СОФИЙСКИ УНИВЕРСИТЕТ
„СВ. КЛИМЕНТ ОХРИДСКИ“
БИОЛОГИЧЕСКИ ФАКУЛТЕТ

SOFIA UNIVERSITY
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Influence of human and environment on the behavior of selected mammal species in captivity- application in zoos and conservation

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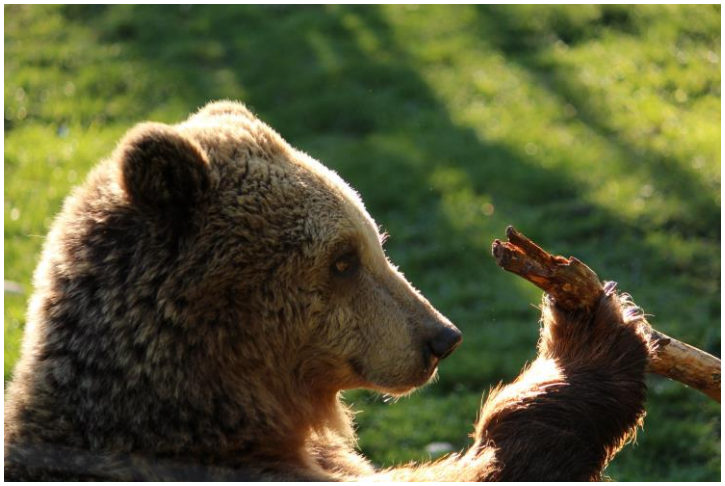

SYNOPSIS

of a dissertation

for the awarding of an educational and scientific degree "Doctor" in

professional direction 4.3 Biological Sciences

Specialty Ecology and Environmental Protection - Behavioral ecology

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Sofia, 2024

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INTRODUCTION

One of the main functions of modern zoos is the protection of endangered and rare animal species. For the improvement of their welfare, reproductive success and increase the effectiveness of conservation and educational activities in zoos, knowledge of animal behavior is of utmost importance. The main factors that influence the behavior of zoo animals are the quality and changes in their surroundings, the individual history of the animals and their interaction with humans. When they are raised in suboptimal conditions, stereotypic behavior is among the usual behavioral manifestations. Providing stimuli such as environmental enrichment in animal enclosures can help avoid or reduce the effect of prolonged stress and increase animal welfare, as well as contribute to the maintenance of the species' natural behavioral repertoire (Coelho et al., 2012).

Brown bear *Ursus arctos* (Linnaeus, 1758) is a species that has traditionally been kept in captivity by human. At the same time, the brown bear is an endangered species in Bulgaria and is included in Appendix II and III of Biological Diversity Act, and internationally in BeC-II; CITES-II; HD – II, IV and with conservation status „Endangered – EN“ in the Red Data Book of the Republic of Bulgaria (Golemanski et al., 2015). Currently, there are about 50 brown bears living in licensed and unlicensed zoos in Bulgaria and in Bear sanctuary Belitsa. Bear exhibits in many zoos tend to be small in size and sparsely arranged. Bears raised in such an adverse environment, especially from an early age, tend to exhibit stereotypic behavior. Zoo professionals employ diverse and creative strategies in attempting to address stereotypic behavior. These include the provision of various incentives, physical change in the environment, provide correct care and development of environmental enrichment programs based on the specific behavioral needs of the species. However, more research is needed to establish the effectiveness of different stimuli on reducing stereotypic behavior in brown bear and to maintain a sufficient species-specific behavioral repertoire. Brown bear is a species that, having been bred in captivity and has lost its fear of man, can hardly be returned to the wild. Constant efforts are required to ensure the good mental and physical condition of individuals kept in zoos and sanctuaries. Assessing the welfare level of captive bears in Bulgaria can lay the foundation for achieving this goal. Nowadays, animal welfare is a priority for modern institutions, such as zoos, aquariums, breeding centers, shelters, laboratories, farms, etc. and is part of the responsible management of the zoo collections. Any change in husbandry and/or enclosure design should be accompanied by a scientific welfare assessment (Ward et al., 2020).

Another important for conservation species in our country is the European souslik *Spermophilus citellus* (Linnaeus, 1766), which is included in IUCN Red List with conservation category „Endangered – EN“, in Appendix II of Biological Diversity Act and with conservation status „Vulnerable-VU“ in the Red Data Book of the Republic of Bulgaria. The species is included in BeC - II and in DH – II, IV. The European souslik is presented in some zoos in Poland, Switzerland, the Czech Republic, Hungary, the Netherlands, Great Britain, etc., but until 2021 it was not present in any zoo collection in Bulgaria. Creating zoo exhibits with European souslik in Bulgarian zoos would have an educational effect and will

also contribute to increasing the effectiveness of the conservation activities for that species. The lack of sufficient knowledge about behavior of the European souslik in captivity in our country determined the need to study its behavior and activity in zoo conditions.

In our country, few studies have been done on the behavior of the Brown bear and the European souslik in captivity. A comprehensive scientific welfare assessment has not been carried out for the brown bear. This knowledge is important for increasing the quality of life of these species in modern zoo collections and for conservation purposes.

AIM AND TASKS

The aim of the present dissertation is to study the behavior of the brown bear *Ursus arctos* in captivity, with an emphasis on stereotypic behavior, the influence of different stimuli and welfare assessment, necessary for the formulation of science-based recommendations to improve husbandry practices, as well as expanding the knowledge about the behavior of the European souslik *Spermophilus citellus* in zoo conditions, important in the planning and implementation of conservation activities for the species.

The following tasks were set for the realization of the goal:

1. Conducting observations on the behavior of the brown bears in captivity and establishing the forms of stereotypic behavior.

2. Offering sound and odor stimuli as environmental enrichment to establish their influence on the behavioral repertoire of brown bear and their effectiveness in mitigating the manifestations of stereotypic behavior.

3. Assessing the welfare of the brown bears in captivity in Bulgaria, by creating and applying a questionnaire to collect information about the individuals and their living conditions.

4. Preparation of recommendations for improving the environment and living conditions of brown bears in captivity in Bulgaria.

5. Preparation of a proposal for changes to Ordinance No. 6 of 23.10.2003 on the minimum requirements and conditions for keeping animals in zoos and breeding centers of protected animal species in the part relating to the keeping of brown bear *U. arctos* .

6. Preparation of a catalog with suitable forms of environmental enrichment for brown bears, applicable in the conditions of Bulgarian zoos.

7. Study of exploratory behavior and behavioral response to a novel object in the European souslik *S. citellus* using the Open Field Test and the Novel Object Exploration Test.

8. Study of the activity of the European souslik in zoo conditions.

On the basis of existing literature data, it can be expected that in brown bears raised in poor environment where the applied husbandry practices are inappropriate, frequent manifestations of stereotypoc behavior will be observed and the level of their welfare will be low. It could also be hypothesized that providing different stimuli in the animal enclosures would reduce the effects of prolonged stress and help maintain the natural behavioral repertoire. The European souslik can be expected to show an ability to adapt to life in zoo conditions and natural behaviors such as hibernation.

I. INFLUENCE OF SOUND AND ODOR STIMULI ON THE BEHAVIORAL REPERTOIRE OF THE BROWN BEAR

I-1. Materials and methods

Animals and housing. The study was conducted from June to October of 2020 and 2021 with a total of 19 brown bears (10 males and 9 females), selected from eight zoos in Bulgaria. In all enclosures there were pools of different sizes and indoor premises for the bears. The description of zoos, individuals and enclosures is presented in Table 1.

For conducting of the experiments each zoo was visited for 3 days. On the first day preliminary observations on the behavior of bears were made with an emphasis on the stereotypic behaviors. On the 2nd and 3rd days experiments with both types of sensory stimulation - auditory and olfactory were performed. Both types of stimuli were natural - sound and odor from conspecifics. No enrichment programs were implemented in any of the zoos, nor were any sensory stimuli offered to the animals prior to conducting this study.

Table 1. Data of animals and enclosures. Explanation: **E1** - the animals participating in Experiment 1, **E2** - the animals participating in Experiment 2; **CAPT** – born in captivity, **UNKN**- unknown origin, **WILD** – from wild nature; Characteristics of the substrate: **C**- concrete, **N**-natural, **SN**-seminatural.

Institution	**Name, sex, age and origin of individuals**	**Participation in experiments**	**Size of the enclosure and number of bears in it**	**Substrate**	**Enclosure furnishing**
Aytos Zoo	Elena - female, 23 years, CAPT	E1, E2	220 m^2 2 bears	C	Empty
	Mia - female, 14 years, CAPT	E1, E2			
Blagoevgrad Zoo	Borko - male, 9 years, CAPT	E2	3 500 m^2 2 bears	N	Seminatural
	Kalina - female, 18 years, CAPT	E2			
Dimitrovgrad Zoo	Mitko - male, 27 years, UNKN	E1	206 m^2 2 bears	SN	Fixed objects
	Ani - - female, 27 years, UNKN	E1			
Dobrich Zoo	Kostadin - male, 9 years, CAPT	E1, E2	3000 m^2 2 bears	N	Seminatural
	Bernadeth - female, 10 years, CAPT	E1, E2			
Lovech Zoo	Javor - male, unknown, CAPT	E1, E2	102 m^2 1 bear	C	Empty
	Svetlio - male, 1.6 years, CAPT	E1, E2	410 m^2 2 bears	C	Seminatural
	Jina - female, 13 years, WILD	E1, E2			
	Velik - male, 19 years, CAPT	E1, E2	155 m^2 3 bears	C	Empty

	Nina - female, 16 years, CAPT	E1, E2			
	Alexandra - female, 8 years, CAPT	E1, E2			
Pavlikeni Zoo	Vasko- male, 14 years, WILD	E1	200 m^2 1 bear	C	Fixed stones
Pleven Zoo	Miladin - male, 11 years, CAPT	E1, E2	560 m^2 2 bears	SN	Seminatural
	Viki - female, 10 years, WILD	E1, E2			
Stara Zagora Zoo	Apolon - male, unknown, CAPT	E2	500 m^2 2 bears	C	Fixed stones
	Nasko - male, unknown, CAPT	E2	500 m^2 2 bears	C	Fixed stones

Note: The two male bears in Stara Zagora Zoo coexist with females that did not participate in the experiments due to their advanced age and limited mobility.

Experimental design and procedure. Experiment 1 - sound of conspecifics. The stimulus was offered to 15 bears (7 males and 8 females) from six zoos. The duration of the signal was 5 minutes and consisted of huffing and grunting sounds. We suggest that they are associated with exploration behavior, as they were registered in this context. The sound recording was played once near the bear enclosures from a GBL Go3 portable speaker. It was placed near the fence of the enclosures, where visitors or staff members do not stand. The sound was played between 10 am and 12 pm in the different zoos. The bears' behavior was recorded 20 min before the sound was played, during its presentation, and 20 min after the sound was stopped.

Experiment 2 - odor of conspecifics.The odor stimulus was offered to 16 bears (8 males and 8 females) from 6 zoos. Urine of a 14-year-old uncastrated male bear from Sofia Zoo was used. This individual did not participate in the study and was unknown to the bears. The urine was collected from the animal's enclosure 1 to 3 days before each visit to some of the zoos. It was stored in a freezer at - 20°C one to three days before the experiment and was unfroze 12 h before the experiment and transferred into a refrigerator bag at 4–7°C in accordance with the recommended procedures for urine storage and transportation (Charteris et al., 2021; Danish et al., 2015). This procedure was repeated with a freshly collected urine sample before each experiment.

During the experiments, a total of 18 cardboard boxes were used in all visited zoos – 9 on the first experimental day and 9 on the second one. The boxes measured 50 X 50 X 30 cm. The cardboard boxes were new and delivered in their factory packaging. These boxes were chosen because they are safe for bears: cardboard is often used as environmental enrichment for zoo animals.The experiment was performed on two consecutive days. On the first day of the experiment, one cardboard box without applied odor per bear enclosure was offered. They were used as a control. On the second day of the experiment, to test the effect of the odor stimulus 20 ml urine was applied on the inside of another, new cardboard box with a syringe. Before providing the boxes to the bears, the urine was absorbed by the cardboard. On the first and second experimental days, the boxes were placed in the outer spaces of the enclosures (Pict. 1). Before putting the odorized boxes in the enclosures, the leftovers of the non-

odorized boxes from the previous day were removed. In preparation for the experiment, we used latex gloves to avoid leaving our odor on the boxes.

On each experimental day, the behavior of the bears was recorded for 20 min before the cardboard box was presented, during the presentation of the boxes, and for 20 min after the end of interactions with the boxes. The end of interactions with the boxes was considered when the animals no longer returned to the box or its pieces.

Picture 1. A brown bear in Stara Zagora Zoo sniffs a non-odorized box in its outdoor enclosure.

To take into account the influence of the sound and odor stimuli on the behavior of bears, the experiments were performed outside the period of feeding and cleaning the enclosures and in the absence of staff members, visitors, or members of our team. An ACME VR06–4 K Ultra HD video cameras were used to record the behavior of the animals during the experiments. Despite the fact that most of the bears lived in groups, the behavior of each of them was tracked separately during the experiments by focal sampling (Altmann, 1974).

Data collection and analysis. During the experiments, the bears showed different behavioral events related to the exploring of the environment and the stimuli, as well as a number of stereotypic behaviors. To assess the significance of the stimuli on bear's behavior, the duration (s) and frequency (number) of a set of behavioral events, demonstrated by all animals before and after the stimuli, as well as variables such as latency to approach objects, were considered (Table 2). For the assessment of behavioral diversity, all behavioral events of animals before, during, and after the stimuli were taken into account.

Table 2. Behavioral variables used for the quantitative assessment of behavior. Explanation: Behavioral events displayed in Experiment 1 are indicated by [1], those in Experiment 2 by [2], and those registered in the both experiments are indicated with [1,2].

Behavioral events and variables	Indicators for quantitative assessment of behavior	Description
Walking [1] (WAL)	duration	The bear walks through its enclosure.
Standing on two/four legs [1,2] (STA)	duration	The bear stands up on its hind legs with or without leaning against the wall of the fence or stands still on four legs, sniffing the air and listening around.
Resting behavior [1,2] (RES)	duration	The bear sits on its hindquarters or lies on its stomach or on one side, visibly calm.
Approaching a cardboard cardboard box [2] (APP)	frequency	Approaching the cardboard cardboard boxes without or with an odor from conspecifics.
Sniffing a cardboard box [2] (SNI)	duration	Sniffing the cardboard boxes without or with applied odor from conspecifics.
Interaction with a cardboard box[2] (INT)	duration	Hitting, eating, carrying, tearing off, rubbing in the cardboard box, playing with the cardboard box.
Latency to approach [2] (LT)	duration	The time from the release of the bear in the outer enclosure to its first approach to the cardboard box without or with an odor from conspecifics.
Stereotypic behaviors		
Pacing[1,2] (PAC)	duration	Walking the same straight path repetitively, with turning points at the same location each time. Three successive identical repetitions were set as the criteria for pacing and continued until the bear changed its path or become inactive for more than 3 s (Anderson, Arun and Jensen, 2010).
Head-tossing [1,2] (HT)	frequency	The bear suddenly throws its head back and turns it (Montaudouin and Le Pape, 2005).
Pacing with head tossing [1,2] (PAC&HT)	duration and frequency	Head-tossing combined with pacing.
Walking in figure-of-eight [1,2] WAL8	duration and frequency	The bear walks around the enclosure, always following the same path and in a trajectory of the figure-of-eight.
Tongue flicking and chewing [2] (TON)	duration	The bear repeatedly and quickly sticks its tongue out and with tossing movements repeatedly touches the upper surface of its muzzle (for 8-10 seconds) and then several times „chews“ its tongue (for 5-10 seconds).
Quick movements with eyes and lips [1,2] (MEL)	duration	The bear stands or sits with its head bowed and blinks unusually quickly and moves its lower lip, sometimes bringing one of its front paws closer to its snout.

Behavioral diversity was calculated using the Shannon Diversity Index -H (Shannon, 1948):

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

where **p** is the rate of each behavior observed divided by the total rates of all behaviors observed, **ln** is the natural log and ∑ is the sum across behaviors, **S** is the number of behaviors performed. It was calculated using the PAST 4 software (Hammer et al. 2001). Shannon's diversity index is one of the most accurate and useful diversity indices and is the most commonly used index to describe behavioral diversity in animals (Miller et al., 2020).

Statistical analysis. To test whether there are statistically significant differences in animal behavior before and after offering sound and odor stimuli, a comparison between the duration of the variables walking before (WALbef) and after (WALaft), resting before (RESbef), and after (RESaft) and standing before (STAbef) and after (STAaft) was done in males and females using the Wilcoxon matched pairs test. To test whether and how bears respond to the olfactory stimulus, the frequency of approaching a cardboard box without odor (APPcon) and with odor (APPod), the duration of sniffing a cardboard box without odor (SNIcon) and with odor (SNIod) as well as the duration of the interaction with the cardboard boxes without odor (INTcon) and with odor (INTod) were compared by Wilcoxon matched pairs test. The duration of the latency to approach the cardboard box without an odor and with odor were also compared for males and females separately. The sex differences in the behavioral response to the sound and odor stimuli, were checked using Mann – Whitney U test. The variables were tested for normal distribution by the Kolmogorov-Smirnov test.

The duration of stereotypic events before and after the stimuli was tested using One sample chi-square test separately for male and female bears to check whether there was a statistically significant difference in the duration of stereotypic behavior before and after offering the sound and odor stimuli. Additionally, a chi-square test of independence (2x2) was applied to prove differences in the frequency and duration of stereotypic behaviors between males and females. Additionally, to check if there was a statistically significant difference in the duration of stereotypic behavior before and after presenting the odor stimulus in male and female bears, the duration of stereotypic behaviors before and after the odorized cardboard box was presented was also tested using the Wilcoxon matched pairs test. The sex differences in the duration of stereotypic behavior before and after presenting the odor stimulus were tested with Mann–Whitney U test. We also tested for any correlation between the duration of stereotypic behavior and the bears' rearing conditions using Spearman Rank Order Correlations. Statistically significant differences were considered at $p < 0.05$. Statistical analyzes were performed using the STATISTICA program, Version 12.0 (StatSoft Inc., Tulsa, OK, USA).

I - 2. Results

Behavioral response of bears to the stimulus "sound of conspecifics". In the period of observations before offering the acoustic stimulus, the animals performed behaviors such as resting, bathing, playing, stereotypies, etc. The behavioral diversity before the sound stimulus was Hbef ♂ = 2.22, S = 12; Hbef ♀ = 2.41, S = 16. When offered stimulus, the attention of bears was attracted by the sound. Under the influence of the stimulus, the bears stood still for longer, listening around and sniffing the air, which led to a relative reduction in the variety of behaviors compared to those demonstrated before the stimulus (H_{aud} ♂ = 2.08, S = 10; H_{aud} ♀ = H = 2.13, S = 12). Nevertheless, some animals expressed fear and they escaped far from the sound. Some bears emitted sounds such as huffing, grunting, puffing, clattering, moaning. Others were digging the ground near the source of the sound. Behavioral diversity after the influence of the sound stimulus was the highest - H_{aft} ♂ = 2.47, S = 16; H_{aft} ♀ = 2.62, S = 19, because bears expanded their behavioral repertoire by sniffing the ground, continued to emit the sounds of clattering and huffing, grunting, and puffing to conspecifics.

Regarding the quantitative analysis, neither in females nor in males statistically significant differences in the duration of the behavioral events WALbef and WALaft, STAbef and STAaft, RESbef and RESaft, manifested before and after the sound stimulus were established (Table 3). However, such a difference was found between males and females in terms of standing on two/four legs (STA). The results showed that males, unlike females, were standing on two hind legs or on four legs, sniffing the air and listening around significantly longer after the presentation of the stimulus (Mann – Whitney U test: U = 8.0, p < 0.05, Table 3).

Table 4. Median and extreme values (minimum and maximum) of behavioral variables in male and female brown bears before and after presenting an sound stimulus. The significance of differences revealed by Mann–Whitney U-test and Wilcoxon matched pairs test is shown: $p < 0.05$. The extreme values are given in brackets. Explanation: n - number of bears, M – males, F – females.

Behaviour	**M (*n*=7)**	**F (*n*=8)**	**Mann-Whitney U test**
	Median (min-max)		**$U_{(MxF)}$, p**
Walking before-WALbef	163.0 (15.0 - 825.0)	210.5 (10.0 - 798.0)	U = 26.0, p = 0.816
Walking after-WALaft	361.0 (146.0 - 1090.0)	280.5 (27.0 - 975.0)	U = 24.0, p = 0.643
Wilcoxon Matched Pairs test (WAL_{bef} - WAL_{aft})	$T_{(MxM)}$ = 7.0, $Z_{(MxM)}$ = 1.183, p = 0.236	$T_{(FxF)}$ = 12.0, $Z_{(FxF)}$ = 0.840, p = 0.840	
Standing on two/four legs before - STAbef	35.0 (15.0 - 375.0)	104.0 (25.0 - 327.0)	U = 14.5, p = 0.118
Standing on two/four legs after – STAaft	361.0 (146.0 - 1090.0)	93.5 (26.0 - 803.0)	**U = 8.0, p = 0.02**
Wilcoxon Matched Pairs test (STA_{bef} - STA_{aft})	**$T_{(MxM)}$=1.0, $Z_{(MxM)}$= 0.197 p = 0.027**	$T_{(FxF)}$ = 14.0, $Z_{(FxF)}$ = 0.560, p = 0.575	

Resting before – RESbef	225.0 (22.0 - 1170.0)	132.5 (12.0 - 1165.0)	U = 20.5, p = 0.020
Resting after -RESaft	366.0 (17.0 - 948.0)	130.0 (25.0 - 1045.0)	U = 26.5, p = 0.862
Wilcoxon Matched Pairs test (RES_{bef} - RES_{aft})	$T_{(MxM)}$ = 7.0, $Z_{(MxM)}$ = 1.183, p = 0.236	$T_{(FxF)}$ = 14.0, $Z_{(FxF)}$ = 0.560, p = 0.575	

During the presentation of the sound stimulus, the attention of all bears was drawn to the sound of a conspecific and they ceased the other behaviors they had been exhibiting until then, such as lying down, staying indoors, bathing, stereotyping, playing, etc. While under the influence of the proposed sound stimulus, the bears exhibited predominantly exploratory behavior – standing still on two hind legs or on all fours, oriented towards the sound source and listening and sniffing the air. In one individual, a 1.5-year-old young bear, an initial startle response was observed, resulting in running away from the sound source. Two male bears and one female bear showed great excitement and, in response to the sound of the conspecific, made sounds such as grunting, pawing, and calls to the conspecific (directed to other bears in the enclosure). Two male bears were observed digging vigorously in the soil near the source of the sound. One of the male bears was observed to rub its back against the fence after the recording ended.

Behavioral response of bears to the stimulus "odor from conspecifics". Before offering the olfactory stimulus, the bears displayed behaviors such as walking, standing, resting, stereotypic forms. Behavioral diversity was Hbef ♂ = 2.43, S = 15, Hbef ♀ = 2.31, S = 14. Offering the cardboard without an odor, we observed an increase in behavioral diversity, compared to before offering the stimulus in males (Hbox without odor ♂ = 3.09, S = 27) as well as in females (Hbox without odor♀ = 3.09, S = 29).

Some of the individuals were cautious and suspicious - walking around the object, pushing and hitting the box with a paw and were sniffing it for a long time. All bears interacted with the box by tearing, rubbing, carrying, bringing it into the pool or indoors, playing, and eating pieces of the box. In tree males and two females urination was observed on and around the box. In some of the bears, we observed clapping and vocalizations sounded as "pulsating threat" and "roar" directed to other individuals in the same enclosure. The latency to approach the box without odor was from 2 to 1160 s. When offering the box with the applied odor, the latency to approach was shorter - from 1 to 414 s. All the bears actively interacted with the cardboard box, but we observed fewer cases of bringing the box with the odor indoors and we did not observe eating pieces of it. In all bears the total duration of interaction with the cardboard box without odor was 8263 s, while the duration of interaction with the box with odor was 4949 s.

Comparing the duration of the behaviors displayed by all male and female bears before and after presenting the odor stimulus, no statistically significant differences between the duration of WALbef and WALaft, STAbef and STAaft, RESbef and RESaft were found either in the females or in the males (Table 4).

Table 4. Median and extreme values (minimum and maximum) of the duration (s) of the behaviors displayed by male and female bears before and after presenting the odor stimulus. The significance of differences revealed by the Mann–Whitney Utest and the Wilcoxon Matched Pairs test is shown: $p < 0.05$. Extreme values are given in brackets. The significance of differences revealed by the Mann–Whitney U test is given in bold. Explanation: n – number of bears, M – males, F – females.

Behavior	**M (*n*=8)**	**F (*n*=8)**	**Mann-Whitney U test**
	Median (min-max)		**$U_{(MxF)}$, p**
Walking before WALbef	277.0 (75.0 - 530.0)	294.0 (25.0 - 865.0)	U = 28.5, p = 0.713
Walking after WALaft	235.0 (123.0 - 350.0)	227.5 (65.0 - 880.0)	U = 31.5, p = 0.958
Wilcoxon Matched Pairs test (WAL_{bef} - WAL_{aft})	$T_{(MxM)}$ = 9.0, $Z_{(MxM)}$ = 1.260, p = 0.207	$T_{(FxF)}$ = 16.0, $Z_{(FxF)}$ = 0.280, p = 0.779	
Standing before STA_{bef}	99.0 (40.0 - 153.0)	97.5 (35.0 - 290.0)	U = 30.5, p = 0.875
Standing after STAaft	119.0 (28.0 - 215.0)	65.0 (41.0 - 134.0)	U = 15.0, p = 0.074
Wilcoxon Matched Pairs test (STA_{bef} - STA_{aft})	$T_{(MxM)}$ = 10.0, $Z_{(MxM)}$ = 1.120, p = 0.263	$T_{(FxF)}$ = 11.0, $Z_{(FxF)}$ = 0.980, p = 0.327	
Resting before RESbef	593.0 (25.0 - 955.0)	124.0 (35.0 - 645.0)	U = 14.0, p = 0.059
Resting after RESaft	375.0 (35.0 - 729.0)	194.0 (15.0 - 900.0)	U = 28.0, p = 0.674
Wilcoxon Matched Pairs test (RES_{bef} - RES_{aft})	$T_{(MxM)}$ = 10.0, $Z_{(MxM)}$ = 1.120, p = 0.262	$T_{(FxF)}$ = 8.0, $Z_{(FxF)}$ = 1.014, p = 0.310	

When comparing the behavioral responses of the males to a non-odorized box and a box with the applied odor, a statistically significant difference was found in how much they stood on two/four legs (STA). Males stood listening to the surroundings and sniffed the air significantly longer, with the non-odorized box, compared to the box with the applied odor (Wilcoxon Matched Pairs test, T = 1.0, Z = 2.380, p = 0.017, Table 5). In contrast to the males, the females stood and sniffed the air and listened to their surroundings for longer when the box with the applied odor was offered (Wilcoxon Matched Pairs test, T = 4.0, Z = 1.960, p = 0.050, Table 5). A statistically significant difference was found in the duration of time the females sniffed the boxes. They sniffed the cardboard box with the applied odor significantly longer than the non-odorized cardboard box (Wilcoxon Matched Pairs Test, T = 1.0, Z = 2.380, p = 0.017, Table 5). In addition, the females interacted with the odorized box significantly longer than with the nonodorized box (Wilcoxon Matched Pairs Test, T = 2.0, Z = 2.240, p = 0.025, Table 5). When the box with the applied odor was presented, the females stood and listened and sniffed the air significantly longer than the males (Mann – Whitney U test: U = 6.5, $p < 0.05$, Table 5).

Table 5. Median and extreme values (minimum and maximum) of the duration (s) and frequency of the behaviors displayed by male and female bears during the presentation of non-odorized cardboard boxes (control) and those with the conspecific odor. The significance of differences revealed by the Mann–Whitney U-test and the Wilcoxon Matched Pairs Test is shown: $p < 0.05$. Extreme values are given in brackets. The significance of differences as revealed by the Mann–Whitney U test is given in bold. Explanation: n – number of bears, M – males, F – females, con - control box, od - box with applied odor.

Behavior	**M (*n* = 8)**	**F (*n* = 8)**	**Mann – Whitney U test**
	Median (min – max)		**$U_{(MxF)}$, p**
Walking: non-odorized box (control) WAL_{con}	230.0 (125.0 – 325.0)	222.5 (105.0 – 982.0)	U = 28.5, p = 0.713
Walking: box with applied odor WAL_{od}	137.5 (15.0 – 571.0)	150.0 (15.0 – 681.0)	U = 4.5, p = 0.430
Wilcoxon Matched Pairs test (WAL_{con} – WAL_{od})	$T_{(MxM)}$ = 13.0, $Z_{(MxM)}$ = 700, p = 0.484	$T_{(FxF)}$ = 11.0, $Z_{(FxF)}$ = 0.980, p = 0.327	
Standing: non-odorized box (control) STAcon	75.5 (25.0 – 235.0)	60.5 (15.0 – 235.0)	U = 22.0, p = 0.294
Standing: box with applied odor STA_{od}	35.0 (15.0 – 178.0)	151.0 (45.0 – 290.0)	**U = 6.5, p = 0.007**
Wilcoxon Matched Pairs test (STA_{con} – STA_{od})	**$T_{(MxM)}$ = 1.00, $Z_{(MxM)}$ = 2.380, p = 0.017**	**$T_{(FxF)}$ = 4.0, $Z_{(FxF)}$ = 1.960, p = 0.050**	
Resting: non-odorized box (control) REScon	70.0 (25.0 – 250.0)	55.0 (13.0 – 270.0)	U = 27.5, p = 0.636
Resting: box with applied odor RES_{od}	54.0 (25.0 – 90.0)	31.5 (10.0 – 81.0)	U = 23.5, p = 0.372
Wilcoxon Matched Pairs test (RES_{con} – RES_{od})	$T_{(MxM)}$ = 7.0, $Z_{(MxM)}$ = 1.540, p = 0.123	$T_{(FxF)}$ = 13.0, $Z_{(FxF)}$ = 0.700, p = 0.483	
Interaction with the non-odorized box INT_{con}	595.0 (90.0 – 1011.0)	414.0 (145.0 – 1135.0)	U = 30, p = 0.833
Interaction with the box with applied odor INT_{od}	171.5 (86.0 – 1175.0)	242.5 (127.0 – 546.0)	U = 27.5, p = 0.636
Wilcoxon Matched Pairs test (INT_{con} – INT_{od})	$T_{(MxM)}$ = 11.0, $Z_{(MxM)}$ = 0.980, p = 0.326	**$T_{(FxF)}$ = 2.0, $Z_{(FxF)}$ = 2.240, p = 0.025**	
Sniffing the non-odorized box SNI_{con}	31.5 (15.0 – 180.0)	35.0 (25.0 – 98.0)	U = 29.5, p = 0.793
Sniffing the box with applied odor SNI_{od}	87.5 (19.0 – 217.0)	79.0 (25.0 – 203.0)	U = 28.0, p = 0.674
Wilcoxon Matched Pairs test (SNI_{con} – SNI_{od})	$T_{(MxM)}$ = 14.0, $Z_{(MxM)}$ = 0.560, p = 0.575	**$T_{(FxF)}$ = 1.00, $Z_{(FxF)}$ = 2.380, p = 0.017**	
Approaching the non-odorized box APP_{con}	5.0 (1.0 – 12.0)	6.5 (4.0 – 12.0)	U = 22.5, p = 0.318
Approaching the box with applied odor APPod	5.0 (1.0 – 18.0)	3.5 (2.0 – 10.0)	U = 28.5, p = 0.713

Wilcoxon Matched Pairs test (APP_{con} – APP_{od})	$T_{(MxM)}$ = 12.5, $Z_{(MxM)}$ = 0.770, p = 0.441	$T_{(FxF)}$ = 6.50, $Z_{(FxF)}$ = 1.610, p = 0.107	
Latency to approach: non-odorized box (control) – LT_{con}	16.0 (2.0 – 1160.0)	9.0 (5.0 – 25.0)	U = 24.0, p = 0.409
Latency to approach: box with applied odor – LT_{od}	15.0 (10.0 – 414.0)	10.0 (1.0 – 180.0)	U = 18.5, p = 0.156
Wilcoxon Matched Pairs test (LT_{con} – LT_{od})	$T_{(MxM)}$ = 17.5, $Z_{(MxM)}$ = 0.070, p = 0.944	$T_{(FxF)}$ = 16.0, $Z_{(FxF)}$ = 0.280, p = 0.779	

Under the influence of the olfactory stimulus, the bears displayed 11 types of new behaviors compared to the behaviors observed before the stimulus was offered. The odor of the conspecific, in combination with the cardboard box on which it was applied, provoked natural behavior and led to an expansion of the behavioral repertoire.

Stereotypic behavior and stimuli. During the preliminary observations on the bears, stereotypic behavior was recorded in 11 bears (7 females and 4 males). During the experiments, 9 bears (6 females and 3 males) showed stereotypic behavior, which was observed before and after the impact of the two stimuli (sound and odor of conspecifics). Observations on the behavior of bears showed that the same individuals exhibited the same form of stereotypic behavior before and after offering the odor and sound stimuli. No stereotypic behaviors were observed during the offering of the sound stimulus. The bears returned to stereotypic behaviors from 16 to 825 s after the end of the sound. During the interaction with the odor stimulus, stereotypic behavior was registered in only one female bear. The animals began to exhibit stereotypic behaviors again within 5 to 620 s after the end of interaction with the odor stimulus.

No significant correlation was found between the duration of stereotypic behavior before the presentation of the odor stimulus and the enclosure sizes (Spearman's rank correlation coefficient, rs = 0.22, p = 0.61 for males; rs = - 0.57, p = 0.14 for females). The one-year-old male bear and the pair of bears in the largest enclosure in Zoo Dobrich (3000 m^2) did not perform stereotypic behavior during the observations. In both males and females, the duration of stereotypic behavior before the presentation of the odor stimulus was greater than after the end of the interaction with the odor-applied cardboard box. A statistically significant difference was found only in males (Wilcoxon Matched Pairs Test T = 1.00, Z= 1.99, p = 0.046). Also, in males, the percentage of time they stereotyped in the 20-minute period varied from 2.9% - 83.7% before the presentation of the odor stimulus to 2.1% - 59.3% after the interaction with it ceased. In females, these percentages respectively ranged from 2.9% - 78.3% before the provision of the odor stimulus to 2.1% - 42.7% after the end of their interaction with it.

Both males and females showed a reduction in the duration of stereotypic behavior after the presentation of the sound and odor stimulus (Figure 1).

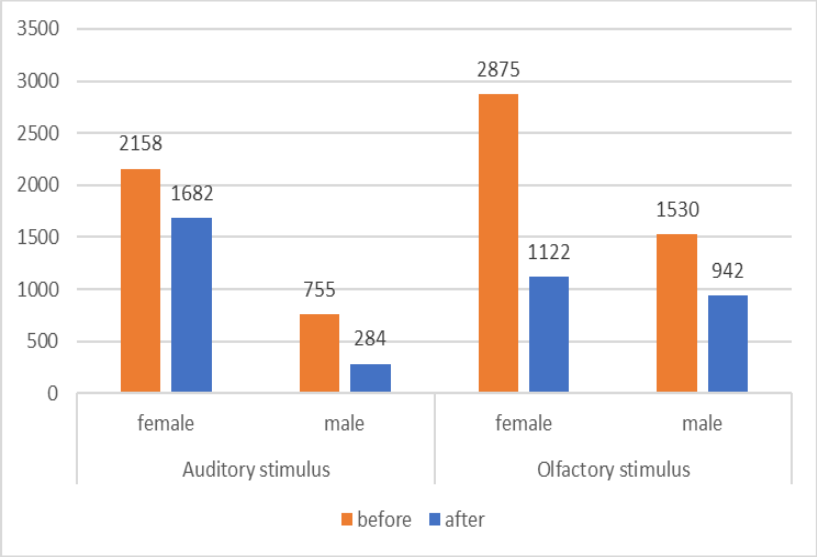

Figure 1. Duration (s) of stereotypic behavior in male and female brown bears before and after the sound and odor stimuli.

Females were found to exhibit more prolonged stereotypic behavior before and after the sound stimulus ($\chi2 = 70.8$, $p < 0.05$) as well as before and after the provision of the odor stimulus ($\chi2 = 92.1$, $p < 0.05$). Females showed more often stereotypic behavior both before and after the sound stimulus ($\chi2 = 2.130$, $p < 0.05$). When comparing males and females regarding the frequency of stereotypic behavior before and after the presentation of the odor stimulus, it was found that females exhibited stereotypic behavior with greater frequency than males ($\chi2 = 0.068$, $p < 0.05$). An analysis of the duration of stereotypic behavior in males and females showed that female bears exhibited stereotypy for a longer period of time than males before and after the presentation of the sound and odor stimulus. Females exhibited longer stereotypic behavior before the presentation of the sound stimulus ($\chi2 = 59.00$) as well as before the presentation of the odor stimulus ($\chi2 = 76.83$). Males also exhibited stereotypic behavior longer before presentation of the sound stimulus ($\chi2 = 213.51$) as well as before presentation of the odor stimulus ($\chi2 = 139.86$).

The total duration of the different forms of stereotypic behavior exhibited by male and female bears before the presentation of both sound and odor stimuli was greater than the duration of the manifestations after the end of the influence of the stimuli. This result shows a tendency that the manifestations of stereotypic behavior decrease under the influence of the proposed stimuli, but more data are needed to confirm whether this tendency exists.

I – 3. Discussion

Sound stimulus. During the playback of the five-minute audio recording of the sound of conspecific, there was a decrease in behavioral diversity compared to the behaviors previously demonstrated by the bears. After hearing the sound, they stood still for a long time, listening and sniffing the air. During the exposure to the sound stimulus, the bears also stopped exhibiting stereotypic behavior. Similar results were obtained by Robbins &

Margulis (2014) in their study on the effect of sound environmental enrichment in gorillas *Gorilla gorilla*. They found that natural sounds led to levels of stereotypic behavior equal to or lower than those in control conditions without sound enrichment. Although all the bears we studied live in zoos where they hear sounds from other animals on a daily basis, including in some cases the sounds of their own kind, they immediately responded to the sound from the recording. Under the influence of the sound stimulus, all bears demonstrated exploratory behavior. They consisted of approaching the source of the sound, standing on two hind legs or standing still and listening and sniffing the air. This behavior can be interpreted as a display of curiosity (Heimbürger, 2014).

The sound stimulus provokes the bears to exhibit natural behaviors such as exploratory and marking behavior. Similar results were obtained by Shepherdson et al. (1989) in providing vocalizations from conspecifics to lar gibbon *Hylobates lar* which resulted in an increase in the number of displays of species-specific behavior.

The youngest individual in the study (a 1.5-year-old male bear), which showed initial startle and flight in the opposite direction of the sound, returned and stood next to its mother 12 seconds after the recording began. This reaction may be due to an instinctive fear of unknown conspecifics. It is known that adult bears, especially males, can be a threat to young bears due to the fact that under certain conditions they commit nonparental infanticide on still maternally dependent young conspecifics (Swenson, 2003; Penteriani et al., 2020). Signs of anxiety were also observed in one of the female bears, who was making moaning sounds to the male she lives with after the onset of the sound stimulus. Three of the bears (two males and one female) were clacking teeth, and two of the females made a series of huffs and grunts in the direction of the sound. These vocalizations may be associated with anxiety in brown bears. Bears are usually quiet, and when the need to vocalize arises, they communicate with grunts and grunts, exhaling air differently, or through a resonant voice (Dolson, 2009; Dolson & Fawcett, 2010; Heimbürger, 2014). According to Heimbürger (2014), “chomping” or “clacking” teeth is merely an expression of fear.

After the end of the sound, both male and female bears showed greater behavioral diversity than before the stimulus was presented. In two of the male bears, part of the behavioral response to the stimulus was intensive digging of the soil near the source of the sound. This may be a manifestation of displacement behaviour. Digging has been found to be one of the typical displays of displacement activities in rats (Mackintosh & Grant, 1963) and dogs (Walker et al., 2016). According to Delius (1967) displacement activities occur in three situations: motivational conflict, frustration of consuming acts and physical thwarting of performance. In the present study, the situation in which bears hear a sound of conspecifics, but without the ability to see or smell it, may be a prerequisite for manifestations of displacement behavior like digging. Rubbing the back against the fence wall after the end of the sound stimulus was also observed and could be the equivalent of tree rubbing by bears in the wild. According to Clapham et al. (2012), tree rubbing by male bears served to communicate their dominance among males. The analysis showed that males stood and listened and sniffed the air statistically significantly longer than females after the end of the sound stimulus. This may mean that they are more impressed by the possibility of a

conspecific being present near their 'habitat'. The use of auditory stimuli as environmental enrichment for bears should be subject of further research because the results showed that they are important in enhancing their behavioral repertoire in captivity.

Odor stimulus. During the interaction with the odor stimulus, the bears exhibited a greater number of behaviors than those observed before being offered the odor by a conspecifics. Before the presentation of the stimulus, the values of the Shannon index – H ranged between 2.43 – 2.31, and during the interaction with the odorized box, H values were 2.99 – 2.87, respectively. Similar index values have been found in other captive animals such as ghost bats *Macroderma gigas* and tamandua *Tamandua mexicana* (Miller et al., 2020). For carnivores, the H values found by Miller et al. (2019) reach 2.01. It has been suggested that when behavioral diversity is greater, the behavioral needs of animals in captivity are likely to be met (Miller et al., 2016). Conversely, when behavioral diversity is low, the animal is more likely to stereotype (Mason & Latham, 2004). In the present study, an increase in the behavioral diversity of the bears was also found after they were offered the odor stimulus (a box with an applied odor). It was expressed not only in an increase in the number of different behavioral cases, but also in the display of some species-specific behaviors such as rubbing, scent marking and exploratory behavior. As Vicino & Miller (2015) point out, that the ability to express species-specific behavior is considered a positive indicator of animal welfare.

During the experiment, increased activity and an increased variety of behaviors were observed both when presenting the non-odorized box and the box with the applied odor. All bears responded to both boxes by sniffing and tearing them with their claws or teeth. Nevertheless, the bears appeared to be more cautious about the control box, sniffing it for longer. Since the zoos where the study was conducted lacked environmental enrichment, the bears' keen interest in the non-odorized box may be explained by its perception as a novel object. Similarly, Stonorov and Lyon (2000) mentioned that after initial timidity, bears often investigated the new object.

Rubbing against the boxes in most individuals studied was observed. Such behavior transfers odors from the environment to the animal's body (Reiger, 1979). There are diverse hypotheses about the functions of scent rubbing: to repel insects, to deter predators or to mask their natural scent to make it easier to capture prey or avoid predators (Charlton et al., 2020). There is also an opinion that animals anoint themselves with less commonly encountered environmental odors in order to make their own odor more distinctive (Gosling, 1982; Gosling & McKay, 1990). We also assume that the novel, unfamiliar odor of the cardboard box and the odor of urine from a conspecific may have provoked them to rub the pieces of cardboard vigorously with their odor in order to reinforce their odor and presence in the enclosure. Likewise, Charlton et al. (2020) established that giant pandas in breeding center in Sichuan Province, China anoint (rub) themselves with relatively novel, strong-smelling environmental odors to signal their competitiveness and occupation of the home range. During the present study, bears urinating on and around the odorized cardboard box was also a manifestation of scent marking behavior, probably induced by the odor of the urine of an unknown male bear. Like other carnivores, the brown bear uses olfactory information to recognize status and kinship (Rosell et al., 2011).

The results showed that female bears sniffed the box with the urine odor longer than males and they interacted with it significantly longer than with the non-odorized box. This suggests that female bears may be more intrigued by the smell of male bear urine than male bears, which in turn are more cautious and suspicious. Male bear urine might be more interesting to female bears because it may signify a potential mate. At the same time, the presence of another male in their territory would be a potential threat to females and their young (Swenson, 2003; Penteriani et al., 2020). In their natural environment, males and females occupy overlapping home ranges (Garshelis, 2009; Todorov et al., 2020). Hence, male bears might consider urine from another male bear as competition for resources: mates, food, and territory.

Growling and roaring in bears are often associated with a threat (Pasitschniak-Arts, 1993). The threatening behavior in some of the bears that live together in one enclosure after the stimulus was presented can be interpreted as a manifestation of hierarchical relations caused by the desire of the dominant individuals to have priority access to the novel object. Brown bears are usually asocial, although a dominance hierarchy can form around concentrated food resources (Bunnell & Tait, 1981). In stable groups in captivity, the agonistic behavior reinforces the hierarchy without necessarily leading to severe conflicts (Figueira et al., 2021). In groups of animals in captivity which habituated to each other such as wolves *Canis lupus*, domestic dogs and crab-eating foxes *Cerdocyon thous*, a low level of aggression with ritualized forms of behavior can be observed, reinforcing a stable hierarchy (Boitani & Ciucci, 1995; Figueira et al., 2021). Therefore, it is likely that the brown bears, subject of the present study, also formed hierarchical relationships in cases where two or three individuals lived together in a common enclosure.

Bears stopped displaying stereotypies during exposure to the odorized cardboard box which may be due to the odor of conspecific, but also as well may be caused by the novel object – the cardboard box itself. Other authors have obtained similar results when offering olfactory stimuli to animals in captivity. For example, Blount & Taylor (2000) showed that clothes sprayed with rose odor presented to captive kinkajous *Potos flavus* led to reduced stereotypic and inactive behaviors among them. Also, Quirke & O'Riordan (2011) found that prey feces introduced to cheetahs *Acinonyx jubatus* in Fota Wildlife Park, Ireland stimulated species-typical behaviors and reduced pacing, while Carlstead & Seidensticker (1991) found that the American black bear *U. americanus* reduced stereotypy and explored the enclosure more when provided with odors from other bears. Possible reasons for the highest percentage of stereotypic behavior displayed by most of the bears in our study could be due to the rearing conditions and their individual characteristics and history. Only a one-year-old male bear and a pair of bears kept in one of the largest enclosures did not show stereotypic behavior. Similarly, in a study on the welfare of bears in zoos in Poland, Ma´slak et al. (2016) found that bigger enclosures were accompanied by lower levels of stereotypic behaviors in bears. At the same time, we established that the individuals who ceased to exhibit stereotypic behavior while the odorized boxes were provided began to exhibit the same forms of stereotypic behavior again after they stopped interacting with them. This lends support to the conclusion of Mason (1991) that stereotypic behavior is persistent and difficult to overcome in animals in zoos. Other authors also suggested that well-established stereotypies are very

difficult to reduce (Koene, 1994; Wemelsfelder, 1993; Montaudouin and Le Pape, 2004). Our results showed that in comparison with males, female bears exhibited stereotypic behaviors for a longer duration both before and after the odorized cardboard box was offered. This suggests more persistent stereotypic behavior in female bears in this experiment. In both male and female bears, the duration of stereotypic behaviors before the odor stimulus was presented was longer than their duration after its presentation. This gives a reason to consider that the urine odor stimulus has value as an environmental enrichment to alter stereotypic behavior in brown bears. However, it remains to be established whether the bears would become habituated to the odor over time and resume stereotypic behaviors at the same levels, or whether the novelty of the enrichment was the factor responsible for the reduction in stereotypic behavior. Further studies, therefore, need to be conducted to establish the above.

Biologically relevant enrichment, such as the odor of conspecifics, can be used as part of a larger comprehensive enrichment program based on an animal's natural history (Babitz et al., 2023). It is therefore of great importance for bears living in captivity to be provided with behavioral opportunities to communicate as a strategy for increasing sensory stimulation (Mason, 2006). The conspecific odor can be included as part of a holistic enrichment program, providing a proactive approach to preventing abnormal behavior from occurring (Mellen & Sevenich MacPhee, 2001; Bacon, 2018). In Bulgarian zoos, where the brown bears live in a stimulus-poor environment, the proposed stimulus will have even greater welfare significance, both due to its novelty and because of the importance of olfactory communication for bears.

II. ABNORMAL BEHAVIOURS IN TWO ADULT FEMALE CAPTIVE BROWN BEARS WITH AN EMPHASIS ON NON-NUTRITIVE SUCKLING

II – 1. Materials and methods

Animals and housing. The objects of the study were two of the three bears living in 2020 in Aytos Zoo, Bulgaria – a mother and a daughter. The mother (Elena) was born in 1998 in Lucky, Kormisosh (a game breeding station) and arrived at the zoo in 2004. In Aytos Zoo she was impregnated by a male brown bear in 2007. The male was briefly brought on a breeding loan and did not live at the zoo any longer. Elena gave birth to her daughter (Mia) in 2008. Since then, the two females have been living together in the same enclosure.

Both bears lived in an 'concrete pit' enclosure, which is divided into two parts with a total area of 250 square meters. Each part measures 10 to 11 m with a concrete base without any vegetation inside. In one of the enclosure parts, there is a pool with measures 2 by 3 meters. The back wall is an "artificial rocks" type, in which there is a den, where the bears hibernated together until the winter of 2020. At that time, they had no indoor premises and insulators. The bears have no view outside of the enclosure and visitors watch them from above, often throwing food inside.

Until the end of September 2020, there was a third female bear in the enclosure. She was over the age of 20 and lived in the other part of the enclosure. The mother and daughter did not allow her to enter their part of the enclosure. Thereafter, this bear died and since then, the bears Mia and Elena have access to the other part of the enclosure (both parts are connected by a passage, which is open all the time and the zoo staff closes it only for cleaning). After the death of the third bear, Mia and her mother separated themselves each in a different part of the enclosure.

The number of visitors in Aytos Zoo varied between 30 and 53/hour in August and 45-100/hour in September. In October their number was 5-10 people/hour. The temperatures during our visits in August and September varied between 23ºC and 28ºC and in October - between 12ºC and 22ºC.

Husbandry procedures The cleaning of the bear facility was once a week. The bears were fed twice a day - at 10 a.m. and 4 p.m., and the food was usually thrown by the keepers from above. There was no fresh water in a separate drinking bowl, the bears used the water in the pools. The bears received weekly portions of powdered milk dissolved in water with the other food. The mother and daughter were fed together and till the death of the third bear were not separated during feeding.

Data collection and analysis Aytos Zoo was visited in August, September and October 2020 for three days and the bears were observed for 6 hours between 10 a.m. and 4 p.m. each day. The total amount of observation time was 18 hours. For the detailed description of the abnormal behaviours, they were video-recorded by video-camera ACME VR06 - 4K Ultra HD and photographed with a photo camera Canon SX730HS. The analysis

of stereotypic behaviours in August and September was based on 65 minutes of video recording each month in a period when both bears performed stereotypic behaviour usually from 11 a.m. to 2 p.m. The observations were made positioned at the visitors' site.

The sequence of the behavioural model and the duration of each separate part of the non-nutritive suckling behaviour were described. The frequency and the duration of all stereotypic behaviours were compared by One sample $\chi 2$ test for statistically significant differences. In addition, the mean duration of stereotypic behaviours performed by Elena and Mia were compared between the months of observation using the Student's t-test. Variables were tested for normal distribution by Kolmogorov–Smirnov test. Data were analysed using Statistica version 7.0 statistical software StatSoft Inc. (2004). In all tests, a significant statistical difference was assumed when $p < 0.05$.

II – 2. Results

During observations in August and September 2020 the two bears (MF and DF) were resting beside the pool, entering the pool and staying in the water, drinking water from the pool, eating food, moving in another part of the enclosure and scratching at surrounding objects. Some types of stereotypic behaviour also were observed such as circling, walking in a figure-of-eight and head tossing. The observed abnormal behaviours in the two bears (MF and DF) are presented in Table 6.

The young female Mia displayed the stereotypic behaviour of walking in a figure-of-eight around the pool, while her mother demonstrated only circling and head tossing. Between 52 and 105 walking in figure-of-eight per hour were registered, the duration of each being 5 - 16 s. One part of the figure eight was the circumference of the pool, and the other part was smaller. The movement was always clockwise. Unlike Mia, the older bear Elena demonstrated circling 15 - 27 times per hour the duration of each being 12-17 s. Performing circling, Elena often showed head tossing (25 - 42 times per hour).

Table 6. Ethogram of abnormal behaviours in the brown bears Elena (MF) and Mia (DF).

Abnormal behaviours	Definition
Walking in the figure-of-eight	Moving around the enclosure, always following the same path (Montaudouin & Le Pape, 2005), in this case in the figure of eight.
Circling	Moving in a circular direction constantly (Veeraselvam et al., 2013).
Head tossing	An individual throws the head back and turns it with accompanying elevation of the front parts of the body upright, sometimes with sitting, usually while pacing at the extreme edges of the path (Maślak et al., 2016).
Non - nutritive suckling	In the present study - false suckling of DF of MF. More details in the text.

A statistically significant difference was found as between the total duration of all walking in figure-of-eight registered in August and September ($\chi 2 = 25.09$, $p < 0.001$, df =1, One sample χ 2 test), as well as between the mean duration of the separate figures-of-eight ($t = 5,46$; $df = 143$; $p < 0,001$). In addition, the frequency of walking in figure-of-eight was almost twice as high in August than in September ($\chi 2 = 13.97$, $p < 0.001$). A statistically significant difference was found in the duration of all circlings and all head tossings registered in August and September. In August, the total duration of all circlings was significantly higher than in September ($\chi 2 = 25.38$, $p < 0.001$, One sample χ 2 test), and the total duration of all the head tossings was also significantly higher in August than it was in September ($\chi 2 = 4,91$, $p < 0.05$, One sample χ 2 test) (Fig. 2). However, no statistically significant difference was found between the mean duration of the separate circling and head tossing events. There was no statistically significant difference in the frequency of these two stereotypes in August and September ($\chi 2 = 1.81$, $p > 0.05$ for circling; $\chi 2 = 1.12$, $p > 0.05$ for head tossing, respectively).

In August we had the opportunity to observe and record the non-nutritive suckling behaviour in the interactions between the two bears with a duration of 310 s. It proceeded as follows: the daughter Mia began pushing with her snout in the upper part of the body under the front limbs at the mammary glands of the mother who was sitting calmly. The daughter bit the fur in the area of the nipple with her lips and began to make suckling movements with her mouth, making the characteristic sound of suckling, which bear cubs produce (humming) and resting (putting) her paws on her mother's belly. There were no signs of lactation and functional suckling. During this time, the adult bear was sitting patiently, showing no signs of pain or anxiety (Picture 2). After the end of the "suckling", the two bears did self-grooming and after that moved on to mutual grooming

We registered the non-nutritive suckling behaviour only once, but according to the zoo staff, it always occurred in this sequence and the daughter was always the initiator. Zookeepers also shared that she initiated the non-nutritive suckling more persistently before the age of 5, but this behaviour continued periodically until the present when Mia was 12.8 years old.

Picture 2. Non-nutritive suckling behaviour in the social interactions of two captive female brown bears.

The bears did not perform any stereotypic behaviour during the observation period in October. Then, the two bears had divided the territory and the daughter was already living in a separate part of the enclosure. The temperatures were lower and the bears were lethargic. According to zoo staff after the death of the third bear, Mia started to enter the emptied part of the enclosure more and more often and at the beginning of October 2020 she was already settled permanently in it. The passage between the two enclosures remained open. In October 2020, we observed how the daughter tried to enter and approach the mother 3 times. That was met by the older bear with anxiety and threatening behaviour expressed through threatening vocalization and paw swing. The vocalization included three types of signals: growl, mouth clapping and a repeating guttural sound. This response of the mother forced the daughter to return to "her" part of the enclosure.

According to information from Aytos Zoo staff in mid-November 2020, the mother entered the den in the old part of the enclosure, where she prepared for hibernation. The daughter continued to try to use the old part of the enclosure and enter her mother's lair but was chased away with a growl. So, from the first days of December Mia entered the den in her part of the enclosure, where she spent most of the time.

II – 3. Discussion

The obtained results confirmed some tendencies about demonstrations of stereotypic behaviour. Circling and walking in figure-of-eight are among commonly observed stereotypies in bears (Montaudouin & Le Pape, 2005). Like other authors such as Lawrence & Rushen (1993) and Fernandez (2010) we also found that long time spent in captivity (for the daughter her entire life), lack of individual space, as well as isolation or poor environment, lead to the manifestation of abnormal behaviour.

Circling around the pool in the cage was observed in both animals. However, with the daughter there is a deviation for one more round (so the figure-of-eight is completed), and with the mother, at a certain place during the round, the tossing of the head is observed. During our observations, most of the time both bears stereotyped simultaneously. We assume that they have evolved the different forms of abnormal behaviours because of the small area they have in which to diverge. Another possible explanation could be that this difference might be due to their personality. As well as Fagen & Fagen (1996) and Quintavalle et al. (2017) pointed out, bears are animals with distinctive personality.

It could be suggested that the lack of a program for environmental enrichment in Aytos Zoo could be a prerequisite for the development of various forms of stereotypies. As mentioned by Wemelsfelder (1993) lack of stimuli often leads to boredom in animals. Boredom can be reduced by environmental enrichment (Meagher & Mason, 2012, Soriano et al., 2019). Therefore, we assume that this is probably also a reason for the manifestation of non-nutritive sucking behaviour.

The results showed that the younger bear Mia was the initiator of the oral stereotypic behaviour of non-nutritive suckling. Hence, it could be suggested that young animals are more likely to develop abnormal behaviour in captivity. Ridley & Baker (1982) mentioned that the effect of captivity seems to be more profound for a young bear than for mature

animals, in terms of the expression and persistence of abnormal behaviours. One of the causes for the abnormal non-nutritive suckling behaviour might be the circumstance that the two females have been living together for 12.8 years - since the birth of the daughter, which would not have been possible under natural conditions. According to Dahle & Swenson (2003) and Mclellan (1994), in brown bears *U. arctos*, offspring separate from their mothers at age of 1–3 (occasionally 4) years. In our latitude – even earlier, around the end of the second year. Besides, the lack of a male brown bear at Aytos Zoo and the lack of any contact with a male relative could also be considered a cause of abnormal behaviour. In this connection Dahle & Swenson (2003) point out that mothers *U. arctos* initiated breakup as they enter estrus, they should be accompanied by adult males during or soon after the family breakup.

The presence of dairy products in the diet of bears, and in particular powdered milk, also could provoke non-nutritive suckling. Thus, the taste of milk could stimulate non-nutritive suckling in calves (Rushen & de Passillé, 1995). Registration of humming sound vocalization in a 12.8-year-old brown bear during suckling complemented the short list of cases in adult bears. According to Peters et al. (2007), the humming sound vocalization is typical for bears in captivity. This gives us reason to assume that for the mother the humming sound had a calming effect and strengthened the relationship with the daughter. In our observations, the animals were in almost continuous tactile contact, sitting side by side. Moreover, after the cessation of suckling, they performed mutual grooming. In many mammalian species grooming is a major social activity and a means by which animals that live nearby may bond and reinforce family links and build companionships (Henazi & Barrett, 1999).

The environmental factors, such as the ambient temperature could have significant effects on stereotypic behaviours causing the expression of various stereotypies. Higher environmental temperature reduced the duration of pacing but increased the frequency of pacing and the duration of head-toss in giant pandas in the study of Liu et al. (2017). It is possible that the higher temperatures during our observations in August and September account for the higher levels of stereotypy in the two bears compared to October.

Assuming that the non-nutritive suckling is a type of infantile behaviour, it could be a strategy to suppress potential aggression by the older bear. In spite of that the daughter's attempts to approach the mother in her part of the enclosure in October, which were met with growls and hostility and the mother did not let her nearby. Therefore, providing rearing conditions that meet the requirements of the specific biological species limit or even cease the manifestations of the stereotypic behaviour non-nutritive suckling. This proved that so far there had been no suitable conditions for the normal process of family breakup. That happened when the first opportunity for the two bears to separate themselves in their own space appeared.

III. WELFARE ASSESSMENT OF BROWN BEARS IN CAPTIVITY IN BULGARIA

III - 1. Materials and methods

Object of study. The study included all brown bears in captivity as of 2021 (n = 47, 22 males and 25 females) in Bulgaria. In the period May - September 2021, all zoos where brown bears were living, as well as Bear sanctuary Belitsa, were visited. At the time of the research, 28 brown bears (14 females and 14 males) lived in 11 zoos, inhabiting 17 enclosures, and 19 bears (8 males and 11 females) lived in 10 enclosures in Bear sanctuary Belitsa. The number and the area of enclosures, the number of bears in them, and their origin and kinship are presented in Table 7. Behavioral observations of each individual were made for 4 hours per day for a minimum of one day according to a prearranged schedule. The focus of the observations were the manifestations of stereotypic behavior, which were recorded with a video camera for a more precise and complete registration of the behavior. The identified cases of stereotypic behavior during the observations were categorized and described. Separately, data were collected for each individual regarding their origin, age, health status, time spent in the organization, breeding, and specifics in their behavior.

Table 7. Information about the brown bears in captivity in Bulgaria and their enclosures. The number and age of individuals are presented at the time of the survey in 2021. When several animals live in the same enclosure and are related, it is indicated. Explanation: **ENC** - enclosure, **O** - outdoor enclosure, **I** - indoor, **M** - male, **F** - female, **CAPT** – born in captivity, **UNKN** - origin unknown, **WILD** - from the wild,* - most likely born in the wild , **SB** - stereotypic behavior.

Enclosure	Enclosure size (m^2)	Number, sex and age	Accommodation and kinship	Origin	SB
Aytos Zoo					
ENC01	O – 110	1 F, 23	alone, mother	CAPT	Yes
ENC02	O – 110	1 F, 14	alone, daughter	CAPT	Yes
Blagoevgrad Zoo					
ENC03	O – 3500, I – 35	1 F, 18	group, mother	CAPT	No
		1 M, 9	group, son	CAPT	Yes
Dimitrovgrad Zoo					
ENC04	O – 200, I – 6	1 F, 27	group, sister	UNKN*	Yes
		1 M, 27	grouop, brother	UNKN*	Yes
Dobrich Zoo					
ENC05	O – 3000, I – 33	1 F, 9	group, not related	CAPT	No
		1 M, 8	group, not related	CAPT	No
Kyustendil Zoo					
ENC06	O – 335, I – 12	1 F, > 30	alone	UNKN	Yes

Lovech Zoo					
ENC07	O – 77, I – 22	1M, unknown	alone	CAPT	Yes
ENC08	O -124, I – 30	1 F, 16	group, mother	CAPT	Yes
		1 F, 8	group, daughter	CAPT	Yes
		1 M, 19	group, father	CAPT	No
ENC09	O – 395, I – 15	1 F, 13	group, mother	WILD	Yes
		1 M, 1.6	group, son	CAPT	No
Pavlikeni Zoo					
ENC10	O – 200, I – 6	1 M, 14	alone	WILD	No
Pleven Zoo					
ENC11	O – 510, I – 50	1 F, 10	group, not related	WILD	Yes
		1 M, 10	group, not related	CAPT	Yes
Sofia Zoo					
ENC12	O – 500, I – 50	1 F, 32	alone	WILD	No
ENC13	O – 2200, I – 75	1 M, 16	alone	CAPT	Yes
Stara Zagora Zoo					
ENC14	O-500, I – 8	1 F, 24	group, mother	CAPT	No
		1 M,8	group, son	CAPT	Yes
ENC15	O-500, I – 8	1 F, 12	group, sister	CAPT	No
		1 M, 12	group, brother	CAPT	Yes
ENC16	O – 120, I – 15	1M, 9	group, brother	CAPT	Yes
		1 M, 9	group, brother	CAPT	Yes
		1 M, 9	group, brother	CAPT	Yes
Varna Zoo					
ENC17	O – 160, I – 10	1 F, 33	alone	CAPT	No
Bear sanctuary Belitsa					
Sector 1	O -27000	1 F, 21	group,not related	UNKN*	Yes
		1 M, 6	group, not related	UNKN*	Yes
		1 M, 5	group, not related	UNKN*	Yes
Sector 2	O – 16000	1 F, 28	group, not related	CAPT	No
		1 F, 33	group, not related	CAPT	No
		1 M, 25	group, not related	CAPT	No
		1 M, 30	group, not related	CAPT	No
Sector 3	O – 4000	1 F, 29	group, not related	CAPT	Yes
		1 M, 11	group, not related	CAPT	No
Sector 4	O – 7000	1 F, 24	group, not related	CAPT	No
		1 M, 12	group, not related	WILD	No
Sector 5	O – 6500	1 F, 14	group, sister	CAPT	Yes
		1 M, 14	group, brother	CAPT	Yes
Sector 6	O – 21000	1 F, 31	group, not related	CAPT	Yes
		1 F, 26	group, not related	CAPT	Yes
Sector 7	O – 3000	1 F, 17	alone	CAPT	Yes
Sector 8	O – 3000	1 F, unknown	alone	UNKN	Yes
Sector 9	O – 3000	1 M, 34	alone	UNKN	Yes
Sector 10	O – 3000	1 F, 34	alone	UNKN	Yes

Data collection. The information, on the basis of which the welfare assessment of the brown bears in captivity in Bulgaria was made, was collected by a specially designed questionnaire based on the questionnaire used by Maślak et al. (2016) to assess the welfare of captive bears in Poland, but adapted for conditions in Bulgaria. The questionnaire consists of five parts, each containing questions based on the Five freedoms concept (Young, 2003). They are related to the following:

1. Feeding and access to fresh water (diet, feeding methods, access to fresh water, control over feeding by visitors, etc.).

2. Environment (area of the enclosures, area per individual, access to the external and internal enclosure during the day, substrate, vegetation, topographic features, enclosure furnishing, temperature conditions, etc.).

3. Veterinary medical services (availability of a veterinarian, number of preventive examinations per year, availability of a preventive program, staff qualification, etc.).

4. Biological and behavioral needs (environmental enrichment, social environment, hibernation conditions, seasonal diet, etc.).

5. Risk of stress (noise level, number of visitors, possibility of withdrawal into an internal enclosure, possibility of separation of individuals living together in one enclosure, etc.).

The questionnaire contains 30 questions, and the answers and collected data for each bear are evaluated on a pre-defined scale with a certain number of points. The maximum score in the questionnaire is 54 points. The questionnaire was completed on site, with some information collected through face-to-face interviews with zoo staff and others through personal observations. A separate questionnaire was completed for each bear, and the information was then summarized for each enclosure in the zoos.

Hibernation is important to the physiological and psychological state of brown bears, and this specific behavior and state of bears is directly related to their welfare. Therefore, an additional study was made about hibernation of captive bears in Bulgaria. The collection of data on the hibernation was carried out during the winter periods of 2021-2022 and 2022-2023, about all bears living in captivity in Bulgaria at that time (n = 46). Data were collected for each individual bear, which included information on the bears' pre-hibernation diet (composition and amount of food provided), hibernation conditions (availability of dens and indoor facilities and provision of den bedding, access by visitors and staff) and about the hibernation of the bears during the indicated periods (whether they hibernate, whether they fell into a temporary torpor or were just inactive, when they went to hibernate and when they woke up).

Statistical analysis. Phi coefficient was used to study the correlation between the two dichotomous variables "sex" and "manifestation of stereotypic behavior" of the bears kept in the zoos and in Bear sanctuary Belitsa. Correlation dependences between a continuous variable and a dichotomous variable were investigated by pointbiserial correlation for the variables: 1. " manifestation of stereotypic behavior" and "score from the questionnaire"; 2.

"manifestation of stereotypic behavior" and "age of bear", 3. " manifestation of stereotypic behavior" and "enclosure size", and only in bears kept in zoos between the variables "manifestation of stereotypic behavior" and " duration of life in the enclosure“. Based on the collected data on bear hibernation for the winter period 2021-2022, the correlation between sex and hibernation was researched by using Phi coefficient. The relationship between the age of the bears and hibernation was also analyzed by pointbiserial correlation. In all tests performed, $p < 0.05$ was accepted for statistically significant differences.

III – 2. Results

Zoos in Bulgaria. It was found that 21 of bears in zoos were born in captivity, 4 were born in the wild and 3 were of unknown origin. The average age of brown bears in zoos at the time of the study was 17 years, with the youngest individual being 1.7 years old and the oldest being 33 years old. The average welfare score of bears in Bulgarian zoos as a result of the completed questionnaires is 21.28 points, with the lowest score being 12 points and the highest being 45 points. The zoo with the highest score is Zoo Dobrich.

The large variation in zoo scores is mainly due to the difference in enclosure area, which scores the most in the questionnaire. Five of the bear enclosures (29% of the total) in the zoos were found to have an area of 150 m^2 (the minimum area for keeping one individual as specified in Ordinance No. 6), with some of them housing more than one individual, i.e. even the minimum standards for the species are not covered. These enclosures receive zero points when evaluating the area indicator. In contrast, other enclosures such as those in Dobrich Zoo and Blagoevgrad Zoo have an area of over 3,000m^2, for which they receive 10 points each.

All bears are vaccinated and dewormed every year according to an established veterinary preventive plan and have access to veterinary care, which is usually given when the need arises. However, periodic full preventive examinations are not carried out in zoos. For 44% of bears in zoos hibernation conditions have been created and they hibernated during the winter period 2020-2021. Manifestations of stereotypic behavior were observed in 18 individuals (10 males and 8 females), which represents 64% of all bears in zoos. No correlation was found between the manifestations of stereotypic behavior and the time spent in the enclosure in bears in zoos ($r = 0.079$; $p = 0.690$).

Bear sanctuary Belitsa. It was found that of the 19 bears living in Bear sanctuary Belitsa, the youngest is 5 years old and the oldest is 34 years old (average age 19.5 years). They were living in 10 enclosures or sectors as they are called in the park. Twelve of the bears in the park originated in zoos, one bear was born in the wild, and six bears are of unknown origin. The average score of the welfare level of the bears in the sectors calculated from the questions in the questionnaire is 46.5 points, with the lowest score being 44 points and the highest being 49 points. All bears in the park undergo a full preventive veterinary check-up every 3 years and are regularly vaccinated and dewormed according to an approved preventive program. Animals have access to highly specialized veterinary medical care, including dental treatment. For all bears, conditions for hibernation have been created -

natural or artificial dens. During the winter period of 2020/2021, almost all bears in the park (95%) hibernated. This is also helped by the fact that the park is closed to visitors every year from the beginning of November to the end of March. The enclosures of the bears are in their natural habitat and have a total area of 93,500 m^2. The smallest has an area of 3,000 m^2, and the largest has an area of 27,000 m^2, with an average of 4,921 m^2 of natural forest per individual. Each enclosure has at least one pool. There is an environmental enrichment program for each individual, which also includes individual training plans for the bears. The park's policy is not to breed bears, so all males are castrated. Visitors are allowed in one-third of the park area, entering only with a guided tour. This eliminates the possibility of unregulated feeding and annoying the bears and regulates the number of visitors entering the park at a certain time.

Based on the results of the completed questionnaires, the following main problems related to the welfare of bears in zoos can be summarized:

• Housing in insufficient space – during the study it was found that the housing area per individual was less than 150 m^2 in 5 of 17 enclosures and a total of 11 bears were living in these conditions.

• Lack of a plan for environmental enrichment, which is not offered in most zoos. Various stimuli are provided from time to time in some of the enclosures, but irregularly and no measures are taken to properly and regularly enrich the environment in order to increase the natural behavior and reduce the manifestations of stereotypic behavior.

• Unsuitable surface – a hard, concrete surface was recorded in 8 of 17 enclosures.

• Periodic full health examinations of bears are not planned and carried out in any zoo.

• For 56% of brown bears in Bulgarian zoos, there are no suitable conditions for hibernation.

• Lack of effective control over unregulated feeding of bears by visitors.

It was also established that in most Bulgarian zoos the zookeepers do not have the necessary qualifications and specialized education, including the bear caretakers. This undoubtedly affects the quality of care provided and the welfare of the bears.

The following main problems related to the welfare of the bears were identified in Bear sanctuary Belitsa:

• Increase in the number of visitors to the park in recent years, which in summer are between 500 and 1000 people per day. This leads to increased disturbance and noise and pollution from the parking lot, which is in close proximity to some of the bear enclosures.

• Although great efforts are made in the park to reduce the occurrence of stereotypic behavior, it was observed in 12 bears (63% of all bears in the park). This is probably the result of the bears being kept in extremely poor and unnatural conditions before coming to the park. Once it becomes part of the behavioral repertoire of carnivores for one reason or

another, persistent stereotypic behavior is difficult to eradicate. The experience shared by park staff is that its manifestations decrease in some cases when the bears are socialized and moved to another sector. For some individuals, there are observations by their caretakers that stereotypic behavior increases in spring and autumn, i.e. there is a seasonal dynamic in its manifestations.

In the analysis of the combined sample of all bears in captivity, no correlation was found between the manifestations of stereotypic behavior and the sex of the bears (phi = 0.036; p = 0.805), their age (r = 0.077; p = 0.616), the size of the enclosure they live in (r = 0.285; p = 0.142) and the score of the enclosure, according to the questionnaire (r = 0.107; p = 0.475).

Hibernation of the bears in captivity in Bulgaria. For all bears in captivity (n = 46) during the winter period of 2021-2022 was found that 9 individuals (2 males and 7 females) out of 27 bears in zoos (13 males and 14 females) were hibernating, or 33% of zoo bears. Of those in Bear sanctuary Belitsa (19 bears - 8 males and 11 females) hibernated a total of 17 animals (6 males and 11 females) or 89% of the bears in the park hibernated. It was also found that 69% of all female bears and 31% of all male bears in captivity were hibernating. However, the analysis showed that the correlation between the variables "sex" and "hibernation" was unreliable (phi = 0.341; p = 0.979). But a positive correlation was proved between the age of bears and hibernation (r = 0.53161; p = 0.00014). Although the correlation between the variables was weak, it appeared that young bears under 10 years of age were less likely to hibernate. Moreover, those up to 20 years of age winter twice less often than older ones.

The diet of captive bears is tailored to the increased needs for food and nutrients in the fall and to the low needs in the winter and hibernation period. But human disturbance is a significant factor affecting successful hibernation. Proof of this is that in Bear sanctuary Belitsa and in Zoo Dobrich, where visitors do not have access to the bear exhibits in winter, the bears hibernate for more than two months. In some of the zoos, bears do not hibernate, but become more lethargic during the winter period. The highest percentage of bears in zoos (45%) hibernated indoors. Some of the bears - 33% - hibernate in semi-natural dens, which are dens dug and fortified by humans in the soil or at the base of trees in the bears' outer enclosures. Usually, such dens are prepared by the keepers in Bear sanctuary Belitsa for the elderly and less mobile bears that cannot dig them themselves (Pict. 2).

Pictire 2. A semi-natural man-made den for an elderly and disabled bear in Bear sanctuary Belitsa.

III – 3. Discussion

It is known that keeping large carnivores as bears in artificially created conditions is a great challenge, which arises from their complex species-specific needs (Blackett et al., 2017; Clubb & Mason, 2003), from their cognitive abilities (Tabellario et al. , 2020), the need for a large territory and specific ecological requirements (McGowan et al., 2010). Bears also have a relatively common occurrence of health problems during their long captive lives (Bourne et al., 2010; Kitchener, 2004). Therefore, the welfare of many captive bears is not at a high level (Maślak et al., 2016). The present study found that the main problems that significantly lower the welfare level and quality of life of captive brown bears are the following: insufficient space, inappropriate surface, lack of environmental enrichment, inappropriate social structure, lack of hibernation conditions, lack of control over visitors' behavior, insufficiently complete diet and lack of fresh drinking water, lack of comprehensive and preventive veterinary care. Similar problems for the welfare of bears in zoos were described in their studies by Maślak et al. (2016), Garssen (2006), Karamanlidis & Zedrosser (2009), Laidlaw et al. (2010), as well as Maher et al. (2021).

Insufficient space in most bear enclosures is one of the main problem for their welfare. National legislation stipulates a minimum area of 150 m^2 per individual, and for each subsequent individual there must be a minimum of 20 m^2 more. This practically means that according to the regulation, if two bears coexist in one enclosure, for each of them the minimum permissible space will be 85 m^2, which is obviously extremely insufficient. In other countries, regulations specifying minimum space for brown bears in captivity have introduced larger areas per individual. In Austria, a minimum of 300 m^2 is required for two individuals (486. Bundesgesetzblatt für die Republik Österreich, 2004), and in Sweden the minimum space for two individuals is 1,500 m^2 (SJVFS, 2003:77). According to other experts, suitable conditions for proper brown bear husbandry are to keep them in enclosures with an area of 4,000 m^2 to 10,000 m^2 depending on the number of bears (Ganslosser, 2004; Kok, 2007; Cuyten & Bos, 2010). By providing such a large area, the natural behavioral

needs of this species can be met. It has been established that their home range in Bulgaria can be up to 605 km^2 (Todorov et al., 2020). The requirements of European Association of Zoos and Aquaria (EAZA) for the minimum area for brown bears in captivity specify 300 m^2 per individual, which is likely to be adjusted in the direction of increasing the area in near future. The small area of the enclosure would lead to the unsatisfied need to travel large distances in bears, which creates a basis for the development of behavioral disorders and especially stereotypic behavior (Clubb & Mason, 2007). In the present study, it was found that in five of the zoo enclosures, the housing area per individual was less than 150 m^2, and a total of 11 brown bears lived under these conditions. On the basis of these data, a reasoned Proposal for the amendment and addition of Ordinance No. 6 of 23.10.2003 on the minimum requirements and conditions for keeping animals in zoos and breeding centers for protected animal species was prepared. It proposes that the minimum area for one individual must be 500 m^2, and for each subsequent one another 500 m^2 must be provided. It is imperative that the minimum space requirements for brown bears in Ordinance No. 6 must be changed, and even if this does not happen, it is necessary to follow global standards and good practices when planning and building new bear enclosures.

Ordinance No. 6 (2003) states that the social structure in which brown bears must live is two animals living together in an enclosure, without explicitly stating what sex they are. It has already been discussed in the present dissertation that bears are solitary animals and gather in groups during the breeding season or when abundant food resources are available. If they live together, then it is about a mother and her cubs, who are together until the cubs are 2-3 years old. The long-term coexistence of a female with her cubs can lead to the development of abnormal behavior, such as the case described in Chapter V of this dissertation with the bears Mia and Elena from Zoo Aytos (manifestation of non-nutritive sucking). Permanent cohabitation of a male and a female bear without the possibility of separating the two animals in different parts of the enclosure can lead to distress, especially in females, and to unwanted reproduction and infanticide. This would be inconsistent with the breeding standards of the species and with maintaining a high level of welfare.

The lack of regular and appropriate environmental enrichment is one of the most frequently observed problem in the husbandry of brown bears in Bulgarian zoos. The reason for this is the insufficient number and qualification of the staff. The fundamental purpose of of environmental enrichment is to provide an opportunity for animals in captivity to exhibit their species-specific behavior (Mellen & MacPhee, 2001). The different types of environmental enrichment are: nutritional (offering food in puzzle feeders, scattered or in different forms), sensory (visual, olfactory or auditory stimulation), social (living with a conspecific, training, living with other species) and cognitive (toys and tools) as well as physical by providing appropriate enclosure furnishing, substrate and relief (Renner & Lussier, 2002). All of these environmental enrichment techniques are widely applicable to bears, and even minimal use of them can significantly increase welfare levels and have therapeutic effects in behavioral disorders (Renner & Lussier, 2002; Law & Kitchener, 2002; Law & Reid, 2010). European Association of Zoos and Aquaria has set out in its standards and requires its members to develop and implement environmental enrichment programs for the different species and individuals in zoo collections. In Bulgarian zoos, such programs are

currently being created and implemented only in Sofia Zoo, which has been a member of EAZA since April 2023. In other zoos, some forms of environmental enrichment such as providing logs, branches, car tires and dispersing food are offered periodically. Bear sanctuary Belitsa staff have developed individual programs for the bears, offering appropriate forms of enrichment, socialization and training to different individuals. This is important for older individuals, especially for blind bears and those with limited mobility.

In some of the zoos, it was found that the bears' diet lacked or contained minimal amounts of food of animal origin. Bread and other foods rich in carbohydrates, but unnatural for bears, predominate. This is partly due to the still widespread prejudice in Bulgaria that providing meat will “enrage” the bear and make it aggressive. Although the brown bear is omnivorous, it is a carnivore and undoubtedly needs food of animal origin. An unbalanced diet can lead to health problems, so there are food charts developed for brown bears that can be used and applied. In addition to the composition and amount of food, it is important in what form and how many times a day it is offered. In the questionnaire, the highest score is given to feeding three times a day, which is offered in the Bear sanctuary Belitsa. In the wild, bears travel long distances and spend a lot of time foraging (Clubb & Mason, 2007). Therefore, hiding, scattering and offering food more often in the enclosure gives the animal the opportunity to exhibit its natural behavior. Feeding once or twice a day (usually in the morning and at the end of the staff's working day) at the same time and in a certain place is a prerequisite for developing stereotypic behavior. The diet can be enriched with vitamins and nutritional supplements. It is especially important that it is adapted to the seasonal needs of the bears. In August and September, they enter a state of hyperphagia and increase their food intake in order to accumulate subcutaneous fat as a source of energy during hibernation (Hilderbrand et al., 1999). After that, the amount of food gradually decreases and during hibernation the bears do not eat. Following these nutritional principles in zoos is one of the conditions for successful hibernation.

Many zoo enclosures still have a hard concrete surface, which is a highly unsuitable environment for bears and can lead to dermatitis on the underside of the paws, skin and arthritic problems. Natural substrate and presence of vegetation are also important for maintaining the bears' natural foraging habits (Law & Reid, 2010). Not to be overlooked is the fact that many of the enclosures that were evaluated lacked fresh drinking water and the bears drank water from their pools. A similar problem was found in zoos in Poland (Maślak et al., 2016).

In all zoos, an annual prophylactic program for deworming and vaccination of bears is carried out, but a medical examination is done only when necessary and when pathological symptoms appear. Periodic complete veterinary preventive examinations of the bears are carried out only in Belitsa Bear Park with the assistance of veterinary specialists from the Foundation „Four Paws“ from Austria. Under full anesthesia, blood samples are taken for research, X-ray and ultrasound scan, eye and dental treatment are performed. Health checkings are usually every 2-3 years to avoid frequent anesthesia, which poses health risks for older individuals. Carrying out such examinations is necessary because bears can have numerous health problems, and therefore monitoring physical health parameters is taken into

account when assessing welfare (Mononen et al., 2021). Common in captive bears are dental problems, injuries from conspecifics in co-housing, and degenerative joint problems in old bears (Bourne et al., 2010).

Bears are particularly prone to develop stereotypic behavior, which is very often expressed in locomotion, such as walking along a certain trajectory. This behavior becomes persistent and mechanical, with the animal increasingly less able to interact with the environment (Sergiel et al., 2012). One of the reasons for its manifestation is the attempt to adapt to the inappropriate conditions in which the animal is placed. The bears move along fixed paths and trajectories in the enclosures, making the same movements. Thus, through a huge number of repetitions, they cover long distances, constantly moving and bending their body in the same way, which can lead to musculoskeletal disorders. The damage to animals from the endless repetition of stereotypic behavior is both mental and physical. Stereotyping can fill between 60 and 80 % of bears' active time (Kolter & Zee, 2008). Various forms of stereotypic behavior were observed in 30 of the bears in zoos and in Bear sanctuary Belitsa. The observed forms of stereotyping were pacing, head tossing, yalking in the figure-of-eight, oral stereotypy, circling, etc. In their study on the welfare of bears in Poland, Maślak et al. (2016) stated that they found stereotyping in all the bears they observed. They describe the same forms of stereotypic behaviors plus one particularly severe case of self-mutilation in which the bear bites parts of its own body.

The ability of bears to hibernate in zoos is an indicator of their welfare level (Maher et al., 2021). It has been found that bears in zoos often only enter a state of mild torpor and do not actually go in deep and prolonged hibernation (Kim et al., 2020). Bears are very sensitive to disturbance, especially in the initial period of hibernation (Friebe et al., 2023). This is probably an important reason why they choose den sites away from human settlements and infrastructures (Swenson et al., 1997; Sahlén et al. 2011, 2015). If disturbed during the winter torpor, bears easily come out of this state. Studies in the wild have shown that disturbance during hibernation can lead to metabolic disorders and changes in activity over several days (Evans et al., 2016). Bears at the zoo in winter experience daily anxiety due to husbandry routines and the presence of visitors. Therefore, hibernation may often not be possible under these conditions, even when bears demonstrate a significant decrease in activity and are lethargic and passive (Fernandez et al., 2020). The ability of bears in zoos to hibernate requires the provision of a secluded and undisturbed environment, as well as the introduction of appropriate husbandry standards (Itoh et al., 2010). The fact that young bears up to 10 years of age are less likely to hibernate and that those up to 20 years of age hibernate less than older ones may necessitate the introduction of different husbandry procedures for young bears during the autumn-winter period.

The accumulated knowledge of the husbandry of captive bears and a better understanding of the needs of these animals has led to the creation of the Large bear enclosures (LBE) concept in Europe, which is presented in the EAZA Ursid husbandry guidelines (Claro-Hergueta et al., 2007). According to this concept, bears should live in an environment that is close to their natural habitat and in a space adapted to their natural needs. A spacious enclosure will offer a variety of stimuli that are biologically relevant to different

bear species and that will allow the animals to display their species-specific behavior in different seasons (Kok, 2007). Therefore, the design of new bear enclosures in zoos should follow LBE standards, and keeping bears in small, concrete cages or in bear pits should be a thing of the past.

IV. HUSBANDRY, BEHAVIOR AND ACTIVITY OF THE EUROPEAN SOUSLIK *SPERMOPHILUS CITELLUS* IN ZOO CONDITIONS

IV – 1. Materials and methods

Animals and housing. The study on the behavior of the European souslik in laboratory conditions and in zoo enclosure at Sofia Zoo were conducted in the period April 2021 - July 2023. The objects of study were seven adult, sexually mature individuals (six females and one male). Their behavior was was studied in Open field test and in Novel object exploration test (NOE test) using focal sampling (Altmann, 1974). After the completion of the experiments, two female souslics were selected for rearing in zoo exhibit.

The sousliks were wild-caught with live traps for the purpose of translocation from a colony in the vicinity of Pazardzhik. Upon arrival at Sofia Zoo, the animals were measured, examined by a veterinarian and treated with Ivermectin for internal and external parasites. They were accommodated in the Quarantine department of Sofia Zoo. The animals were housed in an individual metal cages for rodents in a room with natural daylight and a temperature ranging from 17°C to 25°C. They were housed in separate cages so that they could be observed individually during the adaptation period. A wooden board and nesting material - hay, as well as cardboard shelters were previously placed in the cages. Animals were visited only by members of the research team once a day for feeding and to start the video recording of their behavior. The sousliks were provided with food consisting of seeds (wheat, sunflower), dandelion leaves, cucumber, zucchini, carrot and apple placed in a metal bowl. Water was provided through rodent drinkers mounted on the cages.

Behavior in Open field test and in Novel object exploration test. Both behavioral tests were performed on all seven animals after their accommodation in the Quarantine department, in order to study their exploratory behavior and the risk taking behavior. The tests were carried out according to the activity of the animals and were tests were performed in the morning between 10 and 12 o'clock. The behavior of the experimental animals was recorded for 5 min in both tests by a specially mounted Basler acA1920-155um USB 3.0 camera (164 fps and 2.3 MP resolution) connected to a computer**.**

The analysis of animal behavior during the Open Field test and Novel object exploration test was performed using specialized software EthoVision XT13 with Multiple Body Module and Social Interactions Module of Noldus Information Technology, as well as specialized video recording equipment to the same software. The software allows to track the movement (Track Visualization option) and the location in an experimental arena (Heatmap Visualization option) of each individual. The different behaviors exhibited, such as standing, self-cleaning, jumping, defecation and others can be coded and marked during the analysis of the recordings. The Open field test was always conducted before the Novel object exploration test. The two tests were conducted on different days.

Open Field Test. A specialized square gray plexiglas experimental cage with dimensions of 100 x 100 cm and a height of 50 cm was used to conduct the test (arena with

walls to prevent escape). The arena was conditionally divided into the following sections (Figure 2):

1. "Arena“ - represents the area of the entire experimental cage (1 m^2).

2. "Central zone" - the zone in the middle of the cage measuring 40 x 40 cm.

3. "Peripheral zone" - the zone that lies between the central zone and the outer borders of the cage.

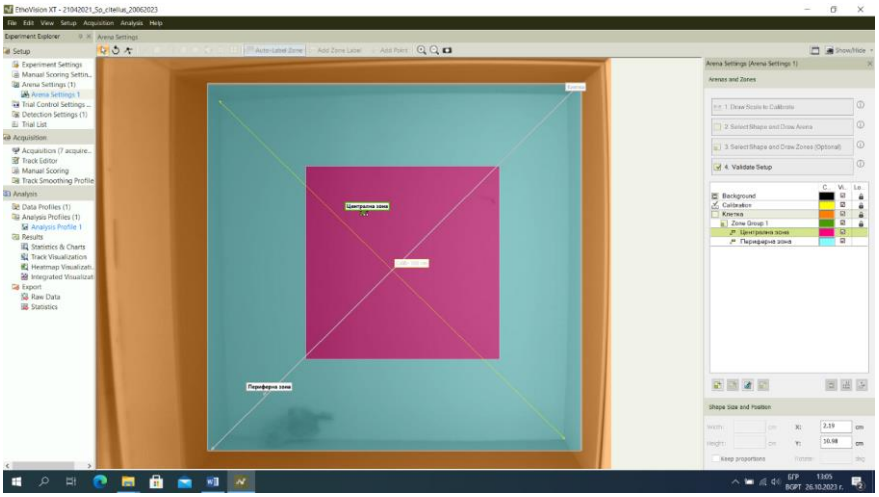

Figure 2. Separation of the area of the experimental cage into „Central zone” and „Peripheral zone”, which are located in the „Arena” when when setting up for the Open field test using the EthoVision XT13 software.

Each individual was placed in the center of the empty cage and allowed to explore the unfamiliar environment within 5 min. When conducting the Open field test, the following variables were considered in the analysis:

• distance covered by the animal in the arena

• distance covered in the Central zone

• movement speed in the arena

• movement speed in the Central zone

• mobility in the arena

• mobility in the Central zone

• immobility in the arena

• immobility in the Central zone

• duration of stay in the Central zone

• duration of stay in the Peripheral zone

To compare the variables "covered distance" and "speed of movement", "mobility" and "immobility" (the animal does not move), as well as "duration of stay in Central zone" and "duration of stay in Peripheral zone" was used paired t test at the significance level of $p < 0.05$. Also compared were the distance traveled in the arena and in the Central zone, the speed of movement in the arena and in the Central zone, the mobility in the arena and in the Central zone, the immobility of the animals in the arena and in the Central zone, and the duration of stay in the Central zone and in the Peripheral zone.

Novel object exploration test (NOE test). This test was conducted in the same experimental cage where the Open field test was previously performed, but on the following day. Animals were placed in the empty cage for 5 min for habituation to the location (training phase) and then removed briefly to place the novel object without them seeing it. The sousliks were then returned into the cage and their behavior was videotaped. The novel object was a 10-centimeter-long green rubber rodent figure. It was chosen in accordance with the dimensions of the souslik and because of its relatively natural shape.

In the analysis of the data from the Novel object exploration test, the following variables were taken into account:

• "latency to first movement" (the duration in seconds from placing the animal in the cage until its first movement)

• "latency time to approach the object" (the duration in seconds from placing the animal in the cage until it first approaches the object)

After conducting the tests with each animal, the experimental cage was cleaned with ethyl alcohol 70% to remove all odors from the test subject. The cage was not used for at least 10 minutes afterwards to allow the scent of the cleaner to evaporate.

Behavior and activity of European souslik in the Quarantine department. Three of the female sousliks, after their arrival at the Quarantine department, were placed in separate special observation cages with glass in the front and the possibility of mounting video cameras on the ceiling. During the first seven days of their stay in quarantine, they were filmed in the cages for one hour a day between 10:00 and 12:00 with an ACME VR06–4K Ultra HD action camera to track their behavior during the adaptation period. This time range was chosen because it is known that the above-ground activity of sousliks in nature is greatest between 9 and 12 h (Katona et al., 2002; Váczi & Altbäcker, 2006). A total of 1330 minutes of video recordings were made over 14 days. Through the recordings, the various behavioral events and states of the animals in the absence of humans were recorded.

Diurnal and seasonal activity of European souslik in the zoo enclosure. After the end of the quarantine period, two female sousliks were moved from the Quarantine department to a specially designed enclosure built next to the building of the Zoo Education center with a southeast exposure. They were accommodated there for the purpose of long-term monitoring of their behavior and study of diurnal and seasonal activity. The sex and the number of individuals in the group was in accordance with the ecological requirements and

social structure of the species and the size of the enclosure. The selection of the two females was made based on the results of the ethological tests.

The enclosure measures 2.5 by 2.5 m and is 4 m high, filled with a soil layer 1.5 m thick (Pict. 3). In order to see part of the underground tunnels dug by the sousliks, three windows of tempered glass were built on the outer walls of the enclosure. The cage was pre-planted with grass, log and pipe shelters were created, and hay was provided for nesting material. The animals were given food and water once a day through a special window in the wall, and their diet consisted of seeds (sunflower, wheat), carrot, cucumber, zucchini, apple, willow twigs, dandelion leaves and blossoms, grass, and boiled egg once during the week. The enclosure was created for scientific and educational purposes and is therefore only visited periodically by school groups and students, and other visitors to the zoo do not have permanent access to it.

Picture 3. The European souslik enclosure in Sofia Zoo.

The behavior and activity of the two female sousliks, moved from the Quarantine department into the enclosure, was monitored by a camera trap Game/Surveillance Camera WK 8A1 (Video- 1920X1080 px; Photo – 8-16Mpx) installed in the enclosure. From the moment sousliks were released into the enclosure, until they went in hibernation in 2021 (the period from 11.06.2021 to 10.10.2021), the clips and photos were systematically downloaded and analyzed to track the animals' 24-hour activity and their various behavioral events and states. Viewing of photo and video data from the camera trap was performed by using Timelapse Image Analyzer 2.0 software. For each frame, the date, time, temperature, moon phase, number of individuals in the frame and, in some cases, notes on the displayed behavior were described. The monitoring continued in 2022 and 2023, and the camera trap materials were reviewed periodically to monitor the condition of the animals and determine when they hibernate (Pict. 4).

Picture 4. A photo taken from the camera trap placed in the enclosure showing the two European sousliks that live there.

IV – 2. Results

Open field test. During the Open field test, the following behavioral events were observed in the seven animals tested: vocalizing, defecating and urinating, jumping, standing, moving or standing at the Peripheral zone of the arena, and passing through the Centeral zone. Statistical analysis showed that there was a statistically significant difference in the distance traveled in the arena compared to the distance traveled in the Central zone only ($t = -7.79$, $p = 0.00023$), in the speed of movement in the arena and in the Central zone ($t = 5.45$, $p = 0.00159$) and in the duration of stay in the Central and Peripheral zones ($t = -6.32$, $p = 0.0007$). No statistically significant difference was found between the mobility and immobility of the animals in the Central zone.

Novel object exploration test. During this test, the sousliks exhibited the following behaviors: sitting still, jumping, vocalizations of alarm calls, attempting to exit the arena, defecating and urinating, approaching the object, touching the object. The animals were cautious of the novel object. Five of the females did not approach the object even once. Only the male souslik and one of the females approached and interacted with it by sniffing and touching.

Diurnal and seasonal activity of the European souslik in captivity. In the first days after the animals were placed in the Quarantine department, they were more active, eating, trying to climb the walls of the cages, digging - mainly in the corners, jumping and trying to open the doors with their teeth. It is noteworthy that immediately after the first day of their placement, they had made nests from the hay provided and spent more time hidden in the nests. All animals vocalized frequently, especially after the first day, making distinctive

whistles and vocal communication. Despite these common behaviors, there were differences between individuals. Manifestations of active behavior predominated in the first two observed females, while in the third female observed passive behavior predominated. The percentage distribution of active and passive behavior in the three observed female individuals is as follows: 52.49 % active behavior and 47.41 % passive behavior. The longest duration behavior exhibited by the three female sousliks was walking, followed by hiding in the nest. The jumps are the shortest. The animals ate, stood still, tried to dig and climb for varying time duration.

In the enclosure at the Educational center in Sofia Zoo, in the first days after their transfer from the Quarantine department, the two female sousliks actively explored the new place. They climbed up the netting to the roof of the cage, went around the corners, sniffed and poked their way into all the pipes, branches and haystacks and started digging holes in the soil. The location of the holes, which were three in number, was chosen as far as possible from the outer walls of the cage - in the inner corner opposite the fence.

The sousliks were observed mouthing hay into the burrows on the sixth day of placement. They were often seen in their natural standing posture, looking around, and their alarming whistle was relatively rarely heard. When people approached from the outside of the fence, when enclosure was opened, and when the food was given, the animals remained hidden. They continued to hide in their holes when disturbed until the end of the observation, which ended on 16.07.2023. But they habituated to the sound of the window being opened and the food and water bowls being placed (after the keeper put the food down and leave, the recordings showed that they came out of the holes and went to the food). They were observed drinking water from a metal shallow bowl which was always kept full. When feeding, they showed a preference for the leaves of dandelions, which are part of their natural food. The two animals were active for the longest time interval during the months of June and August (Table 8). The dependence of the daily activity of the observed sousliks on the ambient temperature is shown in Figure 3.

Table 8. Hourly interval of the daily activity and temperature at which the European sousliks were active in the period June-October 2021.

Month	Daily activity start time	Daily activity end time	Temperature, °C (average, min-max)
June	09:19	21:10	24.4°C (13°C – 32°C)
July	09:22	19:28	26.1 °C (20°C – 32°C)
August	09:09	20:09	28.6 °C (18°C -35°C)
September	09:29	17:11	22.1°C (12°C – 30°C)
Октомври	10:20	17:50	16.2°C (10°C – 22°C)

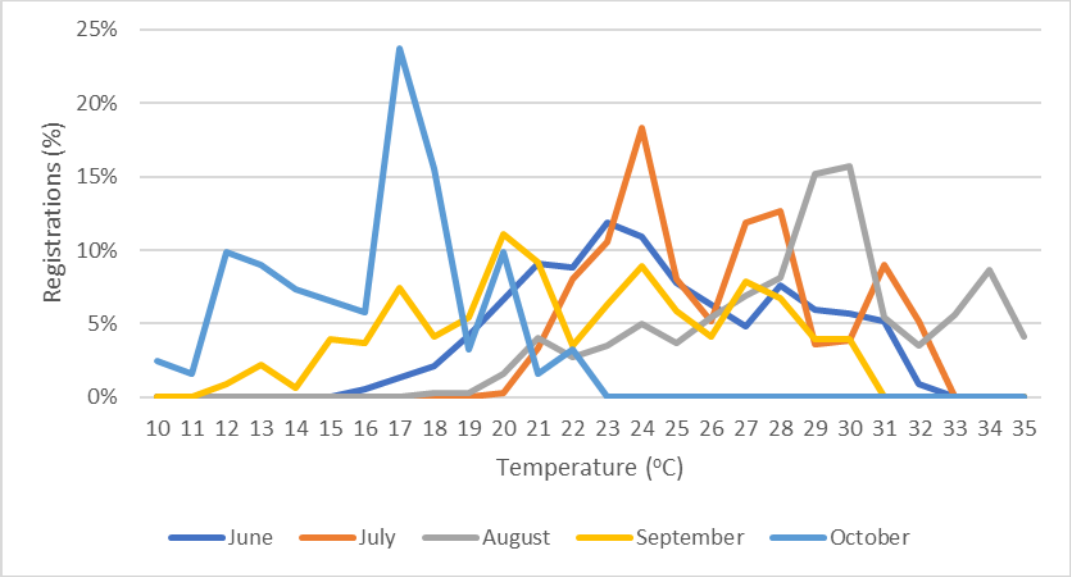

Figure 3. Activity of the two European sousliks in the enclosure versus ambient temperature as recorded by the camera trap in the period June-October 2021.

The two female sousliks were observed to hibernate during the winters of 2021/2022 and 2022/2023. Their date of going in hibernation and date of awakening, which were determined from the camera trap recordings, are listed in Table 9.

Table 9. Seasonal activity of European sousliks in the enclosure for 2021, 2022 and 2023.

Yaer	Date of awakening	Date of going in hibernation
2021	-	10.10.2021
2022	31.03.2022	10.09.2021
2023	17.03.2023 20.03.2023	16.06.2023

IV - 3. Discussion

In the Open Field test, all animals spent significantly more time in the Peripheral zone of the experimental cage than in the Central zone. Furthermore, their movement speed was significantly higher when they passed through the Central zone compared to when they moved throughout the arena. These results confirm that European souslik, like most rodents, is cautious and prefer the peripheral rather than the central area of the experimental cage in the Open Field test (Walsh & Cummins, 1976; Lafaille & Féron, 2014; Sturman et al., 2018; Kraeuter et al., 2019).

Anxiety-like behavior of rodents is frequently accompanied by reduced exploration, seeking shelter, escape, burying, or defecating (Bailey & Crawley, 2009; Simeonovska - Nikolova, 2000). Defecation was observed in all individuals during the experiment and also

prolonged stay in the Peripheral zone and especially in the corners of the cage. The assumption underlying most animal behavioral models of anxiety is that they are part of defense mechanisms essential for animal survival (Hendrie et al., 1996). Many rodent species avoid open microhabitats that are perceived as areas of high predation risk (Dickman, 1992; Jacob & Brown, 2000; Mandelik et al., 2003; Fanson et al., 2010). It is therefore not surprising that the studied sousliks also avoided the Central zone of the arena. Among the anti-predator behavioral responses typical of rodents are reduction of locomotor activity, standing still, vigilance, escape (Dielenberg et al., 2001; Shahaf & Eilam, 2003; Taraborelli et al., 2008), which were also found in the experimented sousliks. European souslik in Bulgaria is the main prey for birds of prey such as eastern imperial eagle *Aquila heliaca,* saker falcon *Falco cherrug,* long-legged buzzard *Buteo rufinus,* white stork *Ciconia ciconia* and 18 other diurnal and nocturnal birds of prey, as well as for corvids Corvidae. In our country, carnivorous mammals such as the steppe polecat *Mustela eversmanii* and the marbled polecat *Vormela peregusna*, the weasel *Mustela nivalis*, beech marten *Martes foina*, the western polecat *Mustela putorius*, the red fox *Vulpes vulpes*, the golden jackal *Canis aureus* as well as domestic dogs *Canis familiaris* and cats *Felis silvestris f. catus* feed on the European souslik (Koshev, 2022).

During the Novel object exploration test, all animals spent part of the time in the cage standing still in one of the corners, with one of the sousliks not even leaving it and making no movements in the arena or toward the novel object. However, there were also differences. Two of the animals (one male and one female) approached the novel object and interacted with it. This confirms that, placed in the same environment and under the influence of the same stimuli, individuals of one species often differ in their behavior (Verbeek et al. 1996; Gosling, 2001). In this test, variations in behavior allow the assessment of individuals' activity (active and inactive - Sih et al., 1992), exploratory tendency (fast and slow - Verbeek et al., 1994) and risk-taking propensity (neophobia and neophilia - Clark & Ehlinger, 1987; van Oers et al., 2004). This helps determine such individual traits as boldness and shyness, which can be decisive in making decisions about inclusion of individuals in wildlife reintroduction programs. The balance between neophilia and neophobia and the greater exploratory activity of individuals is assumed to be a personality trait related to their potential for environmental plasticity and better adaptive capabilities (Richard et al., 2008; Herborn et al., 2010). On the basis of the results of the Novel object exploration test, the more active and "bold" female individual was one of those selected for the zoo enclosure. During the test, the only male in the group also showed active exploratory behavior and neophilia to a greater extent, which is consistent with the results of Hoffmann et al. (2004). According to these authors, male European sousliks are more risk-taking and move further away from their burrows than females.

Immediately after being released together in the enclosure in Sofia Zoo, the two female sousliks socialized and began to dig burrows in the ground. By the sixth day, they were using three entrances – two next to the wall of the building and one closer to the center of the cage. Both animals entered and exited the entrances and apparently used the tunnels they had dug together. In colonies, Eurppean sousliks inhabit solitary burrows up to 2 m deep (Ružić, 1978) where they hide, rest, breed and hibernate (Kachamakova et al., 2019). The

system of underground tunnels in which the souslik lives usually consists of a nest chamber, 16 - 25 cm wide, and in some cases a second chamber that serves as a latrine (Ružić, 1978; Hoffmann & Haberl, 2023). In the enclosure, the two females, probably due to lack of sufficient space, occupied the same tunnels. Whether each animal has its own separate nest chamber in which it rests and hibernates is not known. The two animals could rarely be seen through windows built at soil level because they did not dig passages and tunnels in this part of the cage.

The two sousliks had hay and newly sprouted grass (ryegrass) available in the cage, but preferred hay for nest lining, which was brought into the holes by mouth. One of the most preferred plants by European souslik for nesting material is pseudovina fescue *Festuca pseudovina* (Gedeon at al., 2021), but there was no opportunity to offer it fresh or dried. In the study conducted by Gedeon at al. on nesting material preference of European souslik, they found that animals preferred fresh fescue stalks over dry, due to their better insulating qualities and flexibility. The hay that was placed in the enclosure was from highland pastures and of heterogeneous composition, and the animals not only brought it into the burrows, but were also observed to eat it. When rearing spotted sousliks *Spermophilus suslicus* in outdoor aviary conditions for breeding and reintroduction purpose in Oleni Nature Park in Russia, the animals used for nesting material hay from thin-stemmed grasses, with which they filled the entire nesting chamber (Sapelnikov & Sapelnikova, 2021). Probably, the preferences of the animals in captivity for the nesting material are determined not only by its nature, but also by the amount and its availability. Hay, especially upland meadow hay, is a suitable material that can be provided to ground squirrels for nest building and diet diversification. In the camera trap recordings, the animals were repeatedly seen drinking water from the metal bowl during the summer months. This does not match the observations of zoo staff in Ukraine, Russia and Hungary, who said they did not observe sousliks in captivity drinking water from rodent drinkers or bowls and recommended that the animals' food should always contain fruits and vegetables such as water source. Although the animals had daily access to fresh fruits and vegetables, they occasionally drank water, which is probably related to the high temperatures during the summer months and the relatively dry environment in which they lived. The enclosure has a roof structure and therefore it does not rain inside. In their natural habitats, European sousliks are known to drink water from puddles and natural water sources (Wade, 1930). Regarding the feeding – animals showed the greatest preference for the leaves and flowers of dandelion, which were provided to them during all the time. They always ate the whole amount provided to them, and of the fruits and vegetables there was often an uneaten amount left. European souslik is known to feed on green parts of plants throughout the active season, and these may fully satisfy its water needs (Straka 1959, 1961; Koshev, 2022). Food of animal origin is an important source of protein for the sousliks, but is of secondary importance. In nature they also feeds on eggs of ground-nesting birds (Straka, 1961), so once a week a boiled egg was included in their diet.

The European souslik often experiences the impact of extreme climatic conditions in its natural habitats. A field study by Váczi et al. (2006) proves that ambient temperature has a significant influence on the diurnal activity of the souslik. However, this species, as well as others that live in underground tunnel systems, can avoid high or low temperatures by

choosing the more constant and favorable temperature conditions in their burrow (Hut et al., 1999). Data from the camera trap in the cage showed that in 2021 they were most active in June in the temperature intervals of 16 and 19 °C, in July between 20 and 26 °C and in August between 27 and 31°C. These data can hardly be compared with similar ones from field studies, due to the fact that the two sousliks lived in semi-natural conditions in a cage with a roof, which prevented the sun's rays from reaching the entire surface of the enclosure. On the other hand, the hourly intervals of activity of the observed animals correspond to those established by Koshev & Kocheva (2008) in their study of the daily above ground activity of European souslik in the colony near Knezha, Northwestern Bulgaria. The two females in the cage showed activity with the longest hourly interval during the months of June and August. It can be assumed that this is due to the longer day length in June and the higher average temperature in August compared to the other months (based on camera trap data collected in 2021). In the summer of 2023, temperatures were unusually high - average monthly temperatures in July were between 20 and 28 °C (with a deviation from the monthly norm between +0.5 and +3.5 °C). July 2023 was warmer than July 2022 and was one of the three warmest Julys in the last 25 years (National Institute of Meteorology and Hydrology, 2023). In 2023 the sousliks in the cage were last seen on July 16 and after that they disappeared. This suggests that they may have gone into summer lethargy. Since then, although camera trap monitoring continued until the end of October 2023, the animals were not seen. It is possible that summer lethargy has turned into winter hibernation. For European souslik, it has been found that the duration of hibernation can be from 180 to 240 days (Matějů, 2008). At the southern limit of the species' range, female sousliks are known to wake from hibernation around March 22 (± 9 days), and males - around March 9 (± 8 days). The stars of hibernation can also be on July 21 (± 6 days) for females and August 6 (± 10 days) for males (Youlatos et al., 2007). Also, at higher temperatures during the winter period, it is possible for European souslik to come out of hibernation earlier, in mid-February (Straka, 1961). European sousliks in Northern Greece, Bulgaria, Macedonia and the European part of Turkey (Kryštofek & Vohralik, 2005; Özkurt et al., 2005) have been found to hibernate earlier than in Central Europe (Millesi et al., 1999). This is explained by higher ambient temperatures, which make it more difficult to find food, reduce the water content of the food, and therefore these circumstances lead to earlier hibernation (Schwanz, 2006).

The results of the study showed that European sousliks in captivity hibernate and come out of hibernation at the same times of the year as wild sousliks in our latitudes. However, global climate changes, such as drought, floods and strong storms, as well as the increase in land surface temperatures in recent decades are among the main threats to the existence of the declining populations of European souslik (Hegyeli, 2020; Zidarova & Popov, 2022). Expanding knowledge about the behavior of this species in captivity is important for future conservation efforts and for its preservation in nature.

CONCLUSIONS

1. The brown bear *U. arctos* in captivity exhibits various forms of stereotypic behavior, including the rarely observed "non-nutritive sucking" behavior, resulting from the conditions and husbandry procedures and the personal history of individuals.

2. Sound and odor stimuli from conspecifics affect the brown bear's behavior in captivity, and under their influence the animals exhibit greater behavioral diversity and a tendency to reduce stereotypic behavior.

3. The provision of biologically relevant natural sound and odor stimuli has the potential to be introduced into environmental enrichment programs that may help address behavioral problems in captive brown bears in Bulgaria.

4. The welfare assessment of brown bears in Bulgarian zoos shows that it is at a low level, which is mainly due to housing in insufficient space and unsuitable surface, inadequate diet and insufficient veterinary care.

5. In behavioral tests European souslik *S. citellus*, like most rodents, avoids open spaces, which stems from its way of living and anti-predator behavior. Its behavioral response to a new environment and a novel object reveals individual differences that could have relevance in conservation practice.

6. The European souslik can successfully adapt to zoo conditions and go into hibernation. That allows the species to be reared in captivity for the purposes of various educational and conservation activities.

CONTRIBUTIONS

Scientific contributions:

1. The behavioral response of the brown bear *U. arctos* to natural sound and odor stimuli in captivity builds on the knowledge of the species' behavioral response to such stimuli and their effectiveness in reducing stereotypic behavior.

2. A rare form of stereotypic behavior in brown bear "non-nutritive sucking" is described, which enriches the information about the forms of abnormal behavior in the species, its causes and approaches to their mitigation.

3. The carried out pilot activities for studing the behavior, activity and husbandry procedures of European souslik *S. citellus* in zoo conditions are the beginning of *ex situ* conservation activities for the species in our country.

Applied contributions:

4. An assessment of welfare of brown bears in captivity in Bulgaria was made and recommendations were formulated to improve the conditions and husbandry procedures for the species in Bulgarian zoos.

5. A proposal for changes to Ordinance No. 6 of 23.10.2003 on the minimum requirements and conditions for keeping animals in zoos and breeding centers for protected animal species in the part related to brown bear in captivity has been prepared.

6. A catalog of suitable forms of environmental enrichment for brown bears applicable in the current conditions of Bulgarian zoos has been created.

7. The first zoo exhibit with European souslik in Bulgaria was created in Sofia Zoo for educational and scientific purpose.

RECOMMENDATIONS

Based on the welfare assessment of the brown bears in captivity in Bulgaria, the following recommendations have been formulated:

1. Breeding of brown bears in Bulgarian zoo collections should be controlled and carried out only if it is in accordance with the collection development plan or if it is part of a long-term conservation (breeding) program for the species, in which the zoo participates. In order to avoid inbreeding, it is necessary to carry out genetic studies of breeding individuals.

2. In Appendix No. 1 to Art. 12, para. 1 (amended and supplemented - SG No. 44 of 2009) of Ordinance No. 6 in the part describing the conditions for Carnivora, bears (Ursidae) - brown bears, instead of a minimum area of 150 m^2 per individual + 20 m^2 for each subsequent individual in the enclosure to be determined a minimum area of 500 m^2 per individual + 500 m^2 for each subsequent one. Also, the following should be amended in Appendix No. 1: the social structure in which the bears are kept should not be only in pairs (as is the case in the current version of Ordinance No. 6), but should be single or in pairs; the temperature conditions under which the bears will be reared should not be above 12 °C, and it should be indicated that the species is resistant to winter conditions.

3. Zoos should provide periodic full veterinary medical examinations for the bears, which should also include dental treatment.

4. Zoos should introduce as a mandatory part of brown bear husbandry procedures the regular provision of diverse and appropriate environmental enrichment.

5. The nutritional diet of the bears in these zoos, which offer unbalanced and insufficient nutrition to the bears, should be updated and optimized in accordance with the EAZA Ursid husbandry guidelines (Claro-Hergueta et al., 2007).

6. To improve control over the visitors' behavior towards animals in zoos and to prevent unregulated feeding of bears by visitors.

7. Zoos that keep brown bears should create educational displays around the bear enclosures to inform the public about the biology, behavior and conservation status of the species. The expectation is that better awareness will increase people's conservation culture and lead to a change in their behavior and attitude towards bears in nature and in captivity.

8. To revise the husbandry procedures during the autumn-winter period for bears in zoos where they do not hibernate. To provide a restriction on the access of visitors during this period to the bear exhibits.

9. A periodic welfare assessment of brown bears by using established scientific methods in the institutions keeping these carnivores should be made.

10. Zoos should raise the qualification requirements of bear keepers and assist in the training of staff in order to increase the quality of animal husbandry.

The prepared recommendations will allow the organizations that keep brown bears in Bulgaria to take steps to improve the environment and increase the welfare of the animals entrusted to them. Introducing the species to a near-natural environment and meeting the bears' behavioral and biological needs will greatly increase their welfare. This will also help to convey the right message to the zoo visitors and increase their conservation awareness and empathy for animals. The zoo institutions in Bulgaria will come closer to the good practices for keeping wild animals and to the educational and conservation standards in modern zoos around the world.

PUBLICATIONS

Publications related to the dissertation

Zareva-Simeonova, K., Spasova, V. and Simeonovska-Nikolova, D., 2023. Behavioral responses of captive brown bears *Ursus arctos* to the odor of conspecific urine. Applied Animal Behaviour Science, Volume 267, 2023, 106050, ISSN 0168-1591, https://doi.org/10.1016/j.applanim.2023.106050

Zareva-Simeonova K., Spasova, V. and Simeonovska-Nikolova, D., 2022. Abnormal behaviors in adult female captive brown bears *Ursus arctos* Linnaeus,1758 (Carinivora: Ursidae), with an emphasis on non-nutritive suckling. Acta Zoologica Bulgarica, 74 (4): 535-543, http://www.acta-zoologica-bulgarica.eu/2022/002628

Other publications published during the PhD period:

Todorov V., Spasova V., Simeonovska-Nikolova D., Ihtimansi I.,. Zareva-Simeonova K, Dolapchiev N. and Ganchev R., 2023. Brown bear *Ursus arctos* (Linnaeus, 1758) (Ursidae) den's characteristics in Bulgaria. Acta Zoologica Bulgarica, 75 (3): 331-341, https://www.acta-zoologica-bulgarica.eu/2023/002717

PARTICIPATION IN SCIENTIFIC FORUMS

International scientific forums:

- **Zareva-Simeonova K.**, Racheva V., Simeonovska-Nikolova D., Dimitrov K., 2019. Behaviour of the brown bear, *Ursus arctos*, in captivity – implications for animal welfare and human-bear interaction. VIII International Symposium of Ecologists of Montenegro, Budva, October 2 – 5, 2019, p. 140 - poster, https://www.researchgate.net/publication/336285116_Pesic_V_Ed_The_Book_of_Abstracts_and_Programme_of_8th_International_Symposium_of_Ecologists_of_Montenegro_2-5_October_2019_Budva_Montenegro_207_pp_ISBN_978-86-908743-8-5

- Simeonovska-Nikolova D., Spasova V., Dimitrov K., **Zareva-Simeonova K**., 2020. Is there a future for the Romanian hamster, *Mesocricetus newtoni* in Bulgaria? International Scientific Conference on Restoration of Conservation-Reliant Species and Habitats, 6th NOVEMBER 2020, p. 24, poster RS-8, https://www.uni-sofia.bg/index.php/bul/universitet_t/fakulteti/biologicheski_fakultet2/konferencii/kliment_s_days_2020/international_scientific_conference_on_restoration_of_conservation_reliant_species_and_habitats_resconf_2020

- **Zareva-Simeonova K**., D. Simeonovska-Nikolova, V. Spasova, V. Todorov 2022. Do brown bears hibernate in captivity? International Seminar of Ecology - 2022, Actual Problems of Ecology, 29-30 септември 2022, Online, poster P03_08, https://80fb544f-645e-4a86-bbd9-505bf8716413.filesusr.com/ugd/16129b_59e2c4539a5c4160a88f25fd7d8f1deb.pdf

- Todorov V.,V. Spasova, **K. Zareva-Simeonova**, D. Simeonovska-Nikolova, N. Dolapchiev, R. Ganchev 2022. Brown bear dens in the wild and captivity: implication for conservation and animal welfare. 6th Congress of Ecologists of North Macedonia, with international

participation (Biodiversity and conservation biology), 15-18.10.2022, Ohrid, p. 63, poster, http://congress.mes.org.mk/wp-content/uploads/2022/10/Congress-programme-final.pdf

National Scientific Conferences:

- **Zareva-Simeonova K.**, V. Racheva, D. Simeonovska-Nikolova, K. Dimitrov 2019. Comparative study of the behavior of the brown bear, *Ursus arctos* under different conditions in captivity, Youth Scientific Conference "Kliment's Days" 8th November 2019, Sofia Faculty of Biology, p. 15 – oral presentation http://conf2019.biofac.info/KD_2019_BOOK_OF_ABSTRACTS.pdf

- **Zareva-Simeonova K.**, V. Racheva, D. Simeonovska-Nikolova 2020. Does the stereotypic behaviour in a male Eurasian brown bear *(Ursus arctos arctos)* in captivity is influenced by various stimuli? Scientific Conference "Kliment's Days" 5-th November 2020, Faculty of Biology, p. 57 - poster E&SD-17, http://conf2020.biofac.info/KD_2020_BOOK_OF_ABSTRACTS.pdf

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PROJECTS

1. Project № 12570/08.04.2019 - **„Pilot activities for rearing and breeding of small mammalian species important for conservation"** with project coordinator prof. Daniela Simeonovska-Nikolova, PhD; Financing organization: EMEPA, MOEW.

2. Project № КП-06-Н51/8 - 11.11.2021 - **„ Complex genetic and ethological research for long-term in situ and ex situ conservation of the brown bear, *Ursus arctos*"** with project coordinator ch. assist. prof. Venislava Spasova, PhD; Financing organization: The Bulgarian National Science Fund of Ministry of education and science of Bulgaria.

3. Research project for PhD studens support № 80-10-80/15.04.2019 - **„Impact of visitors, environment and enrichment on the behavior of the brown bear *(Ursus arctos)* in captivity"** with project coordinator prof. Daniela Simeonovska-Nikolova, PhD; Financing organization: Sofia University "St. Kliment Ohridski".

4. Research project for PhD studens support № 80-10-87/15.04.2020 - „**A study of the occurrence of stereotypic behaviour in the brown bear *(Ursus arctos)* in captivity and proposing approaches for its minimization**" with project coordinator ch. assist. prof. Venislava Spasova, PhD; Financing organization: Sofia University "St. Kliment Ohridski".

5. Research project № 80-10-41/22.03.2021 - **„Welfare assessment of brown bears *(Ursus arctos)* in captivity in Bulgaria"** with project coordinator ch. assist. prof. Venislava Spasova, PhD; Financing organization: Sofia University "St. Kliment Ohridski" .