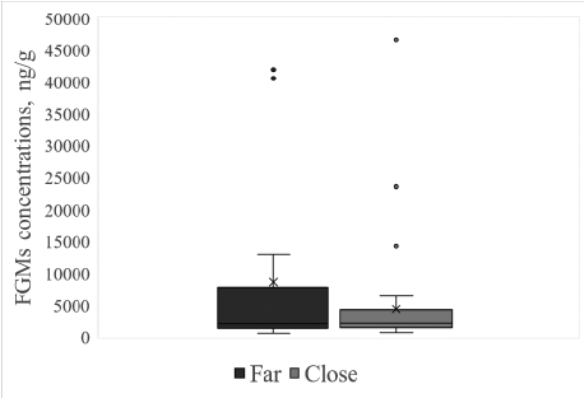

Fig. 4. FGMs levels during the "far" distance from the predator and the "close" distance to the predator of the Amur wildcat: Far — FGMs concentrations of individuals kept at far enclosure. Plotted are the median (horizontal line in the box), mean values (cross), lower and upper quartiles (horizontal box boundaries), and minimum and maximum values (whiskers); dots indicate outliers.

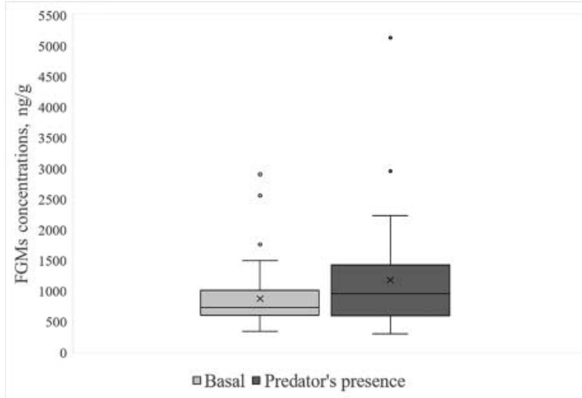

Fig. 5. FGMs levels in caracals before and after the placing of the Eurasian lynx into the adjacent enclosure: Basal — basal FGMs concentrations, Predator's presence — FGMs concentrations after the appearance of the lynx. Plotted are the median (horizontal line in the box), mean values (cross), lower and upper quartiles (horizontal box boundaries), and minimum and maximum values (whiskers); dots indicate outliers.

Caracal

Differences between the absolute FGMs concentrations "before" and "after" the introduction of the predator were significant ($F = 6.06$; df = 1; $p = 0.016$). Mean FGMs level after the appearance of a larger predator nearby (1177 ± 126 ng/g, $n = 4$) was higher than mean baseline FGMs level (824 ± 94 ng/g, $n = 4$) (Fig. 5). We also detected significant peaks in all 4 caracals after placing the lynx into an adjacent enclosure (one animal after 24 h, the other two after 72 h, and the fourth after 60 and 144 h), then mean FGMs levels in each object of the study decreased to baseline values. Individual differences in FGMs levels were significant ($F = 4.06$; df = 3; $p = 0.0096$). The influence of temperature on FGMs levels was not significant in any of the animals ($p > 0.05$).

No significant differences were found between morning and evening samples ($p > 0.05$).

Discussion

This study was designed to investigate the effect of the presence of a larger predator on glucocorticoid levels in typical co-habitants, which may be potential prey or potential competitors, under captive conditions. Exhibitions with different species kept near each other are quite commonly used by zoos to enhance the attractiveness of their collections, attract more visitors to enrich the environment for multiple species simultaneously (Coe, 2001; Leonardi *et al*., 2010). Such exhibits often include birds, groups of primates, or ungulates that coexist sympatrically (Ziegler, 2002; Hardie *et al*., 2003; Probst & Matschei, 2008). However, when it comes to carnivores, species intended for cohabitation need to be selected even more carefully, based on ethical considerations and animal husbandry guidelines (Hediger, 2013). This is particularly relevant for large predators, as there is an increased risk of injuries to other animals and a higher likelihood of lethal outcomes. Even the presence of the predator nearby may affect the well-being of some animals (Wielebnowski *et al*., 2002). Although in our experiment the studied objects were not housed in the same enclosure (they were either in adjacent enclosures or in the same complex), it is known that olfactory, visual and acoustic stimuli from con- and heterospecifics can lead to short-term and sometimes chronic stress (File *et al*., 1995; Hemsworth & Barnett, 2000; Wielebnowski *et al*., 2002; Morgan & Tromborg, 2007).

For example, a negative effect of the presence of large predators on the activity of the adrenocortical system of animals has been shown for the clouded leopard (*Neofelis nebulosa*) (Wielebnowski *et al*., 2002). We hypothesised that due to the greater difference in body mass the Amur wildcat would experience greater stress interacting with lynxes than caracals. Indeed, some Amur wildcats showed short-term increase in FGMs levels, although only one of the six animals showed a significant increase within a week. However, this female differed from all other animals with a higher baseline FGMs level (three times higher) and likely a higher degree of reactivity of the adrenocortical system. Thus, placing a predator in the adjacent enclosure with the Amur wildcat did not lead to a significant change in the activity level of the HPA axis in the animals.

For an arboreal species like the clouded leopard, the presence of structures in the enclosure that facilitate the animal's movement in the vertical axis reduces the stress levels in the animals (Wielebnowski *et al*., 2002). Although Amur wildcats have a terrestrial lifestyle, the animals actively used elevated hiding spots as well as ground shelters in captivity. This likely provided them with a sufficient "degree of comfort" and "feeling of safety", and their glucocorticoid levels did not increase. In general, the presence of shelters and structures in enclosures is an important part of environmental enrichment in captivity, as they provide animals with the opportunity to protect themselves from potential threats (e.g. large number of visitors or conspecifics) and also reduce the likelihood of stereotypic behaviour. For example, young pigs in recently formed groups engaged in less fighting if they had an opportunity to hide (McGlone & Curtis, 1985), and the installation of a camouflage barrier reduced aggression and stereotypic behaviour in gorillas kept in a zoo (Blaney & Walls, 2004).

However, FGMs levels were twice as high in Amur wildcats kept at some distance from lynxes (group "far") than in those kept in close proximity to the predator. This could be associated with the fact that, in the absence of the predator in their line of sight, its vocalization and scent have a significant impact on the behaviour of potential prey (Apfelbach *et al*., 2005). Without visual contact, the animal remains in a state of heightened vigilance and defensive behaviour for a longer duration, leading to increased activity of the adrenocortical system and higher cortisol levels (Popov, 2010). When the animal was kept in close proximity to the predator, it could more accurately "predict" the predator's behaviour, whereas with partial loss of visual contact in distant enclosures, the state of uncertainty in the animal could significantly increase, typically leading to an elevated FGMs level (Popov, 2010; Koolhaas *et al*., 2011).

The situation was different with the caracal. Unlike the Amur wildcat it was more sensitive to the presence of the Eurasian lynx. Prolonged stay in enclosures near a large predator and the inability to fully control the surrounding environment are strong stressors for animals (Anderson *et al*., 2002), leading to a 1.4-fold increase in FGMs levels in caracals after placing the Eurasian lynx into the adjacent enclosure. Interestingly, for mammals, predator scent did not always affect glucocorticoid levels in animals, even when it did influence behaviour and reproduction (Anisman *et al*., 2001).

For the caracal the lynx is more of a competitor than a predator of similar size. During the experiments, no specific observations were made for the animals; however, for one of the caracals, attempts to attack the lynx were noted. As a result, this male showed a 2–2.5-fold increase in cortisol levels after this behaviour was registered (24 hours later). The need to maintain their status during interactions with other individuals very often serves as a reason for an increase in glucocorticoid levels in animals (Creel *et al*., 2013). Perhaps in this situation we can attribute the high stress levels in male caracals to their attempts to establish social relationships with the lynxes. It is known that after the establishment of a social hierarchy in conspecific groups, the stress levels in animals can significantly decrease, whereas during the period of group formation it reaches maximum values (Timmer & Sandi, 2010). Additionally, during mixed-species housing some species demonstrate an increase in aggressive and agonistic behaviour towards members of other systematic groups (Ross *et al*., 2009; Law *et al*., 2021), which confirms our data. In our case, the lynxes were housed in enclosures next to the caracals for only a week, which was undoubtedly insufficient for the formation of stable relationships between individuals.

Conclusions

Thus, the results obtained did not confirm our initial hypothesis: male caracals reacted more acutely to the presence of the Eurasian lynx than Amur wildcats, potential prey. For Amur wildcats, the presence of structures, such as shelters and elevated hiding places, in enclosures and the absence of direct pursuit by lynxes may have been sufficient to reduce the negative impact of the larger predator. This study emphasizes the need for a competent approach to the captivity management of animals, taking into account their individual characteristics and species needs.

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