

Article

Behaviour of Zoo-Housed Red Pandas (*Ailurus fulgens*): A Case-Study Testing the Behavioural Variety Index

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Abstract: The red panda is listed as “endangered” in the IUCN Red List of Threatened Species, due to the rapid population decline. Improving our knowledge on the red panda biology and ethology is necessary to enhance its husbandry and breeding in zoos. Behavioural variety, intended as the presence of a wide array of species-specific behaviour, has been considered a positive welfare index in zoo-housed animals. The aim of this study was to describe the behaviour of two pairs of zoo-housed red pandas, one of them with an offspring, and to investigate the behavioural variability using the Behavioural Variety Index (BVI). Behavioural data from two zoo-living male–female pairs were collected. A continuous focal animal sampling method was used to collect individual and social behaviours of the two pairs. Forty-eight 30 min sessions per subject were carried out. For the BVI, a list of species-specific behaviours previously reported in the red panda was prepared and compared with the behavioural repertoire of the subjects of the study. First, species-specific behaviours were recorded, and no abnormal behaviour was reported. The percentages of time spent on different activities (e.g., routine behaviours, exploratory/territorial behaviours, consumption behaviours, locomotive behaviours, social behaviours, maternal behaviours) were similar to time budgets reported in the red panda, with routine behaviours (resting, comfort and vigilance) being the most performed in both pairs. Moreover, the BVI suggested that each red panda performed on average 73% of the behaviours described in previous literature on this species. In conclusion, studying the behavioural variety of red pandas in zoos can be a useful tool for assessing their welfare as well as improving our knowledge on the behavioural repertoire of a species that is difficult to observe in the wild.

Keywords: red panda; animal welfare; maternal behaviour

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1. Introduction

The red panda (*Ailurus fulgens*) is listed as “Endangered” in the IUCN Red List of Threatened Species, due to the rapid population decline [1]. Given the situation of red pandas in the wild, breeding of this species under human care has become increasingly important to create insurance populations [2]. Improving our knowledge on red panda biology and ethology is necessary to enhance the husbandry standards and breeding success of this species in captivity, as well as for in situ conservation efforts [1]. Past research on red pandas housed in zoological collections has led to improve the husbandry (e.g., diet, housing conditions, group composition) of this species in Europe and to an increase in the total number of births [1]. Moreover, ex situ populations of red panda may allow research opportunities that support wild populations and in situ conservation efforts [1].

Observation of animal behaviour allows us to obtain important information on the psychological and physical health of animals and has become an important non-invasive

tool to measure animal welfare [3]. The assessment of positive as well as negative welfare states has become increasingly important in animal welfare science, with the development of indicators to assess and promote positive welfare of each individual, as well as at the species level. Recent studies on animals in zoos focus on the presence of species-specific, natural and normal behaviours, and the absence of abnormal behaviours, such as stereotypes, as an indicator of welfare [3–7]. For example, affiliative social interactions in solitary species such as bears have been found to reduce the performance abnormal behaviours (reviewed in [4]). Behavioural variety in general has been used to assess the welfare of Northern bald ibises in zoos [7].

Behavioural diversity is intended as richness of behaviours, and it would be linked to a positive welfare state because a high behavioural diversity indicates that we are meeting several behavioural needs of the animal [8]. On the contrary, when behavioural diversity is low, and animals have no opportunities to perform their behavioural repertoire, they can become lethargic and develop abnormal behaviours [9]. Thus, behavioural variety could be lost during challenging situations that could characterize controlled environments and human management, and the presence of different normal and natural behaviours performed by each subject could indicate a positive welfare state [3–7]. In particular, behaviours related to positive welfare can be luxury behaviours, such as play, exploration, and allo-grooming, which are the first ones to be lost during challenging situations and require a sufficient welfare level to be performed by the animals [6,10]. In the red panda, a recent study highlighted that personality could impact the welfare of subjects under human care, with behaviours such as locomotion, exploration, and marking, underlining an active and explorative temperament [11].

In the wild, the red panda is a solitary species, with males and females interacting with each other only during the mating season [12,13]. However, in some cases, individuals can move in pairs or small family groups, with males being more territorial than females, protecting and patrolling their home range to a greater extent [13]. This species can be diurnal, crepuscular, or nocturnal, and activity patterns vary across seasons and between sexes, ranging from 45 to 60% of the day depending on temperature and food availability [14,15]. In particular, red pandas are more active in summer, spring, and autumn, whereas in winter, resting periods increase [14,15]. Red pandas are arboreal and are agile climbers, sleeping and resting in trees [14]. Scent-marking is a relevant behaviour in this species, as pandas mark their trails by secretions from foot glands and mark frequently using ano-genital glands, urine, and faeces [14,16]. Females have a gestation of 114–145 days, with births happening in summer, mainly in June and July in the northern hemisphere [16]. Data collected in zoological gardens suggest that cubs stay in the den for 3–4 months and are completely dependent on their mothers [16]. Indeed, the cubs are altricial and nursing is taxing for female survival, requiring high-energy intake and an increased amount of food [16,17]. Before leaving the mother, juveniles show high levels of exploratory behaviour, practice of locomotory and feeding behaviour, and development of social behaviours through play and interactions with conspecifics [18]. In zoos, the bonds between mother and offspring may continue beyond one year of age of the cub, whereas the length is unknown in the wild [16]. In zoos, male red pandas tend to avoid females after parturition and during the denning period. However, after cubs leave the den, male pandas start to gradually interact with them and may become involved in play sessions [16]. In zoological institutions, red pandas are mostly kept in pairs. On some occasions, red pandas are kept in same-sex pairs or small groups [13]. Given that the red panda is solitary, assessing whether group structures and size meet the welfare needs of all individuals, and allowing the performance of species-specific behaviours are of great importance [1,13]. Moreover, first-year mortality of zoo-housed red pandas is high (36% in countries with warm and humid climates during the species breeding season), probably because mothers leave their cubs unattended due to heat stress [19].

Therefore, it is important to monitor the behaviour of red panda mothers, identifying signals and developing strategies to prevent or reduce infant mortality that can help to enhance breeding success of this species under human care.

The aim of this study was to investigate and describe individual and social behaviour of two pairs of zoo-housed red pandas, focusing on the variety of species-specific normal behaviours performed by the subjects. One of the pairs had offspring and special attention was given to maternal behaviour. To describe the behavioural variety of our subjects, we used the Behavioural Variety Index (BVI) developed and described in our previous research [7]. This index provides a measure of behavioural richness of each individual, quantifying the presence of species-specific natural behaviours described for a species in both wild and captive contexts [7]. Discussing behavioural time budgets of zoo-housed individuals based on data collected on the species as well as analysing the BVI of the subjects could be a useful tool that provides some quantitative and qualitative insights into the welfare of the two red panda pairs [7,18–21].

2. Materials and Methods

2.1. Subjects and Area

The study involved two pairs of red pandas housed in two different enclosures at Parco Natura Viva-Garda Zoological Park, an Italian zoological garden, in October and November 2015. The first pair consisted of one female and one male, Ilosha and Ny'ma. The female of this pair, Ilosha, gave birth to two cubs in July 2015. The second pair consisted of one female and one male, Lin and Maituc. At the time of the study, this pair had no offspring, and the female Lin was contracepted with a Suprelorin implant to prevent pregnancy. The two red panda pairs were housed in two different enclosures. The enclosure of Ilosha and Ny'ma consisted of a 260 m² outdoor area and a 34 m² indoor area; the enclosure of Lin and Maituc consisted of a 358 m² outdoor area and a 34 m² indoor area. Both outdoor areas contained tall and leafy trees, bushes, rocks, horizontal branches linking different trees together, a water pool, small wooden houses, and artificial nests in large tree trunks. Red pandas were fed with fresh bamboo and fruits once a day. Once a week, the diet was supplemented with fresh meat (quail). In both enclosures, fruit was chopped and provided in bowls (two bowls per enclosure, in feeding points placed on trees, at a height of approximately two meters). Feeding time was at 1 PM, whereas bamboo was provided in the late afternoon (5 PM). Both pairs were involved in an environmental enrichment program and were provided with different kinds of stimulation daily (e.g., food-related enrichments such as hanging fruits, sensory enrichment such as cloth with spices or scents). Water was always available. No human–animal interactions were permitted.

2.2. Procedure and Data Collection

A continuous focal animal sampling method was used to collect durations of individual and social behaviour [22]. We collected data only on adult red pandas of the pairs; Ilosha's cubs were not considered to be focal subjects as they were three months old at the time of the study. To minimize human disturbance to the pandas, the observer collected the data on animal behaviour from the visitor path, after a habituation period of ten days. From the visitors' path, the observer could see the enclosure of both pairs from above and was able to follow the focal animal in almost the whole area. Before the beginning of the study, we planned a preliminary period in which the observer learnt to identify the red pandas through features such as the face mask, body size, hair, and tail characteristics. Red pandas were out of sight only when they were in their dens or in the indoor areas or when they were deeply inside the trees' canopy. Information on enclosure use was also recorded, focusing on whether the red pandas were on the ground, or if they were using elevated areas of the enclosure, such as trees and branches. The ethogram of the study is reported in Tables 1 and 2 (maternal behaviours) and was prepared based on preliminary observation of the subjects and on previous studies of the red panda [14–16,23–27]. For each subject, forty-eight 30 min sessions were carried out, for a total of 1440 min per subject

(96 sessions, 2880 min per pair). Per subject, two sessions per day were done, one in the morning (9.30 AM–12.00 PM) and one in the afternoon (2.00 PM–4.30 PM). Data on all subjects were collected by the same observer (M.A.) over a period of approximately two months. The observer started to collect data in a different enclosure every day, so that subjects of each enclosure were observed uniformly over the study period. Moreover, the order in which each subject was observed was counterbalanced over sessions to ensure that observation of each individual was uniformly distributed over the whole observation time.

Table 1. Ethogram of red pandas of the study. Behaviours of the red pandas have been grouped based on their function in five different classes (Behavioural classes) [14,16,23–27].

Behavioural Class	Behavioural Category	Definition
Exploratory/territorial b. (Expl/terr)	Digging	Digging in ground.
	Human-directed b.	Observing visitors, zookeepers or other humans, following them with attention.
	Licking	Olfactory investigation by licking any object (e.g., branches, leaves, enclosure furnishing) or substrate in the enclosure.
	Individual-play/Manipulation	Tactile investigation of objects in the enclosure, biting or chewing objects, interaction with environmental enrichment devices, carrying objects while moving or rolling on the back.
	Interspecific b.	Watching non-conspecifics, following them with attention (e.g., muntjac, bird).
	Scent-marking	Rubbing genitals on ground, objects, or enclosure furnishing.
	Sniffing	Sniffing any object (e.g., branches, leaves, enclosure furnishing) or substrate in the enclosure.
Locomotive b.	Arboreal locomotion	Climbing on trees, walking, or running on tree branches or in the canopy.
	Ground locomotion Standing	Walking or running on the ground. Standing on back two paws.
Routine b.	Hunt/stalk	Hunting, harassing, following a non-conspecific (e.g., birds).
	Individual resting	Lying sleeping, curled in ball, or flat out.
	Comfort	Self-cleaning of the fur, scratching, and stretching.
	Vigilance	Being watchful, alert, while observing the surroundings. Lying, sitting, or standing with head up and eyes open, head or ears moving.
Consumption b.	Eating	Eating food from the bowls (fruit and vegetables) or eating bamboo from the bamboo feeding point.
	Foraging	Looking for food in the enclosure, searching in the grass, digging on the ground, or eating when browsing.
	Maintenance	Drinking at the water pool of the enclosure; urinating or defecating.

Table 1. *Cont.*

Behavioural Class	Behavioural Category	Definition
Social b.	Aggression	Hitting a conspecific with paws, biting a conspecific. Initiating or receiving an aggression.
	Chasing/display	Agonistic behaviours without physical contact: chase, threat (arching the back and the tail, moving the head up and down) or displacing a conspecific. Receiving an agonistic behaviour without physical contact.
	Grooming	Cleaning the fur by licking a conspecific. Grooming could be mutual, received, or done actively.
	Observing conspecific	Watching a conspecific or being watched by a conspecific.
	Sexual behaviour	Courtship (the female moves, marking heavily while the male follows her, marking over her marks) and mating behaviour.
	Sniffing conspecific	Sniffing a conspecific or being sniffed by a conspecific.
	Social play	Playing with another individual by lunging, wrestling, biting softly.
	Social resting	Resting in contact with a conspecific.
Out of sight	Not observed	The individual is not visible.
Abnormal b.	Abnormal b.	Behaviours such as purposeless locomotion, repetitive route in the enclosure, excessive mouth movements (e.g., tongue flicking).

Table 2. Ethogram of maternal behaviours performed by the female Ilosha [Adapted from 16].

Behavioural Category	Definition
Antagonistic b.	The mother displaces, hits, or bites the cub.
Den	The mother and the cubs are not visible, hiding in the artificial nest.
Grooming cubs	Grooming of the cubs.
Nest building	Building a nest with twigs and grass.
Observing cubs	The mother observes and monitor the cubs.
Play with cubs	The mother plays with the cubs by lunging, wrestling, biting softly.
Rest and sleep with cubs	Lying, sleeping with the cubs.
Transport	The mother carries the cubs in the mouth while moving in the enclosure.

2.3. The Behavioural Variety Index (BVI)

To investigate the behavioural variety in the red pandas of the study, we used the method described by Spiezio and colleagues (2018) [7]. After having examined the existing literature, we prepared a list of species-specific natural behaviours (belonging to the wild behavioural repertoire of the species) collected by previous researchers on the red pandas that included exhaustive ethograms of the species [14–16,23–27]. The list prepared for the current study included 24 behaviours that were grouped into 5 classes according to behavioural function (Table 3). Five specific indices were assigned to the resulting classes: consumption (CO; score: 0–3), exploratory/territorial (EXPL/TERR; score: 0–5), locomotive (LOC; score: 0–3), routine (R; score: 0–4), and social (S; score: 0–8). To investigate the presence of species-specific behaviours related to parental care toward the offspring, a

separate class for maternal behaviour (MAT) was prepared. All the indices mentioned above were used to create the Behavioural Variety Index (BVI). When calculating the indices for the red pandas, firstly, each item in Table 3 was scored as 0 or 1, with 1 representing the presence of the behaviour. Then, each index was calculated as the sum of the behavioural items performed by each subject and the indices' score ranged from 0 to the total number of behavioural items found for each class. Each index was calculated for each individual. A BVI was calculated for each subject and resulted from the sum of the five indices. The BVI is calculated based on behaviours recorded in the whole study period, over all sessions.

Table 3. Behaviours of the red panda described in previous research. Behaviours have been grouped in five main groups and the number of behaviours per group were used to develop indices of the red panda behavioural variety. The possible score for each group is calculated starting from the number of behaviours that have been previously collected in the red panda [14,16,23–27].

Consumption (CO)	Exploratory/Territorial (EXPL/TERR)	Locomotive (LOC)	Routine b. (R)	Social (S)	Maternal b. (MAT)
Eating	Digging	Arboreal locomotion	Hunting/stalking	Aggression	Antagonistic b.
Foraging	Licking	Ground locomotion	Resting	Chasing/displacing	Den
Maintenance	Individual play/manipulation/carrying	Standing bipedal	Comfort	Grooming	Grooming cubs
	Scent-marking		Vigilance	Observe consp	Nest building
	Sniffing			Sexual b.	Observe cubs
	Interspecific b.			Sniffing consp	Play with cubs
				Social play	Rest and sleep with cubs
				Social resting	Transport
Score	0–3	0–6	0–4	0–8	0–8

2.4. Data Analysis

In the current study, for each red panda, we collected durations (minutes) of different behavioural categories to obtain time budgets, expressed as percentage of time that an animal spends performing different behaviours [28]. As the sample of the study was small, we used descriptive statistics [29], with a single-case research approach [30,31]. For each session, we calculated the total duration of time spent by each subject performing each behavioural class and category. We grouped data collected for each individual per session to obtain the duration of behaviours per pair per session. Both enclosure use (ground vs. arboreal space) and behavioural time budgets were considered to obtain quantitative information on the time spent performing different activities. We used non-parametric statistical tests (Wilcoxon signed-rank tests) to compare behavioural classes between morning and afternoon sessions for each red panda. In the case of the BVI, we collected occurrences (presence or absence) of different behavioural items and used them to assess behavioural variety, a qualitative measure of the behavioural repertoire (the higher the score, the higher the behavioural variety).

3. Results

3.1. Lin and Maituc Pair

On average, the pair without offspring, Lin and Maituc, spent 81.2% of the observation time on elevated areas of the enclosure and 4.2% of the time on the ground, whereas subjects were not visible to the observer (it was not possible to know whether the pandas were hiding in the canopy or in shelters/dens on the ground) for the remaining time.

Regarding activity levels, Lin and Maituc spent, on average, 42.1% of the observation time being inactive (individual and social resting), 40.3% being active, and 17.6% being not visible to the observer. The most performed class of behaviours was routine behaviours (total duration: 1462.4 min), followed by “not observed” (506.3 min), social behaviours (462.4 min), locomotive behaviours (273.9 min), exploratory/territorial behaviours

(112.9 min), and consumption behaviours (62.1 min) (Figure 1). Median (IQR) durations of each behavioural category performed by each red panda are reported in Table 4.

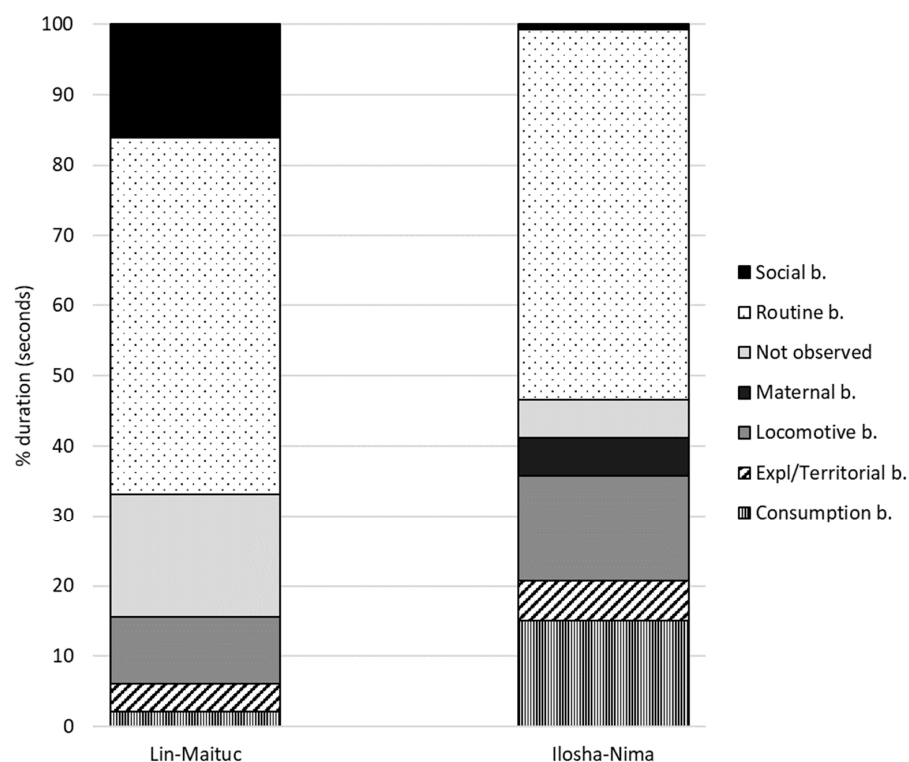


Figure 1. Percentage time spent by the two red pandas' pairs performing different classes of behaviour. For definition of abbreviations and classes see Tables 1 and 2.

Table 4. Median (IQR) duration of behaviours performed by each red panda. Per subject, the table reports the median (interquartile range—IQR) duration of different behavioural categories calculated across session.

		Ilosa	Ny'ma	Lin	Maituc
CONSUMPTION	Eating	568.5 (1161.3)	0 (411.3)	0 (278.5)	0 (7.5)
	Foraging	0 (86.5)	0 (0)	0 (0)	0 (0)
	Maintenance	0 (13)	0 (16.3)	0 (0)	0 (0)
EXPL/TERR	Human-dir behav.	18 (44.3)	49 (117.3)	40.5 (103.8)	32 (91)
	Ind play/manip	0 (0)	0 (0)	0 (0)	0 (0)
	Interspecific b.	0 (0)	0 (2.3)	11.5 (37.3)	0 (13)
	Scent-marking	0 (8.3)	1.5 (38.3)	0 (11.5)	15.5 (76.8)
	Sniff/dig/lick	34.5 (232.5)	0 (14.8)	0 (43.8)	0 (14)
LOCOMOTIVE	Arboreal loc	115 (216.5)	320 (684.8)	184.5 (330)	278.5 (442.3)
	Ground loc	95 (109.5)	81 (525.5)	0 (13.8)	0 (278.8)
	Standing	0 (0)	0 (0)	0 (0)	0 (0)
ROUTINE B.	Comfort	21.5 (226.3)	136.5 (490)	359 (789.8)	253 (717.8)
	Resting ind	811.5 (1783)	1755 (1508.8)	258.5 (1637.3)	1222.5 (2071.5)
	Vigilance	96.5 (270)	191 (253)	197.5 (292.3)	242.5 (440.3)
SOCIAL B.	Aggression	0 (0)	0 (0)	0 (0)	0 (0)
	Chase/displace	0 (0)	0 (0)	0 (0)	0 (0)
	Grooming	0 (0)	0 (0)	0 (0)	0 (0)
	Obs consp	0 (2.3)	19.5 (64.8)	0 (33)	7.5 (35)
	Sexual b.	0 (0)	0 (0)	0 (0)	0 (0)
	Sniffing consp	0 (0)	0 (0)	0 (0)	0 (10.8)
	Social resting	0 (0)	0 (0)	0 (1643.5)	0 (89.3)
OoS	Not observed	73 (350.5)	0 (19)	130.5 (616.5)	36 (631.5)

3.2. Ilosha and Ny'ma Pair

On average, the pair with offspring, Ilosha and Ny'ma, spent 81.7% of the observation time on trees and 15.5% of the time on the ground, whereas they were not visible to the observer (it was not possible to know whether the pandas were hiding in the canopy or in shelters/dens on the ground) for the remaining time.

Regarding activity levels, Ilosha and Ny'ma spent on average 41.2% of the observation time being inactive (individual resting and resting with cubs), 53.4% being active, and 5.4% being not visible to the observer (not observed). The most performed class of behaviours was routine behaviours (total duration: 1516.7 min), followed by locomotive behaviours (434.8 min), consumption behaviours (433.2 min), exploratory/territorial behaviours (162 min), "not observed" (155.6 min), maternal behaviours (155.3 min), and social behaviours (22.4 min) (Figure 1). Median (IQR) durations across sessions of each behavioural category performed by each red panda are reported in Table 4.

3.3. Morning vs. Afternoon Sessions

For Ilosha and Ny'ma, we found no significant differences in duration of behavioural categories performed in the morning and in the afternoon (see Table 5 for median, IQR, and statistical values of Wilcoxon test).

Table 5. Median (IQR) duration of behavioural classes performed by each red panda in the morning and in the afternoon. Per subject, the table reports median and interquartile range (IQR) duration of different behavioural classes (OOS: Out of Sight), calculated across morning (AM) and afternoon sessions (PM) in each study subject. Below, medians and IQR in the table report *V* and *p* values of the Wilcoxon tests between morning and afternoon sessions.

		CO	EXPL/TERR	LOC	OOS	R	S	MAT
Ilosha	AM	100.5 (705)	1 (83.8)	68.5 (255.8)	0 (46.3)	158 (1442.3)	0 (10.5)	24 (498.8)
	PM	176 (1053.8)	31 (113)	104 (232.8)	45 (152.5)	676.5 (1557)	0 (10)	0 (19.5)
		<i>V</i> = 96; <i>p</i> = 0.322	<i>V</i> = 87; <i>p</i> = 0.948	<i>V</i> = 84; <i>p</i> = 0.274	<i>V</i> = 64.5; <i>p</i> = 0.570	<i>V</i> = 88; <i>p</i> = 0.339	<i>V</i> = 40; <i>p</i> = 0.937	<i>V</i> = 102; <i>p</i> = 0.079
Ny'ma	AM	0 (195.3)	36 (144.5)	191.5 (1131)	0 (0)	1301.5 (1579.5)	0 (21.5)	
	PM	0 (139.5)	40.5 (85.8)	75 (285)	0 (0)	1471.5 (819.5)	5.5 (27)	
		<i>V</i> = 55; <i>p</i> = 0.776	<i>V</i> = 126; <i>p</i> = 0.715	<i>V</i> = 183.5; <i>p</i> = 0.064	<i>V</i> = 13.5; <i>p</i> = 0.933	<i>V</i> = 83; <i>p</i> = 0.158	<i>V</i> = 66.5; <i>p</i> = 0.636	
Lin	AM	0 (0)	8.5 (106)	9 (194.8)	0 (351.8)	524.5 (1433.3)	28.5 (1652)	
	PM	0 (258.3)	30 (146.8)	22.5 (236)	0 (192.8)	767.5 (1232.8)	0 (388.8)	
		<i>V</i> = 12; <i>p</i> = 0.062	<i>V</i> = 88.5; <i>p</i> = 0.538	<i>V</i> = 103; <i>p</i> = 0.940	<i>V</i> = 38; <i>p</i> = 0.657	<i>V</i> = 88; <i>p</i> = 0.211	<i>V</i> = 80; <i>p</i> = 0.084	
Maituc	AM	0 (0)	13.5 (241)	6 (581.8)	27.5 (412)	437.5 (1170)	10.5 (226.8)	
	PM	0 (0)	0 (41.8)	0 (219.5)	0 (0)	1662 (1085.5)	0 (43.3)	
		<i>V</i> = 10; <i>p</i> = 0.499	<i>V</i> = 136; <i>p</i> = 0.028 *	<i>V</i> = 107; <i>p</i> = 0.044 *	<i>V</i> = 59; <i>p</i> = 0.117	<i>V</i> = 30; <i>p</i> = 0.005 *	<i>V</i> = 92; <i>p</i> = 0.069	

* Significant difference between morning and afternoon.

For Lin and Maituc, we found no significant differences for the female Lin. The male Maituc performed significantly more exploratory/territorial and locomotory behaviours in the morning than in the afternoon, whereas the opposite pattern was found for routine behaviours (see Table 5 for median, IQR, and statistical values of Wilcoxon test).

3.4. Behavioural Variety

The distribution of the behavioural items reported in red pandas of the study and the five corresponding indices are presented in Figure 2. For consumption behaviours (*C* = 3) and exploratory/territorial behaviours (*EXPL/TERR* = 6), all items were performed by the subjects, with 25 and 75% of the pandas having a score of 3 and 4, respectively. For locomotive behaviours, 50% of the subjects had a score of 2 (*L* = 2) and 50% had a

score of 3, performing all the behavioural items of the class ($L = 3$) (Figure 2). For routine behaviours ($R = 5$), all behavioural items apart from hunting/stalking were found. All subjects performed four items and had a score of 4. For social behaviours, all behavioural items apart from social play were performed by the red pandas, with 50% of the subjects performing 5 behavioural items and 50% performing 6 of them. In summary, the BVI scores revealed that all pandas had a score ≥ 17 and the mean BVI was 17.5 ± 0.6 . In particular, two subjects (the females, Lin and Ilosa) had a BVI of 17 and two subjects (the males, Ny'ma and Maituc) had a BVI of 18.

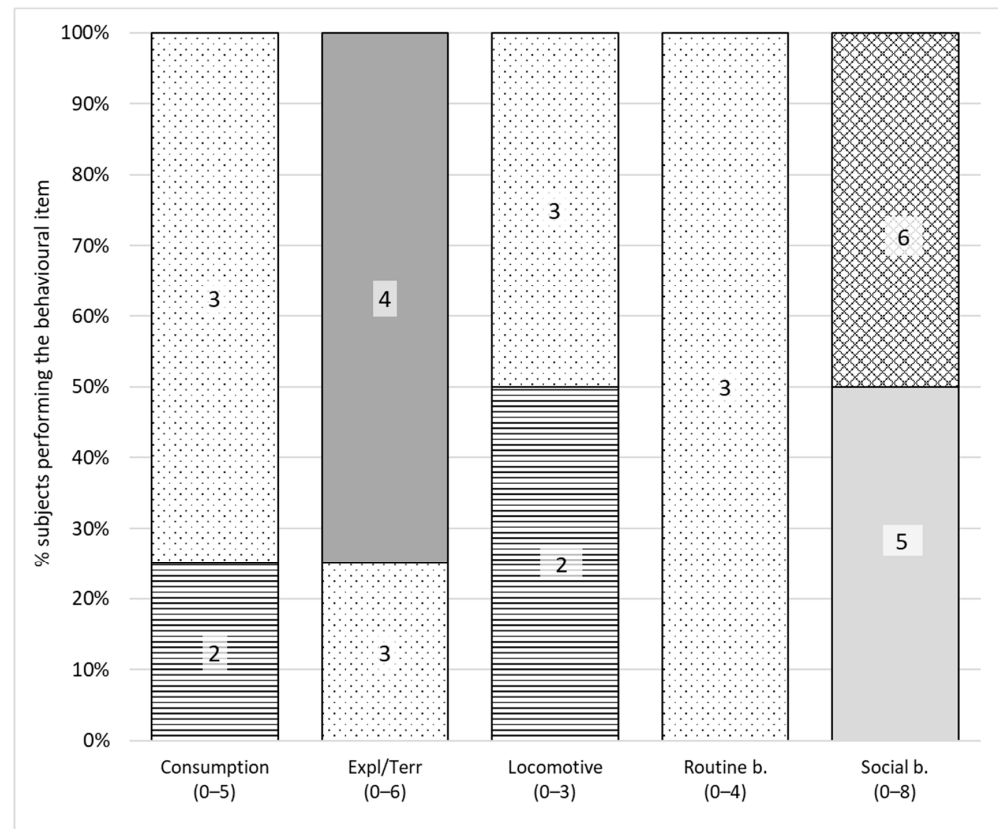


Figure 2. Distribution of scores for the five behavioural indices. Different bar boxes report the number of behavioural items that have been performed per class of behaviours (consumption behaviours, expl/terr: exploratory/territorial behaviours, locomotive behaviours, routine behaviours, social behaviours). Below each bar is reported the name of the class and the score (number of items per class). The y-axis indicates the percentage of subjects that performed the number of behavioural items reported within boxes (and thus had the same BVI score).

3.5. Maternal Behaviour

We investigated the interaction of the female Ilosa with her offspring, describing the average time spent performing different parental care behaviours (Figure 3). The most performed category was “den”, meaning that the female was in the nest box with the cubs (91.2 min, 3.2% of the observation time, median [IQR]: 0 [175.3]). “Den” was followed by rest and sleep with the cubs (26.3 min, 0.9%, median [IQR]: 0 [0]), grooming (23 min, 0.8%, median [IQR]: 0 [33.8]), observe cubs (8.8 min, 0.3%, median [IQR]: 12.5 [38.5]), antagonistic behaviours (3.7 min, 0.1%, median [IQR]: 0 [0]), transport (1.8 min, 0.1%, median [IQR]: 0 [0]), and social play (0.5 min, $\approx 0\%$, median [IQR]: 0 [0]). Based on the BVI analysis, all the behavioural items described for maternal behaviours (Table 2, Table 3) have been reported in the female except for nest building ($MAT = 7$).

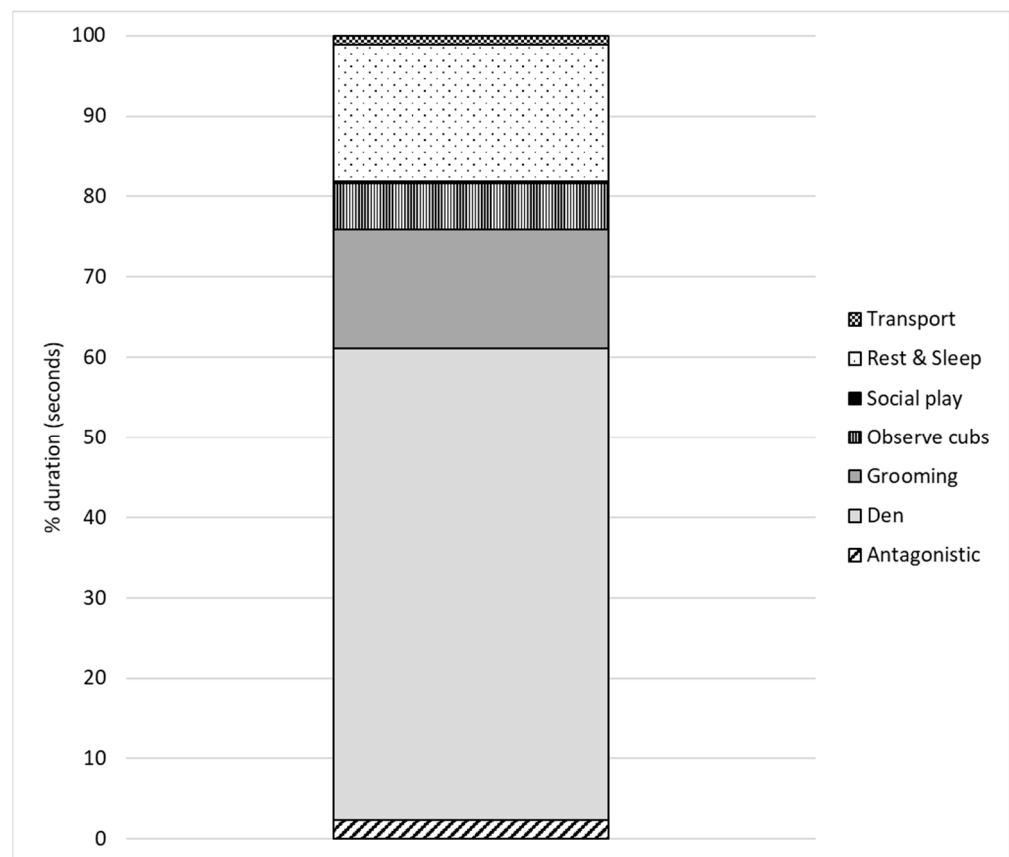


Figure 3. Percentage duration of time spent by Ilosha performing different maternal behaviours.

4. Discussion

First, we observed no abnormal behaviour in any of the pandas of this study. On average, the red pandas' activity time ranged from 40.3% (Lin and Maituc) to 53.4% (Ilosha and Ny'ma) of the total observation time. On the other hand, inactivity was approximately 40% in both pairs (41.2 and 42.1%). These findings agree with previous studies focusing on time budgets of red pandas in zoos, with inactivity ranging between approximately 40 and 50% [26], suggesting that zoo-housed red pandas show daily activity patterns [32]. In the wild, red pandas have been found to be active for 45 to 60% of the day, depending on temperature and food availability, with conspicuous periods of inactivity and long rests, especially in winter. As data collection took place in the late autumn when temperature started to become low, these results seem to agree with data collected in wild red pandas [14,15], suggesting good activity levels of the subjects, together with factors such as temperature and climate, daily husbandry, enclosure design, and environmental enrichment program which appeared to also have influenced the activity level of red pandas of this study.

Red pandas in the wild are arboreal, and in a controlled environment, they have been found to prefer the vertical space, off the ground [14,16,23,33]. The red pandas of this study spent, on average, approximately 80% of the time on elevated areas of the enclosure, specifically on trees, as reported for their wild counterparts. However, Ilosha and Ny'ma spent more time on the ground than Lin and Maituc (approximately 15 and 4%, respectively). This could be due to the presence of the offspring, leading to an increased use of the ground to look for food and to control the territory, especially in the female Ilosha. Indeed, energetic costs of lactation have been found to be particularly high in red pandas: during the lactation period, females might increase food consumption up to 200% above the rate observed during non-lactation [25,34]. This could also partially explain the higher amount of time spent by the pair with Ilosha on consumption behaviour (15%

vs. 2% reported for Lin and Maituc), exploratory/territorial behaviours (6% vs. 4%), and locomotive behaviour (15% vs. 9%), especially ground locomotion and use of the enclosure ground. In particular, the pair with offspring performed more “eating” and “foraging” than the other pair, and these behaviours were frequently performed on the ground rather than on trees.

In both pairs of the study, the most performed behavioural category was routine behaviour (more than 50% of the observation time), including resting, comfort behaviours (grooming, scratching, and stretching), and vigilance, intended as watchful observation of surroundings. This finding is in line with previous study of this species in zoos [11] and in the wild, as the red panda spends several hours resting, as mentioned above, and comfort behaviours are well-represented and may take up to 16% of daily activity [16]. Self-grooming is particularly relevant in this species and takes place mainly after awakening or eating [16,25,35]. When grooming themselves, red pandas spend a lot of time licking their body and limbs, washing their muzzles, stretching, or rubbing their back [16,25,35]. The second most performed behavioural classes were “not observed” and social behaviours for Lin and Maituc (the pair without offspring); consumption and locomotive behaviour for Ilossha and Ny’ma. In the latter pair, these behaviours might be common due to offspring presence, as previously described. In the case of Lin and Maituc, they spent more time in the canopy and in the nest boxes, and were not visible, presumably due to the enclosure design and to different needs in the absence of offspring. The behavioural class “not observed” is particularly relevant in zoos as animals in zoological institutions must have the opportunity to hide or escape from stressors or negative stimuli, such as the presence of visitors [5]. Moreover, they performed more social behaviour, as Lin and Maituc spent time observing each other as well as interacting (grooming, sniffing) and sleeping (social resting) together.

We compared activity budgets of each red panda between morning and afternoon sessions. Overall, we found no significant differences in durations of behavioural classes in different parts of the day, even if the male Maituc performed more exploratory/territorial behaviours and locomotion in the morning than in the afternoon, whereas routine behaviours such as resting and comfort were shown more in the afternoon. This finding agrees with previous literature on wild red pandas, with peaks of activity in the morning (700–1000h) and in the evening (1700–1800h) [27].

To assess the welfare of red pandas, we investigated behavioural variety of the two pairs using the BVI. This tool allows to compare the behavioural repertoire of our subjects with that reported in the species, both in the wild and in controlled environment. Based on our results, the mean BVI was 17.5, meaning that red pandas performed 73% of the behavioural items described in the species. In particular, males of the two pairs performed 71% of the behavioural items whereas females performed 67% of all items. Based on previous literature on red pandas, males scent-mark and patrol their territory more than females [12,13].

In general, subjects of the study performed all behavioural items described in previous literature except for hunt/stalk (routine behaviour) and social play (social behaviour). Regarding hunt/stalk, red pandas are housed in a naturalistic enclosure, and they would have the possibility to prey on small reptiles or even small vertebrates. Yet, red pandas of the study are fed daily with bamboo and fruits and are also regularly provided with meat (quails). Therefore, it is possible that Ilossha, Ny’ma, Lin, and Maituc did not perform hunting and stalking in the data collection period as they did not need to rely on predation. However, red pandas of this study watched carefully and showed interest for birds (e.g., parakeets, corvids) and individuals of other species in their enclosure (interspecific behaviour) and were not indifferent to their presence as well as to the presence of humans, specifically zookeepers (human-directed behaviour). Regarding social play, even in the wild, this behaviour is common among cubs and juvenile subjects or between mothers and offspring, whereas in adults, it can be performed by males and females during courtship and mating seasons [16]. This may be the reason for failing to report this behaviour in our study. However, Lin and Maituc, the pair without offspring, performed some sexual

behaviour, consisting in the male following the female and marking over her scent tracks as well as other social behaviours such as allo-grooming, social resting, and sniffing each other [16]. The percentage of social behaviours showed by Lin and Maituc seems to suggest that housing solitary species in pairs or small groups might promote the performance of a wider array of behaviours, such as affiliative behaviours of Lin and Maituc [5,36]. Positive effects of social housing on solitary species were reported in snow leopards, tigers, and lowland tapirs, showing that housing these animals in pairs or small groups might promote exploratory behaviour and/or reduce the performance of abnormal behaviour [36–40].

Red pandas of the study performed almost all behavioural items in the class exploratory/territorial behaviour, especially scent-marking. These behaviours are typical of the species and represent important indicators of behavioural variability as well as appropriate intraspecific and interspecific communication [12,41]. In addition, these behaviours have been considered as positive welfare indicators in different species, including the red panda, as their presence highlights good health and imply that several needs of the animals are met [10,11]. Measures of variability such as behavioural diversity [42] can be useful to assess overall welfare through the analysis of behavioural changes, allowing to implement positive welfare changes deriving from training, enrichment, or other husbandry practice [28,42,43].

Regarding maternal behaviour, we focused on the interaction of Ilosha with her cubs. Based on our results, the most performed behavioural category was “den” (59% of the observation time), intended as the mother being in the nest box with cubs. Even in the wild, early maternal care takes place mainly in the den and cubs start to leave the den around two months of age. At this stage, they spend approximately four hours a day in the den, although this period can vary based on weather, external temperature, and predator/human disturbance [16]. The second most performed behavioural category was “resting and sleeping” (17%). The mother with her cubs has been found to sleep together in contact, until new-born red pandas are eleven months old [16]. The third most performed behaviour was grooming, intended as the mother licking her cubs. This is one of the most common maternal care behaviours during denning period as the female cleans cubs’ fur and stimulates urination and defecation by licking the ano-genital region of her offspring [16]. The female Ilosha paid particular attention to her offspring, observing cubs for 6% of the observation time, and intervening in the case of threats or cubs vocalizing to ask for consideration. Other behavioural categories such as antagonistic behaviours, cub transport, and social play were performed less frequently (<2% of the observation time), presumably due the age of cubs. Indeed, in the current study, the three-month-old cubs left the den, moved autonomously in the enclosure, and played with each other, with decreased need to be transported, and reduced direct interaction with their mother [16]. Regarding the behavioural variety in maternal behaviour, Ilosha performed all behavioural items described previously as maternal behaviours of red pandas except for nest building. Usually, female red pandas start building the nest several weeks before parturition and continue through the denning period [16]. However, cubs of Ilosha were autonomous and started to spend more time out of the nest, therefore, it needed less or no upkeep, explaining the lack of nest-building activity reported in the current study. Based on our results, the female Ilosha showed almost all maternal behaviours typical of the species, suggesting good reproductive skills and competent parental care behaviour, which need to be preserved in the ex-situ population. Ilosha successfully raised other offspring in years preceding the current study. Thus, behavioural variety and competence reported in Ilosha seems to suggest a positive welfare of that female, leading to good reproductive success and cubs’ survival.

We acknowledge that the current study has limitations since we only observed four red pandas during daytime and in only one season of the year. However, our study provides further information on the behaviour of red pandas in zoos, especially maternal behaviour, and tests the validity of the BVI to measure behavioural variety in animals under human care. Variability measures such as behavioural diversity and enclosure use variability [42]

can be useful to assess welfare as they allow within-subject comparisons of behavioural changes. Thus, future studies could use the BVI as a measure of behavioural variation aside from traditional ethograms, allowing to implement positive welfare changes deriving from training, enrichment, or other husbandry practice [28,42,43].

5. Conclusions

In conclusion, findings of this study highlighted that the red pandas showed no abnormal behaviours, whereas we found different positive behaviours that have been described both in zoological gardens and in the wild. These behaviours need to be maintained in ex situ contexts, to obtain physically and psychologically healthy subjects as well as viable populations. Studies like this are important to improve the knowledge on endangered species biology and needs, enhancing their husbandry standards (e.g., keeping pairs or single animals/stopping reproduction) and breeding success as well as the in-situ conservation efforts. Finally, monitoring the behaviours of pairs housed in different conditions (e.g., female with offspring vs. female with contraceptive implant) might be a valuable tool to make informed decisions about husbandry and management of animals under human care, such as breeding control and social housing of solitary species.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: Caterina Spiezio is employed by Parco Natura Viva as head of the Research and Conservation Department. The category of potential conflict of interest is "Employment". Barbara Regaiolli is employed by Parco Natura Viva as researcher in the Research and Conservation Department. The category of potential conflict of interest is "Employment". All these authors have disclosed those interests fully to the journal, and they have in place an approved plan for managing any potential conflicts arising from the involvement. Janne Weerman and Mariangela Altamura have no conflict of interest.

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