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Behavioural Analysis Of Captive Tigers (*Panthera tigris*): A Water Pool Makes The Difference.

Biolatti Cristina[a*], Modesto Paola[a], Dezzutto Daniela[b], Francesca Pera[d1], Tarantola Martina[d], Gennero Maria Silvia[b], Maurella Cristiana[c], Acutis Pier Luigi[a].

[a]Regional Center for Exotic Animals, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Turin, ITALY; cristina.biolatti@izsto.it, paola.modesto@izsto.it, pierluigi.acutis@izsto.it;

[b]Animal Welfare, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Turin, ITALY; daniela.dezzutto@izsto.it, mariasilvia.gennero@izsto.it;

[c]BEAR, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Via Bologna 148, 10154 Turin, ITALY; cristiana.maurella@izsto.it

[d]Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), ITALY; frap.pera@gmail.com, martina.tarantola@unito.it;

*Corresponding author:

Cristina Biolatti

Regional Center for Exotic Animals

Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle D'Aosta

Via Bologna 148, 10154 Turin, ITALY

Tel. 0039 011 2686292-367; Fax. 0039 011 2686322;

cristina.biolatti@izsto.it

[1]Present address:

Prostock Vets Ltd. Glynhebog, Llysonnen Road, Carmarthen, SA33 5DX, United Kingdom

Abstract

The activity budgets of seven captive tigers (*Panthera tigris*) housed in four zoological gardens (A, B, C, D) were analysed to assess their welfare and to relate it to several variables, including enclosure type, management, and animal history. Behaviours were recorded by instantaneous focal animal sampling at 2-minute intervals. Data were collected by five observers using an ethogram listing 26 behaviours adapted from the literature. To process the data, the activity budgets of each tiger and the overall activity budget were constructed. On the basis of previous literature, some of the behaviours, listed in the ethogram, were labelled as indicators of diminished welfare and some were labelled as indicators of enhanced welfare. Statistical analysis was carried out to determine in which zoo the tigers were more prone to exhibit indicators of enhanced welfare and which feature(s) had a major effect on their welfare. Over 195 hours of data were collected and 5867 observations were recorded. The tigers in zoo A (OR = 4.11, 95% confidence interval [CI] 3.2-5.3) and zoo C (OR = 1.83, 95% CI 1.4-2.4) were more prone to express indicators of enhanced welfare with respect to Zoo D as the reference. Among the variables describing animal peculiarity, daily routine management, and enclosure features, the presence of a water pool with clean water was significantly associated with enhanced welfare (OR = 2.04, 95% CI 1.4-3.04). The data suggested that none of the tigers displayed consistent signs of stress and that all experienced a basic welfare status. An essential feature that helped to enhance good animal welfare was a water pool in the enclosure containing clean water.

Key words

Panthera tigris, zoo, enhanced welfare, activity budget, behavioural analysis.

1. Introduction

The tiger (*Panthera tigris*) is currently listed as endangered by the International Union for Conservation of Nature, due to poaching and a decline in its home range by over 50% during the last three generations (21-27 years) (http://www.iucnredlist.org/details/15955/0, last accessed on 13/10/2015). At present, the wild population count is approximately 3000 individuals, of which fewer than 2500 are mature and potentially of reproductive age (http://www.iucnredlist.org/details/15955/0, last accessed on 13/10/2015). Paradoxically, tigers are one of the most commonly exhibited species worldwide: they reproduce well in captivity (Brown, 2011), are tolerant to heat and cold (Shoemaker et al., 1997), and figure among the charismatic megafauna thanks to their great potential to attract zoo visitors (Brown, 2011; Skibins and Powell, 2013). Four out of six zoological gardens located in Piedmont (area 25,400 km^2 in northwest Italy) host tigers, although providing adequate welfare to these animals is extremely complicated because the captive environment is dramatically different from what the animals experience in the wild (Clubb and Mason, 2007). A good example is the home range: wild tigers tend to occupy extensive territories (ranging from 7 to 1000 km^2, with a reported median home range area of 48.40 km^2), which cannot reasonably be provided in captivity (Breton and Barrot, 2014; Clubb and Mason, 2007).

Pacing is the main and most frequent form of stereotypy that big cats develop when kept in zoos (Clubb and Mason, 2007). Because animals that typically occupy large home ranges in the wild tend to fare worse in captivity and are much more vulnerable to welfare problems (Clubb and Mason, 2007; Szokalski et al., 2012), research has been dedicated to finding the causes (Chosy et al., 2014; Lyons et al., 1997; Mohapatra et al., 2014) and how to prevent or reduce such behaviour (Bashaw et al., 2007; Breton and Barrot, 2014; Jenny and Schmid, 2002; Miller et al., 2008; Skibiel et al., 2007). During the last decade, many zoos have undertaken efforts to reduce abnormal behaviours by restructuring enclosure architecture and improving animal management.

Nonetheless, ensuring adequate welfare entails not only fulfilling essential needs and preventing the onset of stereotypies, but also encouraging animals to express their most complete behavioural repertoire, providing resources towards which animals are motivated only when more immediate deficiencies have been met (Hemsworth et al., 2015; Maple and Perdue, 2013; Yeates and Main, 2008). Promoting positive experiences is one way to give captive wild animals a better quality of life, safeguard and possibly improve their health status (Boissy et al., 2007; Maple and Perdue, 2013).

77 Such experiences may also benefit zoo visitors. As visitors become increasingly attuned to animal welfare,
78 they expect to see animals engaged in natural behaviours and can recognize the main stereotypies when
79 displayed. For example, people who watched a video of a tiger pacing perceived the animal as receiving
80 lower levels of care than visitors who viewed a tiger resting (Miller, 2012). In addition, the study participants
81 were less interested in supporting zoological facilities after seeing the video of a tiger pacing. In contrast,
82 visitor interest was closely related to activity in cat exhibits: it was found to be greater when the cats were
83 active (Margulis et al., 2003). Watching animals displaying positive behaviours may increase a visitor's
84 connection to wildlife and knowledge about a certain species, turning it into an educative experience that
85 fosters an interest in conservation. Education and conservation are among the main purposes of modern zoos,
86 as required by law in the European Union (EU Council Directive 1999/22/EC).
87 The aim of the present multi-zoo study was to assess the welfare of tigers housed in four different zoological
88 gardens located in Piedmont. Specific attention was paid to identify in which zoo tigers were more prone to
89 perform behaviours considered as indicators of enhanced welfare. Another goal was to detect features
90 (regarding enclosure type, management and animal history) statistically linked to the expression of indicators
91 of enhanced welfare. The main challenge was to identify features that could be easily implemented in zoo
92 settings, to be recommended to facilities already hosting or willing to host big cats.
93
94 **2. Material and methods**
95 *2.1 Animals, enclosures, management*
96 Four zoological gardens (A, B, C, D) were involved in the study. Zoos C and D are safari parks, where it is
97 possible to drive through the enclosure where tigers are displayed. All zoos are privately owned. The study
98 population was seven captive tigers (SIL, TOA, TOB, MPO, FPO, MMU, FMU), three males and four
99 females. All animals were captive born. Tiger signalment, host zoo, history, and medical history are reported
100 in Table 1. All were adult individuals (age range 4-17 years, mean 7.86 years, SD = 4.49) and all, but one
101 (SIL), were housed in pairs. Both members of the pairs were included in the study.
102 The tigers were on exhibit in their outdoor enclosures every day, from morning to late afternoon, with on-
103 exhibition time varying between zoos and seasons. All enclosures can be considered naturalistic. The areas
104 ranged from 700 to 10,000 m^2. A glass separated the tiger enclosure from the public point of view in Zoos A
105 and B partly or entirely, respectively. Trees, logs, elevated platforms, and water pools were the main

106 furniture. Though all enclosures had a water pool, the water level was low, dirty or muddy in some. Data on
107 the quality of shade, presence of areas visually inaccessible to visitors' sight, and presence/quality of
108 enclosure furniture were recorded (Table 2).
109 Table 3 presents the main characteristics of animal management: time that the animals can spend in the on-
110 exhibition area, diet and feeding routine, and presence/absence of enrichment plans. None of the zoos
111 participating the study had a routine enrichment plan (i.e., planned provision of different types of enrichment
112 to animals) in place.
113
114 *2.2 Ethological study*
115 An ethogram composed of 26 behaviour items (Table 4) was adapted from the literature (Bashaw et al.,
116 2007; De Rouck et al., 2005; Lyons et al., 1997; Miller and Kuhar, 2008). Five observers performed the
117 ethological study. To standardize recording of the behavioural patterns and to refine the ethogram, an
118 informal period of preliminary observation was conducted. It consisted of an, 8-hour session of *ad libitum*
119 sampling method (Martin and Bateson, 2007) carried out on each animal in the study, by all observers.
120 Behaviours were verbally described in the form of long-hand written notes. Moreover, different
121 measurement techniques, points of view and sampling intervals were tried out. Data obtained during the pilot
122 were discarded and not used for further analysis.
123 All five observers were involved in the main data collection. Before beginning the study, between-observer
124 reliability was assessed by having the observers simultaneously watching a 60-minute video recorded during
125 the pilot observation. Within-observer reliability was assessed by having them watching the same video (the
126 one utilized for the between-observer reliability evaluation) during two separate sessions carried out on two
127 different days. Behaviours were recorded by instantaneous focal animal sampling at 2-minute intervals
128 (Martin and Bateson, 2007), using a gridded checklist with the columns denoting the different behaviour
129 categories and the rows denoting successive time sample intervals. At the end of each sample interval, at the
130 signal of a stopwatch, the observer recorded the behaviour on the relative row of the checklist. The data were
131 then progressively transferred onto a computerized spreadsheet (Microsoft Excel 2003. Microsoft. Redmond,
132 WA, USA). The reliability of the observation method was assessed using Cohen's Kappa (Stata 13.0 SE®;
133 StataCorp, College Station, TX, USA).

Each zoo was visited five times from April 2012 to October 2012, and observations were taken from public-viewing areas when the animals were on exhibition. In zoos C and D observations were taken from a car inside the enclosures. Three visits were made on work days and two on holidays, with a maximum of six hours of observation. Each cat was observed for eight/twelve 30-minute sessions a day. The sessions began between 9.30 AM and 11.00 AM.

Behaviours were recorded as described for the assessment of within and between-reliabilities and were collected by all the five observers.

2.3 Data analysis

To process the data, the activity budgets of each tiger and the overall activity budget were constructed by calculating the percentage of time each behaviour was observed over the whole observation period. In some circumstances at the moment of the sampling, the observer was not able to see the animals because of a visual barrier (e.g. cars or a large group of people staying between the tiger and the observer, animal staying in spots that were blind for the observer only). These records were excluded form further analysis. On the other hand, the clear choice of the animals to hide in shelters or behind vegetation was recorded as "intentional out of sight" and considered for analysis.

On the basis of peer reviewed papers, published in the last 15 years, some of the behaviours, listed in the ethogram, were labelled as indicators of diminished welfare (pacing, aggression, flee, avoid) and some were labelled as indicators of enhanced welfare (self-grooming, immersion, affiliative behaviours, intentional out of sight, interaction with environment). Table 5 describes the rationale of our choice and the literature references. A behaviour that we could not consider for the further statistical analysis was "vocalize" (making a sound with the voice). Even though it has been suggested that vocalizations could be markers of both diminished and enhanced welfare (Boissy et al., 2007) and Miller and colleagues (2013) used some sounds as indicators of welfare, the observers involved in the present study could not be sure of every vocalization, since the used view points did not give the chance to hear all the sounds that tigers might have emitted.

Any behaviour expressed for less than 20 minutes of total time was excluded from further statistical analysis, because the inclusion in the model of those sparce data would have added rumors and casuality to the output.

A multinomial logistic regression model was then fitted in order to determine in which zoo the tigers were more prone to exhibit behaviours labelled as indicators of enhanced welfare. In order to identify which

aspects had a major effect on tiger welfare, animal peculiarity, daily routine management, and enclosure features potentially influencing the welfare status of captive tigers were listed, categorised, and analysed using a mixed-effects models for binary responses (Stata 13.0 SE®; StataCorp, College Station, TX, USA). The variables used in the models and their categories are listed in Table 6. To assess which of the enclosure features were associated with indicators of enhanced welfare, a multilevel mixed-effect logistic regression was fitted using the single individual as the grouping variable (Stata 13.0 SE®; StataCorp, College Station, TX, USA). All the outcomes of the dependent variables, given the random effect, have been treated as dummy variables, according to a Bernoulli distribution (Rabe-Hesketh and Skrondal, 2012). Zoo D was taken as the reference zoo, because it was the institution where the maximum number of observations was taken. This choice allowed the best precision of the estimates and the narrowest confidential interval.

3. Results

Before beginning the study, the observers reached a within-observer reliability in the range of 0.72 (95 % CI 0.54–0.9) to 1 (95 % CI 0.82-1) and a between-observer reliability of 0.95 (95 % CI 0.9-1). Over 195 hours of data were collected. Of the total of 5867 observations recorded, 809 were excluded from further analysis because the animal was not visible due to a visual barrier between observers and animals (i.e., cars, people) as described previously.

Overall and single tiger activity budgets are reported in Table 7. The main item on the activity budget was the time spent sleeping (32.64%, range 24.18 to 41.84%), followed by resting (27.5 %, range 9.47 to 47.51%), and walking (17.3 %, range 6.18 to 28.09%).

All the tigers displayed indicators of diminished welfare for 0.69% (range 0.13 to 1.99%) of the total observation, with pacing accounting for 0.43% (range 0.00 to 1.36%) of time. No episodes of aggression with physical contact between the tigers were observed. Indicators of enhanced welfare were observed in 11.74% (range 6.67 to 21.70%) of recordings. The percentage of time that the tigers spent in displaying behaviours considered as indicators of either diminished or enhanced welfare is reported in Table 7.

The behaviours excluded from further analysis because performed less than 20 minutes of total observation time were: avoid, run, body shake, flee, jump, stretch, aggression and scratch.

Table 8 presents the results of the multinomial logistic regression model. Behaviours labelled as indicators of enhanced welfare were more often expressed by the tigers hosted in zoo A (OR=4.11, 95% CI 3.2-5.3) and zoo C (OR=1.83, 95% CI 1.4-2.4) than those in zoos B and D.

Among the variables describing animal peculiarity, daily routine management and enclosure features, the presence of a water pool with clean water was significantly associated with the expression of indicators of enhanced welfare (OR=2.04, 95% CI 1.4-3.04).

4. Discussion

To assess welfare of captive tigers an observational study was carried out, being the least invasive and intrusive form of welfare assessment and because behaviours, as recognised indicators of the presence of pleasant and/or unpleasant feelings, can be employed as a means to assess both diminished and positive well-being (Dawkins, 2004; Szokalski et al., 2012). Behaviours can be considered as powerful welfare indicators because they reflect an animal's first attempts to cope with a stressor and so may indicate a situation where welfare is at risk at an early stage (Dawkins, 2004).

The multi-zoo approach was chosen in order to enlarge the sample size and properly weigh the variables analysed. As a matter of fact it is suggested to perform multi-zoo studies to tease out the effects of a number of variables and to find out about the prevalence of phenomena in which researchers are interested (Hosey et al., 2009). Few studies have been published on tigers' welfare so far (Breton and Barrot, 2014; De Rouck et al., 2005; Pitsko, 2003).

The present multi-zoo study may have some limitations: the study sample was small and some variable could not be inserted in the analysis either because records were not available (i.e. hand rearing/parental rearing) or because they were not present in the studied zoos (enrichment plans).

These limitations notwithstanding, the high number of observations (totalling more than 195 hours) provided a good basis for constructing the activity budgets, and a firm starting point for deriving a meaningful outcome from statistical analysis. Indeed, the analysis of the variables by means of a model adjusted for all parameters, so as to avoid introducing any confounding variables, allowed to obtain statistically significant results.

The obtained activity budgets showed that the observed tigers spent most of their time sleeping (32.64%) and resting (27.50%), as expected given that felids are often inactive (Karanth and Sunquist, 2000). The third

most frequently observed behaviour was walking (17.30%). Wild tigers, being wide-ranging species, travel a minimum of 4.86 km every day looking for food and controlling their territory (Clubb and Mason, 2007). In such territorial animals kept in captivity, walking different routes across an enclosure may mirror the animal's need to perform locomotory behaviours and to patrol their home range (De Rouck et al., 2005).

In all the studied zoos none of the tigers displayed consistent signs of poor welfare. Pace was observed for only 0.43% of the total observations. This datum is shared by only two previous studies. Observers of a group of six female tigers recorded percentages of pacing of 0.47% of all scans when housed at night in a group and of 0.61% when housed at night alone (Miller et al., 2013), and the three tigers involved in the study by Lyons and colleagues (1997) did not pace at all. Conversely, the majority of studies in this field reported higher values: between 4.67 and 23.91% in 15 animals hosted in nine European zoos (De Rouck et al., 2005), 16.43% in a review by Clubb and Mason (2007), nearly 60% in two tigers observed before a feeding enrichment experiment (Bashaw et al., 2003), and an average of 23.02 ± 14.27% recorded in 19 captive tigers in Nandakanan Zoological Park (India) (Mohapatra et al., 2014). The tigers we observed thus appeared to enjoy a basic welfare status, which may be the result of the efforts that the zoos made to reduce abnormal behaviours by adopting adequate enclosure architecture and animal management. Furthermore, the multinomial logistic regression model revealed that zoo A and zoo C were those facilities where animals were more prone to express behaviours considered as indicators of enhanced welfare. Accordingly, the statistical analysis focused mainly on identifying the variables that potentially enhance tiger welfare.

The results of the model showed that the presence of a water pool with clean water was the only variable significantly associated with indicators of enhanced welfare. Water pools are structural features that international guidelines recommend to be present in tiger enclosures (Backer, 2006; Shoemaker et al., 1997, Tilson et al., 1994). The presence of a water body has been already observed to increase exploratory behaviours and reduce stereotypic pacing (Pitsko, 2003). Moreover entering in a pool can elicit a natural behaviour, since wild tigers like water and can swim for kilometres (Bracke and Hopster, 2006; Mazak, 1981). In the present study, the presence of a water pool was classified as "present, with a low water level or dirty water" and "present, with clean water", because this difference in the management of the pools was noticed during the informal pilot observation session. It should be highlighted that it is not only the presence of a pool but the quality of the water that makes the difference in playing a key role in tiger welfare. As a matter of fact the tiger SIL, housed in zoo A, performed the behaviour "immersion" more consistently than

all the other animals (6.60% rather than less than 2%). The subjects MPO and FPO, housed in zoo C (a facility where tigers statistically performed more indicators of enhanced welfare and where the enclosure is provided with a pool with clean water) did not seem to make remarkable use of this feature, thus it can be inferred that the only presence of a water pool with clean water encourages tigers to perform behaviours considered as indicators of enhanced welfare. The statistical association, stated by the present study, is reliable because the multilevel mixed-effect logistic regression carried out has taken into account all the possible confounders and the results are not fortuitous. Furthermore, it is accurate, given the point and interval estimate.

Another result of the mixed-effect model was that enclosure size was not correlated with tigers' display of indicators of enhanced welfare. In their recent study, Breton and Barrot (2014) observed tigers in 14 enclosures ranging from 21.25 m^2 to 35,865 m^2, eight of them around or less than 1000 m^2, and quantified the influence of enclosure size on the distances covered and paced. One of their main results was that enclosure size was negatively linked with pacing. This finding is in agreement with those of Clubb and Mason (2007), but is in contrast with the results of Lyons and colleagues (1997) who observed that total size of enclosures was not a major factor in pacing activity. Breton and Barrot (2014) recommended to build or to modify existing enclosures to provide tigers with more than 1000 m^2. In the present study, the size of two out of four enclosures was smaller than 1000 m^2 (Zoo A: 850 m^2, Zoo B: 700 m^2) but the animals were observed to perform pacing in less than 0.5% of the total scans. On the other hand enclosure size resulted not linked with indicators of enhanced welfare. Prefacing that all the outdoor exhibits in the present study were naturalistic ones, it can be concluded that, with regard to enclosures that are not barren, quality, also in terms of complexity, of space is more important than its quantity, as supposed by Lyon and colleagues (1997) and stated by Maple and Perdue (2013).

5. Conclusion

The tigers observed in this study appeared to experience a basic welfare status. However, access to a water pool with abundant clean water substantially enhances their overall welfare. The costs and staff commitment for the installation and maintenance of this essential feature in already existing or newly planned zoos hosting tigers are surely outweighed by the benefits.

Conflict of interest

None

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362 **Tables**

363 Table 1: Animal signalment, known history, and hosting zoo. ♂: male; ♀: female.

Animal	Zoo	Subspecies	Sex	Age (years)	Origin	Medical history
SIL	A	Siberian x Bengal	♂	17	Circus	Exocrine pancreatic insufficiency, chronic arthritis
TOA	B	Siberian	♀	9	Born in place	Chronic lameness right forelimb
TOB	B	Siberian	♀	9	Born in place	
MPO	C	Siberian	♂	5	Born in place	
FPO	C	Siberian	♀	5	Born in place	
MMU	D	Siberian	♂	6	Born in place	
FMU	D	Siberian	♀	4	Born in place	

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Table 2: Enclosure features: quality of shade, presence of areas visually inaccessible to visitors, and presence/quality of enclosure furniture.

Zoo	On-exhibit area (m^2)	Quality of shadow	Elevated platform	Water pool	Log	Visually inaccessible area
A	850	Half shade	No	Present: clean water.	Yes	Yes
B	700	Full shade	No	Present: low water level or dirty water or filled with mud	Yes	Yes
C	10000	Half shade	Yes	Present: clean water.	No	No
D	6000	Half shade	Yes	Present: low water level or dirty water or filled with mud	Yes	Yes

370 Table 3: Tiger management: time that animals are allowed to spend in the on-exhibition area, diet and

371 feeding routine, presence/absence of enrichment plans.

Zoo	Time of on-exhibit stay	Diet and feeding days	Enrichment plans
A	From 10:00 AM to 6:00-7:00 PM	Beef meat (every evening)	No
B	Spring - Summer: from 6:30 AM to 7:00 PM Autumn - Winter: from 8:00 AM to 5:00 PM	Beef, chicken, rabbit meat (six days per week)	No
C	Spring - Summer: from 11:00 AM to 6:00 PM Autumn - Winter: from 11:00 AM to 5:00 PM	Beef meat (Wednesday, Saturday, Sunday)	Rare
D	Access to the on-exhibition area always possible	Beef and chicken meat (Wednesday, Thursday, Saturday, Sunday)	No

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374 Table 4: Ethogram: observed behaviours, in alphabetical order, and their description.

Observed behaviours	Description
Affiliative behaviours	Any non-aggressive social interactions with other animals, including allogrooming and play one another
Aggression	Striking with paws, biting, pouncing, charging, threatening other tiger or animal keeper
Avoid	Changing direction when moving towards to or being approached by another cat or person
Body shake	Moving the body in short, quick movements
Drink	Consumption of water
Eat	Eating, licking, or chewing food
Flee	Running away from a tiger, human or something else
Grass	Consumption of grass
Immersion	Entering a water pool with any part of the body other than the mouth
Interaction with environment	**Spray**: horizontal ejection of urine against vertical surface **Mark**: claws being drawn or cheeks being rubbed over any non-animal surface **Sniff**: taking air through the nose in repeated small sniffs **Flehemen**: grimacing facial expression with the tongue out of the mouth while drawing scent over the facial glands **Listen**: lifting up and turning ears in direction of a noise **Play**: any playful behaviour directed towards an object
Jump	Leaping with all four legs off the substrate from one point to another
Intentional out of sight	Animal, on purpose, is in an area which is visually inaccessible to observer (mainly staying in a shelter or behind vegetation).
Pacing	Walking on a distance back and forth, immediately after this distance has been paced once in both directions
Rest	Lying down or sitting with eyes opened, focused on object, animals or humans
Roll over	Animal on one side and completely rotates to the other side while lying down
Run	High speed forward locomotion
Scratch	Rubbing own skin with claws
Self-grooming	Licking and/or biting any part of own body (no physical indicators of overgrooming)
Sleep	Lying down with eyes closed
Stand	Animal is upright, supported on all four extended legs, but not in motion
Stalk	Slow walking movement, with all legs slightly bent, and eyes focused on a specific item
Stretch	Extended forelegs, depressed body from standing position
Urinate / Defecate	Send urine or faeces out of the body

Vocalize	Making a sound with the voice
Walk	A symmetrical gait in which each foot is on the ground more than half the time in a specific direction
Yawn	Fully extending then closing the jaw, with eyes closed

375 Table 5: rationale of the choice to suggest particular behaviours as indicators of diminished or enhanced welfare and the literature references.

Category	Behaviour	Rationale	References
Indicators of diminished welfare	Pacing	Pacing is the main stereotypy reported in tigers. It likely reflects present or past inadequate environment and is one of the most commonly used measures of compromised animal welfare, for tigers in particular. As any other stereotypy, pacing should always be taken seriously as a warning sign of potential suffering, but never used as the sole index of welfare.	Bashaw et al., 2003; Bashaw et al., 2007; Clubb and Mason, 2007; Maple and Perdue, 2013; Mason et al, 2007; Mason and Latham, 2004; Mellor, 2015a; Miller et al., 2013.
	Aggression	Aggression, not related to hunting behaviours, is expressed when animals appear frustrated, threatened or otherwise irritated and, probably, feeling anger. It is deemed as a long-term symptom of stress. We suggest it as an indicator of a social issue between members of the group or between animals and humans. Aggression, in all its meanings, has already been used to monitor interactions between female tigers forced to be housed together.	Bashaw et al., 2003; DEFRA, 2012; Hemsworth et al., 2015; Hosey et a., 2009; Mellor, 2012; Mellor, 2015b; Miller and Kuhar, 2008.
	Flee and Avoid	Both behaviours reflect a state of fear in response to aggressive threats or intimidating situations, especially if both intense and prolonged. Also aversive handling can elicit these behaviours. Moreover the freedom from fear is one of the "five freedoms".	DEFRA, 2012; Hemsworth et al., 2015; Hosey et al., 2009; Mellor, 2012.
Indicator of enhanced welfare	Self-grooming	Miller and colleagues (2013) suggested self-grooming as a potential enhanced welfare indicator in tigers. It has been proposed that it may be performed when social grooming is not possible, to reduce arousal. On the other hand, self-grooming may also occur as a displacement activity, i.e. with a relaxation effect, and overgrooming has been recognised as an abnormal form of behaviour in felids because it appears to be repetitive, non functional, and may lead to self-inflicted physical harm. The ethogram we used specified the behaviour item groom as "licking and/or biting any part of own body (no physical indicators of overgrooming)" so as to discriminate a normal action from a stereotypy.	Boissy et al., 2007; Miller et al., 2013.
	Immersion	In nature, wild tigers like to enter in water ponds. If in zoo settings they enter a water pool, they are expressing a natural behaviour. The combination of natural conditions and animal preferences are the reasons that lead some authors to address positive natural behaviours (pleasurable and promoting biological functioning) as a good indicator of enhanced welfare.	Bashaw et al., 2003; Bracke and Hopster, 2006; Maple and Perdue, 2013; Mazak, 1981.

	Affiliative behaviours	Affiliative behaviours represent positive interactions that initiate or strengthen the bonds between animals. Allo-grooming is though to play a major role in reinforcing social bonds and reducing tension in a group of animals. Moreover, the play behaviour is a sign that animals feel relatively safe or unstressed.	Boissy et al., 2007; DEFRA, 2012; Hemsworth et al., 2015; Mellor, 2012; Mellor, 2015a; Mellor, 2015b.
	Intentionally out of sight	Providing conditions that allow animals to hide or escape from potential dangers has been associated with lowered stereotypy. Indian leopard have been seen to occupy the central and back areas of their enclosure in high visitors-presence days. If animals have the opportunity to be out of sight intentionally and get this chance, it may be inferred that they exert control on their environment and make the choice to reduce the level of their own stress.	Clubb and Mason, 2007; Clubb and Vickery, 2006; Mallapur and Chellam, 2002.
	Interaction with environment	This group of behaviours comprises actions that tigers engage in order to obtain information from the environment (sniff, flehemen, listen) and leave their own signature on (spray and mark), other than playing with it. Exploring the territory provides animals with information on their home range that is vital both in wild and captive environment. It is a behaviour that most species of animals are motivated to perform, fulfilling a behavioural need. Moreover it helps animals to gradually approach to new objects and situations: explore the environment may give back information about ongoing changes, so it may be easy for animals to cope. Playing with objects available in the environment may have the same rationale than playing with another animal, thus being a sign that animals feel relatively safe or unstressed.	Boissy et al., 2007; DEFRA, 2012; Hemsworth et al., 2015; Mellor, 2012; Mellor, 2015a; Mellor, 2015b.

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382 Table 6: List and categories of variables that describe animal peculiarity, daily routine management, and

383 enclosure features analysed using a mixed-effects models for binary and binomial responses.

Variable	**Category**
Is there the possibility for the visitor to drive through the enclosure where animals are displayed?	No. Yes.
Day of observation	Weekday. Weekend/holiday (high visitor turnout)..
Weather conditions	Sunny. Cloudy. Drizzle. Heavy rain. Fog.
Temperature	5 – 9 °C. 10 - 14 °C. 15 – 19 °C. 20 – 24 °C. 25 – 29 °C. 30 – 34 °C. 35 – 39 °C.
Enclosure area	Less than 1000 m^2. More than 1000 m^2.
Amount of time the animal spend on exhibition	6 – 9 hours. 10 – 13 hours. Access to the on-exhibition area always possible.
Group	Single or couple. More than two tigers.
Shadow	Absent. Half shade. Full shade.
Water pool	Absent. Present: low water level or dirty water or filled with mud. Present: clean water.
Elevated platform	Absent. Present.
Log	Absent. Present.
Interaction with keepers	No interaction. Antagonistic interaction. Positive interaction.
Medical history	Absent. Chronic. Acute.
Observation time slots	10:00 AM – 11:59 AM. 12:00 AM – 1:59 PM. 2:00 PM – 3:59 PM. 4:00 PM – 6:30 PM.

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386 Table 7: Tiger's activity budget (%) and total observations made per animal, overall activity budget (%), and
387 percentage of behaviours labelled as indicators of diminished or enhanced welfare.

Behaviour	**Number of observations per behaviour / Total observation (%)**							
	Animal							**Overall**
	SIL	**TOA**	**TOB**	**MPO**	**FPO**	**MMU**	**FMU**	
Aggression	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.02
Affiliative behaviours	0.00	0.34	0.47	1.00	0.81	0.62	0.79	0.53
Avoid	0.00	0.00	0.16	1.00	0.00	0.00	0.00	0.16
Body shake	0.42	0.00	0.00	0.00	0.13	0.00	0.00	0.10
Drink	0.94	0.92	0.63	0.43	0.00	0.16	0.00	0.49
Eat	0.00	0.00	0.00	13.25	7.80	0.93	3.93	3.50
Flee	0.00	0.00	0.00	0.57	0.00	0.00	0.00	0.08
Grass	1.36	1.03	1.26	0.28	0.67	0.62	0.00	0.81
Immersion	6.60	0.11	1.57	0.28	1.08	0.00	0.79	1.74
Intentional out of sight	8.49	0.46	0.00	0.00	0.00	2.18	2.95	2.25
Interaction	1.47	2.64	3.14	1.14	1.48	0.62	1.38	1.72
Jump	0.00	0.23	0.16	0.00	0.13	0.00	0.00	0.08
Pace	1.36	0.34	0.00	0.43	0.00	0.31	0.20	0.43
Rest	9.43	31.84	32.03	16.81	36.02	47.51	25.34	27.50
Roll over	0.00	0.34	0.47	0.28	0.13	0.16	0.98	0.30
Run	0.00	0.00	0.31	0.57	0.00	0.16	0.00	0.14
Scratch	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.02
Self-grooming	5.14	3.10	4.40	6.84	10.48	4.98	3.14	5.50
Sleep	34.17	41.84	24.18	28.63	31.59	26.64	39.29	32.64
Stand	1.36	4.14	4.08	3.42	2.96	4.98	5.89	3.62
Stalk	0.00	0.34	0.47	0.43	0.00	0.31	0.98	0.32
Stretch	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.04
Urinate / Defecate	0.73	0.00	0.16	0.57	0.27	0.31	0.39	0.36
Vocalize	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.02
Walk	28.09	11.26	26.22	23.65	6.18	9.35	13.75	17.30
Yawn	0.42	0.80	0.16	0.43	0.00	0.16	0.20	0.34
Total indicators of diminished welfare	1.36	0.34	0.16	1.99	0.13	0.31	0.20	0.69

Total indicators of enhanced welfare	21.70	6.67	9.58	9.26	13.84	8.41	9.04	11.74
Total observations	954	870	637	702	744	642	509	5058

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389 Table 8: Results of the multinomial logistic regression model. Zoo D was considered as reference.

Dependent variable	**Independent variable**	**Odds Ratio**	***p-value***	**95% Confidence Interval**	
	Zoo				
Enhanced welfare	D (ref.)	1	-	-	-
	C	1.83	0.000	1.41	2.37
	B	1.06	0.691	0.80	1.39
	A	4.11	0.000	3.18	5.30

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