ACTIVITY BUDGET OF TWO GIANT PANDAS AT PAIRI DAIZA ZOO (BELGIUM) AND THE POSSIBLE INFLUENCE OF ENVIRONMENTAL FACTORS

by

Claire VANHOOMISSEN

Promoters:

Prof. dr. Christel Moons
Prof. dr. Tim Bouts

Research report as part of the Master's Dissertation

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PREFACE

I thank my promoters, Christel Moons and Tim Bouts, for their presence and their advices, my proofreaders, John Maddox, Marie Van Parijs, Nazaré Storms and Kim Vervliet, for their time and their fine work, my parents Christine and Marc, who always support me, my grand-parents Sylvie and Robert, who warmly welcomed me during my observations and the panda's keepers Tania, Robin, Quentin, Mr Strong and Liu Yong, for their kindness.
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SUMMARY

Since the giant panda (Ailuropoda melanoleuca) is one of the most endangered species, its conservation should be monitored with great care. The captive population might therefore be considerably valuable even though conditions in captivity may, despite the efforts of most zoos in that field, not always be optimal (stressors, lack of control by the animals, monotony), compromising animal welfare. In order to increase well-being, behavioural monitoring at the individual level is implemented, furnishing baseline information on animal behaviour. Evaluation of welfare and consequences of management adaptations are thus possible, especially through activity budgets. This study focused on two giant pandas living at the zoological park Pairi Daiza, in Belgium. After two months of behavioural observation, activity budgets for both animals in two different seasons (Summer and Winter) were built. The influence of different environmental factors (season, temperature, relative humidity, sound and visitor presence) on the pandas’ behaviour was equally investigated.

Visitor presence, sound and relative humidity were found to have a significant impact on panda activity budgets, whereas temperature did not. Activity budgets were considerably different between Summer and Winter. However, two disturbing events interfere with the interpretation of these results: the female was in pseudopregnancy in Summer and a fight occurred between the two pandas just before the Winter observation month. Regardless, this study has provided some valuable information on the behaviour of these two specific pandas, which can be used as basis for further research and allow informed husbandry and management decisions.

Key words: Giant panda - behaviour - activity budget- environmental factors
SAMENVATTING

De reuzenpanda (*Ailuropoda melanoleuca*, verder panda genoemd) is een van de meest bedreigde species in de wereld. Er worden veel inspanningen gedaan om deze diersoort zowel in situ, in het wild, als ex situ, in gevangenschap, van uitsterven te redden. Wanneer de dieren in gevangenschap worden gehouden, kan dit schadelijk zijn voor het welzijn van de dieren. Naast vele mogelijke stressfactoren, zoals het geluid, de bezoekers, de interactie met de verzorgers, leven de dieren in een monotoon en beperkt milieu. Hierdoor kan het welzijn van de dieren aangetast worden. De dierentuinen worden zich hier steeds bewuster van en passen hun werkwijze aan om zo het welzijn van hun dieren te verbeteren. Kennis over de soortspecifieke eigenschappen is belangrijk, maar het is ook belangrijk het management op het individu zelf af te stellen. In deze masterproef worden de twee reuzenpanda’s van Pairi Daiza, een dierentuin in België, bestudeerd om kennis over deze twee specifieke dieren op te doen. Er wordt activiteitsbudgets (gedefinieerd als de tijd dat een dier aan bepaalde activiteiten, zoals slapen en eten, besteed (uitgedrukt in procent)) vrijgemaakt om verder onderzoek te doen om zo informatie over deze dieren in te winnen. Deze informatie kan dan later gebruikt worden om gerichte beslissingen over management te nemen. Bovendien wordt de invloed van verschillende omgevingsfactoren zoals seizoen, temperatuur, relatieve vochtigheid, geluid, bezoekers en bouwwerken, op het gedrag van de panda’s onderzocht.

De twee panda’s, een volwassen mannetje en een volwassen vrouwtje, zijn gedurende twee maanden geobserveerd door een waarnemer. De observatieperiode duurde vierentwintig dagen. De eerste observatieperiode was in augustus 2015 (zomer periode) en de tweede in november-december 2015 (winter periode). Het gedrag werd volgens een vooropgesteld ethogram opgenomen, met behulp van een computerprogramma. Dagelijks werden de temperatuur (in °C), de relatieve vochtigheid (in %), het geluid (in dB) en het aantal bezoekers (in % van ruimte bezet door bezoekers) op regelmatig tijdstippen gemeten. Verder werden de dieren 6 uur geobserveerd. Ook de locatie van de dieren in het verblijf werd geregistreerd om het gebruik van hun verblijf te evalueren. De winterobservaties werden echter verkort door een onvoorspelbaar incident. De twee panda’s hadden de deur tussen hun twee kooien opengebroken. Daarop volgde een gevecht waarna de panda’s van verblijf wisselde. Daarom telde de winter maar achtien dagen observaties.

Vanuit de verkregen gegevens uit het onderzoek, wordt de relatieve duur (in %) per dag van elk gedrag berekend. Daarvan werden vier activiteitsbudgets, een per dier per seizoen, opgesteld. Locatiebudgets werden op dezelfde manier opgebouwd, met de relatieve duur (in %) per dag van de tijd die elke panda per locatie besteedde. Er werden drie statistische testen uitgevoerd om het effect van de verschillende factoren op de meeste voorkomende gedragingen te bepalen. Het effect van de bouwwerken kon niet getest worden door het feit dat de werken enkel in de winter werden uitgevoerd en niet tijdens de zomer. De invloed van seizoen- en klimaatomstandigheden (temperatuur en relatieve vochtigheid) werden eerst getest. Vervolgens, in de tweede test, werd het effect van geluid en aanwezigheid van bezoekers op het gedrag van de panda’s vastgesteld. De derde en laatste test
werd toegevoegd omdat het vrouwtje schijndrachtig was tijdens het begin van zomerobservatie. Ze toonde tijdens de eerste zestien dagen specifieke kenmerken van schijndracht zoals rode en gezwollen melkklieren, verminderde eetlust en lethargie. Er werd een test uitgevoerd om het verschil in gedrag te bepalen tussen deze periode en de laatste acht dagen, waarin ze niet meer schijndrachtig was. Alleen de meest voorkomende gedragingen werden getest: rust en zelfverzorging.

Het seizoeneffect, waaronder het geluid en het aantal bezoekers, bleek een significant effect te hebben op meeste gedragingen. Er kon toch geen echt bewijs van verminderd welzijn worden aangetoond. De invloed van temperatuur bleek niet significant te zijn. Relatieve vochtigheid beïnvloedt wel bepaalde gedragingen. Het rustgedrag van het vrouwtje was significant hoger tijdens de vermoedelijke periode van schijndracht. Deze twee verstoringe elementen, de schijndracht en het deurincident, belemmeren de interpretatie van de resultaten. Niettemin beschikt de dierentuin nu over een basis om het gedrag van hun panda’s te evalueren en om het effect van komende aanpassingen in het management te beoordelen.
INTRODUCTION

The giant panda (Ailuropoda melanoleuca, hereafter mostly panda) is one of the most endangered species on the planet (Lü et al., 2008), with the last estimation of population size around 1800 individuals (Qiu, 2015). Many efforts are thus focused on its conservation, in situ, i.e. in the wild as well as ex situ, i.e. in zoos and breeding centres (Peng et al., 2007). The captive panda population is believed to be undoubtedly valuable for the giant pandas in the wild (Ellis et al., 2006). This value may be regarded in different ways: as an ambassadorial value, an educational value, an insurance value, a funding value or even a value for scholarly knowledge.

However, a captive environment may not be optimal and therefore compromise animal welfare, leading most zoos, increasingly concerned about their animals, to implement changes in order to raise this welfare (Liu et al., 2006; Steinman et al., 2006; Whitham and Wielebnowski, 2013). Notwithstanding, their approach tends to be resource-based and not animal-based, mainly focusing on what they provide, i.e. environment and management practices rather than directly focusing on the animal. In order to promote physiological, emotional and mental well-being, a direct approach should be developed to monitor and improve animal well-being (Whitham and Wielebnowski, 2013). Because behavioural variation between individuals may be truly high, an individual-based approach to management should be taken (Watters et al., 2009; Whitham and Wielebnowski, 2013). In this thesis, activity budgets of two pandas as well as the possible influence of different environmental factors on their behaviour are studied in order to contribute to behavioural monitoring.

The introduction is divided into five parts. Firstly, some information is given about both panda biology and threats to the species. Secondly, behavioural monitoring is discussed in addition to its link with animal welfare. Thirdly, the importance of activity budgets and some features of the pandas’ activity budgets are presented. Fourthly, the influence of various factors on these activity budgets as well as their influence on welfare in general are discussed. Lastly, a summary disclose the purpose of this study.

1  PANDA BIOLOGY AND THREATS TO THE SPECIES

The giant panda is classified as a carnivore of the family Ursidae (Ellis et al., 2006; Spady et al., 2007; Lù et al., 2008). Besides some bears’ characteristic, like a solitary life-style (Swaisgood et al., 2003), its diet is particular, mainly herbivorous with bamboo accounting for more than 99% of its diet in the wild (Rybiski Tarou et al., 2005; Ellis et al., 2006; Hansen et al., 2010; Wei et al., 2015). This animal relies on a poor food source (bamboo contains mainly cellulose, hemicellulose and lignin) despite its typically short digestive tract of the order Carnivora (Hansen et al., 2010; Wei et al., 2015). Specialized gut microbiota seems to account for the possible cellulose and hemicellulose digestion even though efficiency is particularly low (Zhu et al., 2011). However, bamboo is an accessible and abundant resource for which the giant panda does not have many competitors (Wei et al., 2015). Furthermore, a
Panda’s morphology is well adapted to this specialized diet, with its pseudo-thumb that allows it to grasp bamboo shoots, its large and specific skull, its strong masticator muscles and its particular teeth which improve mastication (Rybiski Tarou et al., 2005; Ellis et al., 2006; Zhang et al., 2009; Wei et al., 2015; Wei et al., 2015). Pandas are not only morphologically well-adapted to their herbivorous diet, they have similarly developed characteristic foraging strategies (Wei et al., 2015; Zhang et al., 2015). They spend a considerable proportion of their time eating, selecting the bamboo with great care, probably guided by its nutritional composition. Pandas maximize their energy intake while minimizing the energy expenditure, by daily short-distance movements in old-growth forests with mild inclination, which provides them with a smooth passable field and easy access to bamboo (Rybiski Tarou et al., 2005; Hansen et al., 2010; Wei et al., 2015; Wei et al., 2015; Zhang et al., 2015). They also show seasonal movement according to the different bamboo species available. In captivity, pandas are also offered steamed cake, fruits and vegetables, reducing their bamboo consumption, still up to 14 kg a day (Ellis et al., 2006; Swaisgood et al., 2006b; Liu et al., 2015). Another distinction of pandas is their lack of hibernation (Owen et al., 2005). Unlike other bears, their primary source of food, bamboo, remains abundant during winter. In addition, this low-energy diet may force them to keep feeding, in order to maintain a sufficient food intake (Zhu et al., 2001).

Giant panda habitat has been lost, fragmentized and deteriorated, signifying one of the most important threat to the species. Wild pandas are only present in China, in six different mountain ranges, mostly within reserves (Ellis et al., 2006; Liu, 2015; Liu et al., 2015; Wei et al., 2015; Wei et al., 2015). They usually stay between 1500 and 3000m in altitude (Zhu et al., 2001; Liu et al., 2015), adapted to this mostly cold environment thanks to their thick fur. They conversely suffer in warm temperatures because of their incapacity to use evaporative cooling and passive heat loss (Ellis et al., 2006). Human pressure, through forest harvesting and livestock grazing for instance, as well as climate change, might compromise the survival of pandas by hazarding their habitat (Liu, 2015; Qiu, 2015; Wei et al., 2015). The restricted area pandas have at their disposal could probably be a great risk to panda populations in cases of bamboo-die off (Ellis et al., 2006; Wei et al., 2015), a recurrent phenomenon of flowering (every 40-100 years) which causes a die-off of all plants. Pandas have survived until now to large-scale events (reducing their unique food supply) by selecting other bamboo species as food over the flowering kind and by travelling longer distances. So, habitat degradation and fragmentation limit the ability of the giant panda to respond to this threat. Population isolation and small population size can also be considered as disadvantages for the survival of pandas as a consequence of low genetic diversity (Liu, 2015; Wei et al., 2015; Wei et al., 2015).

Problematic reproduction (lack of sexual interest, failed mating, etc.) has long been thought to be responsible for the pandas’ decline whereas population is now believed to have a positive growth rate (Swaisgood et al., 2006b; Wei et al., 2015). A better knowledge about panda physiology and behaviour have led to a better understanding of its specific features (McGeehan et al., 2002; Swaisgood et al., 2006b; Wei et al., 2015), contributing to an increased success of panda reproduction
in captivity (Owen et al., 2004; Zhang et al., 2004; Swaisgood et al., 2006b). Especially, reduced stress and more natural enclosures have promoted the numerous births per year. The following paragraphs present the most relevant information about panda reproduction.

Giant pandas are seasonal breeders, a common trait of the ursids (Spady et al., 2007). The female panda undergoes only one estrus per annual mating season (i.e. seasonal mono-estrus) (McGeehan et al., 2002; Ellis et al., 2006; Spady et al., 2007). This unique fertile period is short, from 24 to 72 hours and is characterized by multiple, short copulations (McGeehan et al., 2002; Ellis et al., 2006). Then, fetal growth is onset as pandas undergo delayed implantation, or embryonic diapause. The corpus luteum, in a dormancy phase, is reactivated at the time of implantation. The active luteal phase is not in every case triggered by a gestation since the female undergoes spontaneous pseudopregnancy, which is undistinguishable from pregnancy. Behavioural, physiological and hormonal changes associated with pseudopregnancy mimic the changes experienced during gestation while no conceptus is present in the womb. This ensures a well prepared uterus for implantation and a similar luteal activity for a preprogrammed duration identical to this of normal gestation (Steinman et al., 2006; Spady et al., 2007; Kersey et al., 2010; Willis et al., 2011). The female panda shows in both cases decreased appetite, lethargy, nest-building and cradling behaviours as well as a swollen, reddened vulva and enlarged mammary glands (Steinman et al., 2006; Kersey et al., 2010).

The female gives birth to one or two immature cubs in the late summer (Zhu et al., 2001). They weigh from 75 to 175 grams, which is thus around 0.12 - 0.18% of the mother’s weight. The female panda will only take care of one cub, abandoning one of them in case of twins. In captivity, raising of the two cubs is possible thanks to human care (Snyder et al., 2003). One cub remains with the mother while the other is placed in an incubator and fed by bottle. The cubs are regularly alternated throughout the day. This is called pear-rearing, as opposed to mother rearing. At 6 months old, the captive cubs are taken away from the mother in order to reduce the inter-births interval, which is around two years in the wild (Zhu et al., 2001; Snyder et al., 2003; Swaisgood et al., 2006b; Spady et al., 2007).

2 WELFARE AND BEHAVIOURAL MONITORING

The environment of animals in captivity is very different from that in the wild. The constant changes and challenges of the wild life are replaced by fixed schedules, permanent care from the keepers and easily accessible food that does not require much foraging (Swaisgood et al., 2003; Liu et al., 2006). The animals lack control over their environment (for instance avoidance behaviour compromised) and they have few opportunities to express their normal behaviour (no labour to access a nice shelter for example) (Liu et al., 2003; Swaisgood et al., 2006b) leading to the exhibition of more rigid and repetitive behaviours. Since resources required for the full expression of behaviours are likely to be insufficient (lack of space as well as lack of naturalistic stimuli), survival needs are expressed in priority, inhibiting other behaviours such as reproduction (Swaisgood et al., 2003; Peng et al., 2007). In view of this, behavioural diversity in zoo animals is thought to be as important as health or nutrition to reach an acceptable level of well-being.
Besides the poor environment and the lack of behavioural opportunities, captivity also places the animals in presence of many potential stressors: visitors, noise, construction works, an unsuitable climate and interactions with the keepers, which may be damaging to the animal if they trigger chronic stress (Owen et al., 2004; Owen et al., 2005; Swaisgood et al., 2006a). Stress is defined as the physiological and behavioural response to an aversive stimulus (Sapolsky et al., 2000). It is supposed to increase the chances of animal survival in acute situations. However, when the animal is constantly exposed to stressors and fails to habituate to these, it can lead to chronic stress, causing, inter alia, a decrease in immunity or reproductive failure (Owen et al., 2004; Zhang et al., 2004; Owen et al., 2005; Liu et al., 2006). The identification of these negative elements and their reduction is thus believed to play a notable role in animal well-being.

Recognising the stressors tends to be difficult except when they cause a modification in the animal’s behaviour, which can then be diagnosed (Owen et al., 2004; Owen et al., 2005; Watters et al., 2009). In light of this, the study of behaviour seems to be inevitable to evaluate well-being.

However, reactions of animals in negative situations are multiple and variable (avoidance of the negative stimulus, development of stereotypies or change in activity levels (Swaisgood et al., 2006a)), depending on the stimulus and on the sensitivity of the animal. Consequently, behaviour evaluation is important both at a species level and at an individual level, hence the importance of case-studies in order to promote optimal psychological welfare (Owen et al., 2004; Watters et al., 2009).

When its behavioural needs are not met, the giant panda is likely to develop stereotypies. These repetitive and apparently functionless behaviours tend to appear in 62% of pandas in captivity, especially exhibiting pacing, head tossing, pirouetting and cage climbing (Liu et al., 2006; Swaisgood et al., 2006b). It is believed that these behaviours are the consequence of the limited and monotonous environment. They are also mostly observed as feeding anticipation, the animal becoming more agitated while waiting for food. In the wild, 55% of their time is devoted to bamboo consumption but in captivity, pandas’ foraging strategy is possibly disturbed (Swaisgood et al., 2003). Clumps of already broken-off bamboo in a restricted enclosure and easy-to-process food supplementation provoke a decrease in foraging and feeding activity (Zhang et al., 2015). In light of this, the time normally required for these activities should be fulfilled, leading to the emergence of stereotypies (Swaisgood et al., 2006b). Indeed, animals are not only motivated to obtain resources, their motivation is also based on the performance of the behaviour developed to obtain it. Therefore, captive environment should give the possibility to animals to engage in normal behaviour (Swaisgood et al., 2003; Peng et al., 2007). Furthermore, occurrence of stereotypies could be dissociated from its initial cause. Animals then perform stereotypies in diverse situations, without the original eliciting stimuli (Mason, 1991). These stereotypies are considered as emotionally neutral and pure motor automatism. This emancipation has, inter alia, already been detected in bears (Vickery and Mason, 2004). Established stereotypies can thus be indicators of past welfare problems.

Behavioural monitoring is an interesting tool to evaluate the causes of behaviours (Swaisgood et al., 2006a; Watters et al., 2009). This technique is similar to health monitoring, which has already found its
place in many zoos. For health monitoring, animals are, inter alia, weighed and their food intake is measured on a regular basis, providing valuable baseline information in order to detect any health problem. Behavioural monitoring should provide similar background information, about behaviour. It should thus be possible to identify normal behavioural patterns as well as modifications to these patterns, which are often linked with physical, social, psychological problems due to different possible causes (poor environment, stressors, ...)

3 ACTIVITY BUDGETS

Behavioural monitoring may be performed by building activity budgets. Activity budgets reveal the time allocated for each activity of the animal throughout the day. This division of their time into different activities appears to be variable and influenced by different factors (Resende et al., 2014). As Zhang et al. (2015) declare, activity patterns may furnish “key information about an animal's biology, such as foraging strategy or evolutionary adaptations, and about physiological responses to environmental cues”. Quality and quantity of food, reproductive status or weather are believed to be determining for the activity budgets.

To evaluate behaviour, it is first critical to recognize the natural variations in panda biology. Some studies have been conducted to identify the activity patterns of the giant panda, both in the wild and in captivity (Johnson et al., 1988; Liu et al., 2003; Owen et al., 2005; Zhang et al., 2015). These can be used as a basis for interpreting the results of this study. Nevertheless, panda studies may often be problematic due to the small sample size, compromising statistical significance and impeding much extrapolation. Even though these studies have provided panda activity budgets, it is essential to keep in mind that their results, based on few individuals, may not reflect the population reality.

The effect of season has been shown to have a great influence on panda behaviour in several studies (Johnson et al., 1988; Owen et al., 2005; Zhang et al., 2015). A panda's activity tends to peak in the spring and then to drop in summer to reach its lowest level in August-September. Afterwards, it seems to rise steadily in October to December and to remain at a relatively high level until it peaks again in May and June. Seasons are characterized by different factors, from changes in climatic condition to photoperiod.

Photoperiod is believed to be the fundamental factor determining the circannual gonadal rhythm in bears, leading to modifications in their behaviour (Owen et al., 2005; Spady et al., 2007). Zhang et al. (2015) suggest that the reproductive status has a major influence on panda behaviour, which is supported by the findings of Owen et al. (2005), at least for the female. Major changes tend to happen during estrus (more activity and less feeding). Pregnancy and pseudopregnancy also seem to affect the female behaviour, as well as lactation. The seasonality of panda reproduction may thus be the explanation of its seasonal behavioural patterns.

Moreover, seasons are distinguishable by different climatic conditions: temperature, relative humidity, solar radiation, air movement or precipitation may have an influence on the behaviour of mammals
Pandas are believed to prefer cold conditions (Ellis et al., 2006), probably due to their poor evaporative capacities, well-known in bears because of their large body, short limbs, a thick under-fur, a substantial layer of fat and absence of sweat glands (McLellan and McLellan, 2015). These features could lead to a decrease of activity in hot conditions (Garshelis and Pelton, 1980; Schwartz et al., 2010) even though these findings are controversial (McLellan and McLellan, 2015). Nonetheless, temperature should be measured together with relative humidity, which influences heat dissipation by impeding evaporative loss (Brody, 1956; Bohmanova et al., 2007).

Furthermore, changes in climate through the different seasons also affect food quality and availability. Pandas are not only seasonal breeders, they also exhibit seasonal movements into their habitat (Liu et al., 2015) as well as seasonal shifts in bamboo plant part consumption (Hansen et al., 2010). Forage type, quality and quantity may also be influenced by the seasons (Zhang et al., 2015).

Gender can also affect activity budgets, with males eating more and sleeping less than females (Liu et al., 2003). A behaviour can also be the consequence of a stressor or a change in internal motivation of the animal (Owen et al., 2005). It is therefore important to conscientiously analyse each modification in order to distinguish the underlying cause of the change. Interpretation of activity budgets should be conducted carefully. Swaisgood et al. (2006a) underline the importance of assessing stress on basis of the individual, in order to identify the chronic factors to which the panda fails to habituate. The following paragraphs provide an overview of the possible effect of some other environmental factors which may impact panda behaviour and well-being.

4 STRESS AND ENVIRONMENTAL FACTORS

Animals in zoos are exposed to numerous stressors which may decrease their well-being. One of the utmost factors is visitor presence, providing visual, olfactory and auditory stimuli (Liu et al., 2006; Fernandez et al., 2009; Quadros et al., 2014). Big institutions also frequently perform construction, deconstruction or restoration works which may similarly impact the animals (Swaisgood et al., 2006a). On top of this, animals are sometimes kept in geographical locations far away from their origins, consequently in a completely different climate (temperature, humidity, photoperiod,...) although the panda’s adaptation ability and associated problems are not well-known (Spady et al., 2007).

The effects of visitor presence on zoo animals have been studied by numerous authors (Davey, 2007; Fernandez et al., 2009; Quadros et al., 2014). Public exhibition has to be taken into account for the giant panda since this iconic and rare animal attracts large crowds (Swaisgood et al., 2006a). It is suggested that visual stimuli from visitors may be positive, neutral or negative depending on the species. It is thus important to identify this effect in order to diminish it if it is damaging (Davey, 2007). The crowd can be characterized by different features: its presence or absence, its density, its size, its position, its activity, its proximity or even the occurrence of flashes from cameras (Davey, 2007). It is in
any case believed that visitors become harmful to any species if visitor interaction exceeds a specific threshold.

Visitors are not only visual stimuli, they also may be very loud. Similarly, construction works can create high sound levels. Therefore, noise should be taken in consideration as an environmental factor, whenever it originates from visitors or construction works (Owen et al., 2004; Powell et al., 2006; Quadros et al., 2014). It is defined by different features which may presumably all be of importance to panda behaviour: amplitude, frequency and duration. Owen et al. (2004) and Powell et al. (2006) studied the effect of noise on panda behaviour. They both suggest that, even if noise has been shown to have an impact on pandas (changes in activity levels and more abnormal behaviours), this impact may presumably be negligible. Indeed, modifications in behaviour due to noise were minor as well as their welfare did not seem to be affected since response’s magnitude was low. Noise appears to be less meaningful in the captive environment than other factors increasing welfare (for instance: enrichment, better husbandry practices,...). Although Owen et al. (2004) and Powell et al. (2006) do not advance any reason to raise concerns about sound level, they recommend noise monitoring and a decrease if possible.

5 ABOUT THIS STUDY

The goal of this study is to build activity budgets for two pandas at one specific animal park, Pairi Daiza (Belgium), in two different seasons. These budgets will thus indicate in which activities these pandas are involved throughout the day in Summer and in Winter. This will provide baseline information about panda behaviour to be used in the evaluation of future adaptations to their environment. The effect of some environmental factors on these activity budgets will similarly be studied. The goal is to identify the impact of temperature, relative humidity, sound and visitor presence, on the pandas’ behaviour, especially their effect on behaviours commonly used as welfare indicators (for instance stereotypies).

For the purpose of this study, five different hypotheses are tested (H₅):

- There is a difference in panda behaviour between summer and winter conditions
- Alterations in climatic conditions (temperature, humidity) affect the behaviour of the pandas
- Alterations in noise levels affect the behaviour of the pandas
- Alterations in amount of visual stimuli (visitor presence) affect the behaviour of the pandas
- Occurrence of construction or demolition work affect the behaviour of the pandas

The null hypothesis (H₀) is obviously that these factors do not have any influence on the behaviour of the animals.

Additionally, the use of their enclosure by the pandas will be studied through construction of location budgets.
MATERIAL AND METHODS

1 SUBJECTS AND STUDY AREA

The subjects of this study were two giant pandas, a six-year old intact male Xin Hui and a six-year old intact female Hao Hao, both born in July 2009. They were housed since February 2014 at the Belgian animal park Pairi Daiza, Domaine de Cambron in Brugelette. The pandas originate from China. The male was peer-reared with his twin whereas the female was a singleton and mother-reared.

Each panda had an enclosure divided in a cave, i.e. indoor, and an outdoor grass yard. The two enclosures were adjacent and similarly built, having approximately the same shape and slope as well as containing analogous elements. They are illustrated in figure 1 and in the appendix. The caves were made with rough concrete walls and concrete floor with some fixed small stones. The female cave contained a wooden construction, a wooden swing and an area with big stones. The male cave contained two wooden constructions and one big stone. Two massive windows delineated each cave to allow visitors to look at the pandas in their cave. A glass door was the separation between the two caves. A small space between the door and the floor allowed the pandas to smell each other. Consequently, they had visual and olfactory contact through the window. Other windows were placed in the wall between the cave and the yard for an outside view and incoming daylight. Another door lead to a smaller cage called quarantaine, where keepers performed their medical training. This cage was the only passage way between the indoor cave and the outdoor yard, accessible thanks to small doors. No observation was performed in this cage, as pandas usually only passed through it.

The caves were under controlled atmosphere with constant temperature and constant relative humidity. They were also insulated against external sound, so the pandas did not perceive visitor-generated noise. The outdoor yards for the male and female panda consisted of a long strip of grass, separated from each other by a bamboo fence and bamboo plantations. Figure 2 shows one of these yards. The pandas could see each other along the first meters of the fence or when they were both up high, for example on the higher points of the wooden constructions. Pandas could probably also detect olfactory signals outside. An artificial creek separated visitors from the yard flow. Each yard contained some specific items: some trees, a pool of water (called pandacuzzi), a hill of stones, a small cavern and two wooden constructions (later referenced as wooden construction 1 and wooden construction 2). A swing was also present in the female panda yard. These specific items are depicted in the appendix.
Figure 1 Schema of the two giant pandas enclosure, with the cave on the left (light grey shading) and the yard on the right. The specific items are indicated with a blue letter (in the cave) or a blue number (in the yard): A= wooden construction, B= big stone(s), C= second wooden construction, only in the male cave, 1= hill of stones, 2 = pandacuzzi, 3 = wooden construction 2, 4 = wooden construction 1, 5 = cavern, 6 = swing, only in the female cave. The different yard areas used by the observer for indication of location are separated with green lines and numbered with roman numerals. The locations from which the observer watched the animals are indicated with an orange X. Windows inside the cave are represented by dotted lines.

Figure 2 The male giant panda's enclosure seen from above.
The pandas did not always have free access to the two parts of their enclosure (cave or yard). The doors were opened and closed following a fixed daily scheme, controlled by the animal keepers. The pandas spent the night in their cave and went in the yard at around 08:00, upon the arrival of the keepers. Bamboo was placed beforehand in each yard. The doors between the outdoor yard and the quarantaine (and thus the cave) were then closed for a few hours (variable from day to day). Bamboo was placed in the cave. At approximately 12:00, the doors were opened again. At the end of the day, around 17:00 the doors were closed for the night with the pandas each in their caves. Some exceptions in the fixed schedule happened during the observations, with pandas restricted to some parts of their enclosure.

2 DATA COLLECTION

2.1 EXPERIMENTAL DESIGN

Observations were conducted for twenty-four days during two seasons in 2015, in August (Summer) and November-December (Winter). Pandas were observed six days per week during four weeks. The non-observation day was randomly chosen. Weekend days always consisted of observation days during Summer, as visitors presence was expected to be higher on Saturdays and Sundays.

Each day, direct live observations were carried out during six periods of one hour, from 09:10 to 17:45 (start time of the six periods: 09:10, 10:30, 11:45, 14:00, 15:20, 16:40). Each hour was divided in four sessions of fifteen minutes. When time was needed for the observer to reposition herself to continue watching an animal, the observation was momentarily suspended and then resumed. Focal animal sampling was carried out for 15 min per animal, randomizing whether the male or the female was observed first. When both animals had been observed, the observation was repeated again in a random order, so that each animal was observed for 30 min total during a one-hour period. The randomization technique used was to blindly draw a pawn from a set of two, where one represented observation of the male, the other of the female.

Both continuous (15 min) and instantaneous recording (interval of 5 min within a 15 min observation session) were used. One observer was responsible for the observations and she positioned herself on the visitor walkway (usual locations visible in figure 1). If the panda changed its location during an observation (went outside when the observation began inside and inversely), it was recorded as 'Out of Sight' unless it was clear for the observer that the animal would remain in that location. In the latter case, the observer also changed her location in order to pursue the observation from a clear vantage point.

2.2 BEHAVIOUR AND ANIMAL LOCATION

Behaviour and location of the pandas were continuously recorded during each 15-min session. In addition, interval recording of panda behaviour and panda location was performed every five minutes.
from the beginning of each interval, so at 0, 5 and 10 min. The computer program used was The Observer version 10.5 (Noldus, Wageningen, The Netherlands).

Behaviour observations were based on the ethogram in Table 1, adapted from Liu et al. (2003), Powell et al. (2006), Quadros et al. (2014). Behaviours were considered as mutually exclusive. If two behaviours did happen simultaneously when vocalising or eliminating, the observer recorded only the one that happened first.

Table 1 Ethogram of the giant panda

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion</td>
<td>Any type of displacement of the body by movement of the limbs (walking, running, climbing)</td>
</tr>
<tr>
<td>Resting</td>
<td>Animal in any posture with its eyes closed or not paying attention to its environment</td>
</tr>
<tr>
<td>Being vigilant</td>
<td>Animal stationary in any posture paying attention to its environment or actively scanning/checking its environment</td>
</tr>
<tr>
<td>Feeding</td>
<td>Handling and ingesting food</td>
</tr>
<tr>
<td>Drinking</td>
<td>Ingesting water</td>
</tr>
<tr>
<td>Foraging</td>
<td>Exploring the enclosure for food</td>
</tr>
<tr>
<td>Investigating</td>
<td>Exploring the enclosure, unrelated to food, including sniffing, licking, biting objects or substrates and manipulating with paws (not play or feeding)</td>
</tr>
<tr>
<td>Startling</td>
<td>The response to a sudden external disturbance.</td>
</tr>
<tr>
<td>Vocalising</td>
<td>Producing vocal sounds</td>
</tr>
<tr>
<td>Playing</td>
<td>Rolling and summersaulting with manipulation of objects, such as food dishes, bamboo stalks, tree branches, or toys provided by the keeper</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>Scratching and licking of pelage</td>
</tr>
<tr>
<td>Ano-genital marking</td>
<td>Rubbing the ano-genital area around or up and down the surface of an object or on the wall</td>
</tr>
<tr>
<td>Urine marking</td>
<td>Urinating while in a squat, leg-cock, handstand, or standing posture on the wall or ground</td>
</tr>
<tr>
<td>Urinating</td>
<td>Producing urine (not urine marking)</td>
</tr>
<tr>
<td>Defecating</td>
<td>Producing faeces</td>
</tr>
</tbody>
</table>
Performing stereotypies

Repetitive behaviours morphologically identical repetitions, with no obvious goal or function. Examples: pacing, circling, self-mutilation, head bobbing. The male panda had developed a complex stereotypy during which he paced, rocked (shift of the body from side to side, remaining stationary (Liu et al., 2006)), performed door-directed behaviour (mostly pirouetting, defined by Liu et al. (2006) as standing on his hind legs and spinning back and forth), looked at the visitors, went in his pandacuzzi and drank. These elements were performed in a repetitive and predictable pattern, sometimes during hours. Therefore, these behaviours were recorded as stereotypies when they were part of this scheme, mostly easily recognizable.

Performing door-directed behaviour

Waiting restlessly at the door separating the indoor and outdoor enclosures (alert, investigating or manipulating the door, scratching at the door, pirouetting)

Other

Any behaviour not listed above

Out of sight

When the animal is not in the field of vision of the observer

To determine the use of the available enclosure surface, the location of each panda in its enclosure was recorded in parallel with behaviours, as modifier to the behaviour. Each enclosure was divided in seven area locations, one indoor in the cave and six outdoor in the yard (areas separations visible in green on figure 1). The animal was considered in one area when more than half its body was placed in the specific area. Besides the areas, each specific item with which the panda could climb on or otherwise interact (big stones, trees, pool of water, hill of stones, small cavern, swing and wooden constructions) were identified as separate locations. Consequently, aside from seven area locations, there were nine item locations visible in blue in figure 1 (three indoor and six outdoor for the male enclosure, two indoor and seven outdoor for the female enclosure, as the animals' enclosures were not completely similar (more items inside for the male, outside for the female). Trees in the outdoor enclosure were recorded as one item location. The animals were recorded as being on item locations as soon as they had one paw or more on the item or when they showed interest directed to this item.

2.3 VISITOR PRESENCE

Interval sampling was used to record visitor presence, every five minutes (at 0, 5 and 10 minutes of each 15 min session). The observer estimated the percentage of space occupied by visitors, from 0% when the only human presence was the observer to 100% when the available space was totally occupied by visitors. Inside, only visitor presence at the window where the observer was present could be measured because visitors at the other window could not be seen and shifting vantage point was not possible.
During the summer observations, the park was opened to public (the first hour of observation was conducted before the park opened) whereas the park was closed to visitors during the second month of observation. The only human presence in December, besides the observer, were thus animal keepers, workers and guests invited by the park.

2.4 SOUND PRESSURE

Sound pressure was recorded to evaluate disturbance due to visitor presence or occurrence of construction and demolition work. Every five minutes, at 0, 5 and 10 minutes of each session, the observer took the maximum decibel pressure over five seconds using a decibel meter (model S1351 of Captelec, Croix, France). The sound measured was an approximation of the sound the pandas perceived as it was measured while standing on the visitor walkway and not in the panda enclosure, which was logistically not feasible. Sound measurements were only performed when the pandas were outside in the yard, as inside in the cave, the pandas were insulated from visitor noise.

2.5 TEMPERATURE AND RELATIVE HUMIDITY

Temperature and relative humidity of the outdoor enclosures was measured at the beginning of each 15 min observation session, thus four times per one-hour observation period. A thermometer/hygrometer (TFA, model 45.2032, Wertheim, Germany) was placed on the fence of a panda enclosure (approximately one meter in height) and values were recorded just before the start of each observation occurring outside. In the cave, temperature and relative humidity were under control of a specific program. They were maintained at the values of 19°C (Summer) or 13.5°C (Winter) for the temperature and 86% for the relative humidity.

2.6 OCCURRENCE OF CONSTRUCTION OR DEMOLITION WORK

Minimal construction work was performed during the summer observations when the park was open to public. Influence of construction was thus considered insignificant during this period. Main construction and demolition work took place during the winter observations and was recorded daily in a binomial fashion (absence or presence of works).

3 STATISTICAL ANALYSIS

3.1 DATA TREATMENT

Observations provided the daily duration of each behaviour wherein pandas were involved per day. By dividing this duration by the total duration of observation per day (thus the duration of observation minus the time that the time animals were out of sight) and multiplying by 100, we obtained the percentage of time pandas spent for each behaviour. These relative duration were calculated for each 15-min session and for each day. Using this information, activity budgets were constructed.

The proportion each panda spent per location was calculated, on a similar way used for behaviour: the total duration on one place was divided by the total duration of observation (thus the time of
observation less the time “out of sight”) and multiplied by 100, to obtain a percentage. The time spent outside was also determined, by adding the relative duration of the outside locations.

3.2 STATISTICAL TESTS

The hypotheses were tested by an external specialist in statistics. Three tests were performed in order to identify the effect of the different factors on panda behaviour: the first one assessed the effect of season and climatic conditions (temperature and relative humidity), the second one tested the influence of noise level and visitor presence and the last test was performed in order to identify the impact of the pseudopregnancy on two behaviours of the female giant panda, resting and self-grooming. The two first tests investigated the effects of the different components on only frequently occurring behaviours of the two giant pandas, i.e. locomotion, resting, performing stereotypies, performing door-directed behaviour, being vigilant, feeding, foraging, drinking and self-grooming. Other behaviours of the ethogram were observed at too low levels (less than 1% of the observed daily activity budget) to allow relevant statistical analysis. Therefore, these behaviours were not taken into account in the statistical analysis.

Effect of season and climatic conditions

The first test analysed the effect of season and climatic conditions on panda behaviour thanks to a longitudinal analysis. As temperature and relative humidity were recorded at the beginning of each session (i.e. each quarter of an hour), the investigation of their effect was based on the proportional duration of each behaviour during the session. The effect of season was tested simultaneously.

The data has a complex nested structure with observations performed during a quarter, part of an hour, itself part of a day. To take this structure into account into the model, the following model was fitted: relative humidity and temperature were entered as fixed effects in the model. Season (summer and winter) and order (first or second quarter observation of the animal in a hour) were also entered in the fixed model. Period (i.e. hour), session (i.e. quarter) and day were entered as nested random effects into the model (i.e. session nested in period, period nested in day and day as such). The model also accounts for the fact that all measurements within one animal are correlated.

The results present the modification of average duration of each behaviour in function of the different factors. For temperature and relative humidity, results show the effect of increase of every 1-unit (respectively 1 Celsius degree and 1 percent relative humidity) on the average duration of the behaviours (increase or decrease and amplitude). For season, the average duration of behaviour in summer is compared to the average duration of the same behaviour in winter (higher or lower and amplitude).

Effect of sound level and visitors presence

This test assessed the influence of sound level (dB) and visitors presence (% of space occupied by visitors) by a longitudinal analysis. Sound level and visitors were recorded instantaneously every five
minutes. Behaviours were similarly instantaneously entered, recorded as absent (0) or present (1). They were mutually exclusive, so that only one behaviour could be recorded per instantaneous sample point. The analysis was performed on this basis, with, for each sample point (every five minute of each session), a behaviour associated to a sound level and a percentage of visitors presence. The response was thus in 0/1 format implying a logistic type of regression. The data followed the similar complex nested structure as the first test. This was thus likewise taken into account in the longitudinal logistic model. The following model was fitted: season (summer or winter), sound (in dB), visitors (in percentage of space occupied), order (first or second quarter of a hour observation) and sample point (0, 5 or 10 minutes of the session) were entered as fixed effects in the model. Period (i.e. hour), session (i.e. quarter) and day are entered as nested random effects into the model (i.e. session nested in period, period nested in day and day as such). The model also accounts for the fact that all measurements within one animal are correlated.

The main focus was dedicated to the effect of sound and visitors on the behaviour. The occurrence of each behaviour was determined in function of two different levels of sound and visitors whilst fixing the other covariate i.e. visitors and sound respectively at its mean level. By comparing the chance of occurrence of the behaviour between lower and higher level of dB and percentage of place occupied, it could be determined if a panda was more likely to increase or decrease a behaviour (with a certain factor expressed through the odds ratio) if its environment was louder or more occupied by visitors whilst fixing the other covariate. Correlation between sound and visitors has also been checked. Moreover a vast amount of missing observations for sound and to a fewer extent for visitors lead to the construction of models where a relatively large number of observations was removed. In light of this, two additional models for each behaviours were made: one with removal of sound as fixed effect and the second one with removal of visitors as fixed effect. By also entering season, order and sample point into the models, it corrected for possible effects of them.

**Effect of pseudopregnancy of the female**

The third and last test analysed the effect of the reproductive status of the female on her behaviour. A comparison of the behaviour of the female during the first sixteen days of the summer observations with her behaviour during the last eight days of the same period was carried out. Anecdotal evidence suggested resting and self-grooming were most likely to be affected, so only these two behaviours were tested. Pseudopregnancy was entered into the model as fixed effect whereas day (correlation between different days within one animal should be taken into account) was entered into the model as a random effect. This model thus takes into account the within-subject correlation between all measurements. For this purpose, the best structure for the variance/covariance matrix was found. A p-value was calculated for the effect of pseudopregnancy on resting and self-grooming. Least squares means and differences were also calculated.
RESULTS

The initial schedule of observations could not be followed as planned for the Winter season. The day prior to the start of the Winter observations, the pandas had succeeded to break and open the glass door between their caves. As a result, they fought and the male injured lightly the female before the keepers were able to separate them. To resolve the problem as soon as possible, the keepers were forced to put the male in the female enclosure and vice versa. Some days were needed to get back to the pandas’ normal daily routine, after having the broken door repaired. Observations were thus postponed and the winter observation period counted only eighteen days.

In addition, construction only took place during the Winter observation period, when the park was closed. Within this period, construction occurred on all days but four, which did not allow for an investigation of the effect of construction on panda behaviour.

This chapter is divided into two sections. The descriptive statistics reveal the activity budgets of both animals in the two seasons. Locations use and environmental factors features are examined. The second section, the inferential statistics, analyse the activity budgets so that the different hypotheses about influence of environmental factors on panda behaviour are tested.

1 DESCRIPTIVE STATISTICS

1.1 ACTIVITY BUDGET

As explained above, daily relative durations (%) were calculated for each behaviour. The average of these durations was determined for each animal in each season, leading to four activity budgets. These can be seen in figures 3 to 6.

![Figure 3: Average daily activity budget (%) of the female giant panda during the Summer](image-url)
Figure 4 Average daily activity budget (%) of the female giant panda during the Winter

Figure 5 Average daily activity budget (%) of the male giant panda during the Summer

Figure 6 Average daily activity budget (%) of the male giant panda during the Winter
The activity budget of the female in the summer is considerably different from the other activity budgets, which is mostly because she spent a considerable amount of time resting. The observer found out from the keepers that the female was in pseudopregnancy. She indeed exhibited the particular characteristic of this period described by Steinman et al. (2006) (she had not been inseminated so pregnancy could be excluded): decreased appetite, vulvar swelling and colouration, mammary gland enlargement and lethargy. Consequently, all other behaviours occurred relatively less frequently. The only behaviours performed more than 1% of the total observation time were feeding, self-grooming and locomotion. The activity budget of the female in the winter is more comparable to the activity budgets of the male, with resting and feeding durations together accounting for more than 60% of a panda day. The rest of the day, both pandas were mostly engaged in locomotion, foraging and performing stereotypies.

Figures 7a and 7b illustrate the differences between the activity budgets of the female in both seasons for frequently occurring and rarely occurring behaviours, respectively.

![Figure 7a Activity budget of the female giant panda for frequently occurring behaviours (> 1% of her time) expressed as daily average % (± SD) in Summer (grey bars) and Winter (black bars).](image_url)
Figure 7b Activity budget of the female giant panda for rarely occurring behaviours (< 1% of her time) expressed as daily average % (± SD) in Summer (grey bars) and Winter (black bars).

As resting represented more than 75% of the time of the female in the summer, nearly all other behaviours were considerably lower in summer than in winter, with the exception of self-grooming, investigating and playing. Feeding was noticeably increased in winter as it nearly tripled. Performing stereotypies and door-directed behaviour were likewise remarkably higher. As standard deviations reveal, behaviours accounting for a larger percentage of panda daily time were more homogenous than behaviours accounting for less time.

The activity budgets of the male in both seasons are more similar to each other. However, some differences may be detected. These can be seen in figures 8 a and b, two bar charts analogous to these of figures 7a and 7b.
Figure 8a Activity budget of the male giant panda for frequently occurring behaviours (>1% of his time) expressed as daily average % (± SD) in Summer (grey bars) and Winter (black bars).

The male was much more engaged in stereotypic behaviour in the summer than in the winter. Nonetheless, his time involved in door-directed behaviour increased dramatically in the winter. Both pandas actually showed much more interest towards the door separating their two caves during this period. The male spent also more time feeding during the winter, on the contrary of foraging. Other considerable differences can be seen in the average duration of investigating and self-grooming, which were lower in winter than in summer.

Variations are, as for the female, higher in rarely occurring behaviours than in frequently occurring behaviours.

1.2 ANIMAL LOCATION

Table 2 illustrates the time each panda spent outside (in the yard).

Table 2 Average percentage ± SD of time the giant pandas spent in their yard in both seasons, Summer and Winter

<table>
<thead>
<tr>
<th>% time outside</th>
<th>Female</th>
<th>Summer</th>
<th>23.49 ± 21.78</th>
<th>Winter</th>
<th>59.44 ± 24.80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td></td>
<td>42.24 ± 7.47</td>
<td></td>
<td>44.67 ± 19.67</td>
</tr>
</tbody>
</table>
The male spent approximately the same time outside in both seasons, with an average slightly over 40%. Nonetheless, the standard deviation is much higher in winter, with a variation of nearly 20%. Measures for the female are remarkably different, with an average time outside that nearly tripled in winter (around 20% to nearly 60% of its time). The standard deviation is analogous to this of the male in winter, which depicts a large variation between days.

Figure 9 and 10 illustrate, in function of the season, the average time per location and the standard deviation associated. The different locations are presented in figure 1 and illustrated in the appendix.

![Figure 9 Locations budget of the female giant panda, expressed as daily average % (± SD) of occupation of each location in Summer (grey bars) and Winter (black bars). The item locations are indicated with a number or a letter (referring to these used in figure 1).](image-url)
Figure 10 Locations budget of the male giant panda, expressed as daily average % (± SD) of occupation of each location in Summer (grey bars) and Winter (black bars). The item locations are indicated with a number or a letter (referring to these used in figure 1).

Some similarities may be found in the use of enclosure space between males and females. Two area locations (cave area and yard area 1) and one item location (the wooden construction 1, the two-stages square) are considerably occupied by both pandas. More variations occur for other locations.

For the most part, however, occupation of enclosure space is surely different between the male and the female. Besides the three preferred locations mentioned in the previous paragraph, two other ones (both in the cave) were preferably used by the female: the wooden construction and the big stones. Her location budget is also clearly different between summer and winter. She spent a lot of time (more than 60%) on the big stones in the cave during summer. Occupation of other locations are consequently remarkably reduced during this period. As she spent more time outside during winter, she spent more time in the yard area 1 (where bamboo was usually placed) and on the wooden construction 1 (the two-stages square). Similarly, the cave area was much more occupied during winter.

The location budget of the male has less variation between summer and winter. The remarkable difference in the cave is the total negligence for the big stone during winter, while he spent nearly 5% of its time during summer. Most differences are visible outside. During winter, the male panda left over the yard area 1 in profit of the yard area 2 (where bamboo was mainly placed) and the wooden construction 1. He also spent less time in the other area of his enclosure, especially the pool of water (where it performed stereotypies during summer).
1.3 ENVIRONMENTAL FACTORS

Pandas were exposed to different environmental factors which are presented in table 3.

*Table 3 Summary of the average measurements and standard deviation of the environmental factors which may have an influence on the behaviour of the giant pandas at Pairi Daiza.*

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Temperature outside (°C)</td>
<td>22.12 ± 3.41</td>
<td>9.03 ± 3.63</td>
</tr>
<tr>
<td></td>
<td>21.42 ± 4.69</td>
<td>8.22 ± 2.58</td>
</tr>
<tr>
<td>Relative humidity outside(%)</td>
<td>70.79 ± 14.38</td>
<td>97.48 ± 4.00</td>
</tr>
<tr>
<td></td>
<td>75.82 ± 16.08</td>
<td>98.96 ± 2.50</td>
</tr>
<tr>
<td>Visitor presence (% of space occupied)</td>
<td>32.34 ± 9.88</td>
<td>0.51 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>43.39 ± 12.58</td>
<td>0.57 ± 1.12</td>
</tr>
<tr>
<td>Sound pressure outside(dB)</td>
<td>68.61 ± 1.43</td>
<td>62.63 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>66.99 ± 1.73</td>
<td>63.53 ± 1.51</td>
</tr>
<tr>
<td>Total construction or demolition days / total days observation</td>
<td>0/24</td>
<td>14/18</td>
</tr>
</tbody>
</table>

Temperature, relative humidity and sound pressure are similar for both animals. The average temperature measured when pandas were outside in summer is greatly different from the one in winter, with more than 20°C against less than 10°C. Variation for these measures may be considered low and relatively constant. Conversely, relative humidity is higher in winter than in summer (nearly 100% and around 70%). The standard deviation is, however, much higher in summer than in winter. On the other hand, sound pressure is slightly more elevated in summer than in winter. In addition, standard variation is low. All these values correspond to measurements taken when pandas were outside, since temperature and humidity were maintained constant in the cave (19°C in summer, 13.5°C in winter and 86% relative humidity), as well as sound pressure was considered insignificant in the cave.
The distinction between visitor presence in summer and winter is clear, as the park was opened during summer observations and closed during the winter. The only human presence besides the observer in December consisted of the keepers, workers and guests invited by the park. Visitor presence during summer was superior on the male side than on the female side. Nevertheless, the standard deviation is quite big for this measurement.

Important construction and demolition works took only place during the winter. They were conducted every day of the week with the exception of Sunday and, sometimes, Saturday. This explains the much greater number of days of work in winter. These works were surely the same for both pandas. As a result, only one measure (similar for the female and the male) appears in the table.

2 INFERENTIAL STATISTICS

2.1 GENERAL OVERVIEW

A summary of the results on the effect of season, temperature, relative humidity, sound and visitors is illustrated in table 4. Results are considered significant if the p-value is above 0.05.

The results of the columns Season, Temperature and Relative humidity come from the statistical test on effect of season and climatic conditions whereas the results in the columns Sound and Visitors come from the statistical test on effect of sound level and visitor presence. Correlation between sound level and visitor presence was tested and illustrated in figure 11, with the measures made at the male (represented by a 1) side in blue and the measures at the female (represented by a 2) side in red. Pearson correlation coefficient equals 0.62499, with p-value < 0.0001. A high correlation implies that both variables contain the same information. This might imply that a model with both sound and visitors leads to one of both variables not having a significant effect on behaviour whilst this would be the case if one of both (redundant) variables is removed. This is the reason why models with removal of fixed effect (or noise level or visitor presence) were built. The results of these additional tests are not presented in this table but are discussed in point 2.3 “Results for sound and visitors”.
Table 4 Effect of different environmental factors on some behaviours of two giant pandas.

<table>
<thead>
<tr>
<th>Season</th>
<th>Temperature</th>
<th>Relative humidity</th>
<th>Sound</th>
<th>Visitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(compared to winter)</td>
<td>For temperature increase</td>
<td>For relative humidity increases</td>
<td>For sound increase</td>
<td>For visitors presence increase</td>
</tr>
<tr>
<td>Locomotion</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td>-</td>
</tr>
<tr>
<td>Resting</td>
<td>↑</td>
<td>-</td>
<td>↑</td>
<td>-</td>
</tr>
<tr>
<td>Performing stereotypies</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↓</td>
</tr>
<tr>
<td>Performing door-directed behaviour</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Being vigilant</td>
<td>↓</td>
<td>-</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Feeding</td>
<td>↓</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Drinking</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
<tr>
<td>Foraging</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↓</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>↑</td>
</tr>
</tbody>
</table>

Significant (p-value < 0.005) modifications in behavioural duration in function of season, temperature and relative humidity and modification of behavioural occurrence in function of sound level and visitors (↓ = decrease, ↑ = increase).
Figure 11 Correlation between visitor presence (expressed in % of space occupied) and the sound level (in dB)

2.2 RESULTS FOR SEASON AND CLIMATIC INFLUENCES ON PANDA BEHAVIOUR

The table below (table 5) illustrate the results obtained from the statistical test on effect of season and climatic conditions.
<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Least squares mean of behaviour duration (in % of time observation)</th>
<th>Effect of season: Difference summer minus winter</th>
<th>Effect or relative humidity: For every one percent increase</th>
<th>Effect of temperature: For every one Celsius degree increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Change of the behaviour mean duration (in %)</td>
<td>Change of the behaviour mean duration (in %)</td>
<td>Change of the behaviour mean duration (in %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Locomotion</td>
<td>7.5</td>
<td>-4.3213</td>
<td>0.0012*</td>
<td>-0.2457</td>
</tr>
<tr>
<td>Resting</td>
<td>46.7</td>
<td>20.8871</td>
<td>&lt;0.0001*</td>
<td>0.5337</td>
</tr>
<tr>
<td>Performing stereotypies</td>
<td>11.58</td>
<td>-1.3431</td>
<td>0.9081</td>
<td>-0.09122</td>
</tr>
<tr>
<td>Door-directed behaviour</td>
<td>2.24</td>
<td>-3.9813</td>
<td>&lt;0.0001*</td>
<td>-0.0389</td>
</tr>
<tr>
<td>Being vigilant</td>
<td>1.16</td>
<td>-0.5617</td>
<td>0.0383*</td>
<td>-0.02137</td>
</tr>
<tr>
<td>Feeding</td>
<td>23.12</td>
<td>-11.1439</td>
<td>0.0012*</td>
<td>0.07956</td>
</tr>
<tr>
<td>Drinking</td>
<td>0.81</td>
<td>0.2156</td>
<td>0.8116</td>
<td>0.00089</td>
</tr>
<tr>
<td>Foraging</td>
<td>3.08</td>
<td>-0.6591</td>
<td>0.7009</td>
<td>-0.05382</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>1.22</td>
<td>1.4904</td>
<td>0.1170</td>
<td>-0.02899</td>
</tr>
</tbody>
</table>

The relative mean duration of each behaviour is expressed (in percentage) in the second column. For season, the mean duration change of each behaviour is exhibited, with summer compared to winter. For climatic conditions, the relative mean duration change is shown in function of every one-unit increase. * indicates p-value < 0.05.

The effect of season is found significant for five behaviours: locomotion, resting, door-directed behaviour, being vigilant and feeding. These changes in behaviour duration are mostly consequent if compared with the least squares mean of behaviour duration. For instance, the absolute decrease of 0.56% of being vigilant, which could be considered as negligible, is proportionally big in comparison of
the general low percentage of being vigilant mean duration. All behaviours appear to be reduced in summer, except for resting, which undergoes the biggest absolute change (increase of more than 20% in summer compared to winter). Even if it is not found significant, the effect of season on self-grooming tend to be spectacular.

The same behaviours, with the exception of feeding, are similarly shown to be affected by the relative humidity. In like manner, all these behaviours tend to decrease with the relative humidity increase, aside from resting. Changes may appear small, they reflect a modification induced by an increase of only one percent relative humidity. As this could vary from 0 to 100, behavioural changes induced by relative humidity could be 100 times higher than these presented in the table.

On the other hand, the effect of temperature does not appear to be significant, on any behaviour.

2.3 RESULTS FOR SOUND AND VISITOR PRESENCE

The statistical results of the test on sound and visitors are presented in the table underneath (table 6). Values come from the model with both fixed effects, sound and visitors. If a result was only found significant in the additional models (with removal of one effect), the result is discussed below. Similarly to sound, results are expressed by one-unit increase (thus one decibel and one percent of space occupied by visitors). However, both sound and visitor presence may vary much more than one unit. Indeed, most sound records were between 50 dB and 85 dB, as well as visitor presence could vary from 0 to 100%. Therefore, the odds of occurrence may ultimately be much more different than expressed in the table. For example, the odds for door-directed behaviour = 1, which is 0.867 times smaller for 1 dB increase (around 65dB), becomes 0.240 times smaller for 10 dB increase. Similarly, this odds is 0.982 times smaller for a 1% space occupied by visitors increase but is 0.574 times smaller for an increase of 30%.

The effect of sound is found significant for every behaviour in the model with both effects, sound and visitors, with the exception of locomotion and resting. However, if effect of visitor is removed, the effect of locomotion is found significant (odds ratio = 1.049, p-value = 0.0362). The effect seems to be particularly high on door-directed behaviour, being vigilant, drinking and self-grooming. Door-directed behaviour and being vigilant are inhibited by a sound increase, as opposite to drinking and self-grooming, increasing with the sound.

Locomotion, foraging and self-grooming are significantly and clearly increased with visitor presence, as opposite to resting, performing stereotypies and performing door-directed behaviour. Being vigilant, feeding and drinking are found significant in the model with only visitor effect. Their odds ratio, is then respectively 0.984 (p-value = 0.0010), 1.028 (p-value < 0.0001) and 1.027 (p-value < 0.0001). Thus being vigilant seems to decrease with an increase in visitor presence, as opposite to feeding and drinking, which tend to increase.
The behavioural difference for each one-unit increase is lower for visitor presence than for sound. However, effect of visitors is not in any case lower because variation in visitor presence can be higher than variation for sound. Indeed, sound mostly vary of 30 dB as visitor presence may vary of 100%.

Table 6 Effect of sound and visitors on some giant panda behaviours (* = significant result).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Effect of sound:</th>
<th>Effect of visitors presence:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>odds ratio if sound rises in 1 dB around 65 dB</td>
<td>odds ratio if space occupied by visitors rises in 1% around 14.4%</td>
</tr>
<tr>
<td></td>
<td>Odds for behaviour</td>
<td>p-value</td>
</tr>
<tr>
<td>Locomotion</td>
<td>1.004</td>
<td>0.8752</td>
</tr>
<tr>
<td>Resting</td>
<td>0.983</td>
<td>0.3495</td>
</tr>
<tr>
<td>Performing stereotypies</td>
<td>0.960</td>
<td>0.0413*</td>
</tr>
<tr>
<td>Door-directed behaviour</td>
<td>0.867</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Being Vigilant</td>
<td>0.822</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Feeding</td>
<td>1.067</td>
<td>0.0031*</td>
</tr>
<tr>
<td>Drinking</td>
<td>1.308</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Foraging</td>
<td>0.898</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>1.196</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

2.4 EFFECT OF PSEUDOPREGNANCY ON THE FEMALE BEHAVIOUR

The effect of pseudopregnancy was tested on two behaviours, resting and self-grooming. The duration of self-grooming (on a daily basis) appeared to be ,18% smaller during the eight last days of the summer observation than during the previous sixteen days. However, this effect is not significant (p-value = 0.4979). Conversely, the effect of pseudopregnancy on resting behaviour was significant,
causing a decrease of 16% of resting duration in comparison with the presumed period of basal sexual activity (p-value < 0.0001).
DISCUSSION

The statistical analysis has provided many significant results. With the exception of temperature, environmental factors are shown to have a significant impact on the activity budget of pandas, at least on the behaviours they performed most frequently. However, some of these effects appear to be biologically irrelevant. The interpretation of these results is discussed below.

1 ACTIVITY BUDGET AND INFLUENCE OF SEASON

Amongst all the environmental factors, season appears to be one of the most relevant factor for a change in behaviour. In the summer, pandas rest more, with a difference of 20.9% in their mean duration of resting. Subsequently, the duration of locomotion, stereotypies, door-directed behaviour, being vigilant, feeding and foraging decreased, in a substantial way, and most of all for feeding (a decrease of 11.4%). However, the statistical analysis has been performed on data of both pandas, together. If the seasonal activity budget of the male is analysed, the duration of resting is not really different between summer and winter, with 41.2% and 40.4%, respectively, of his time dedicated to rest. His activity level thus seems to be the same in August and in December, which is consistent with the findings of Johnson et al. (1988) and Zhang et al. (2015) who did not detect a change in activity levels during these two months. This also corresponds to resting for 43% of day observed in the wild by Johnson et al. (1988) but is much higher than measures made on pandas in captivity (ranging from 9% to 34%) by Powell et al. (2006) and Liu et al. (2003).

The considerable contrast of resting duration between summer and winter in the statistical test likely comes from the enormous change in the female’s behaviour. She devoted nearly 80% of her time to resting in summer, presumably due to her pseudopregnancy, since female pandas tend to show a reduction in activity a month before parturition (Zhu et al., 2001). In winter, this time was reduced to 40%. Although her resting behaviour was shown significantly impacted, no reliable test (for instance, endocrine analyses) has been performed to confirm the pseudopregnancy of the female, nor the start and end date. The division of the period in sixteen and eight days was only based on behavioural observation, not on objective hormonal data. Therefore, no definitive conclusion can be drawn for the behaviour of the female in summer. Her reproductive status may have affected all her activity budget in this season. Furthermore, such a discrepancy should obviously have an impact on all the statistical results. It could also be the cause of variation in other results. Therefore, the results should be interpreted with care.

For example, self-grooming is, with drinking, the only behaviour appearing to have increased in summer. This change, even if not significant, could be a consequence of the reproductive status of the female. In summer, the female frequently licked her vulva, with self-grooming occupying on some days up to 10% of her daily time. However, this behaviour was not found significantly impacted in the test for pseudopregnancy.
Another heavily altered behaviour is feeding. Its mean duration is significantly 11.4% smaller in summer versus that in winter. By comparing its mean duration between the two activity budgets, the female panda of Pairi Daiza tripled her time dedicated to feeding from summer to winter. This is probably another consequence of the female’s pseudopregnancy, as female pandas display a period of hyperphagia followed by a time of decreased appetite (Owen et al., 2005) during pregnancy and pseudopregnancy. A similar but smaller change can be seen in the male budget, that switched from a mean feeding time of 20% to 26%. It can thus be supposed that the pseudopregnancy of the female is not the only cause of this significant change. Owen et al. (2005) suggest that colder temperatures play a beneficial role in panda activity and appetite. It could consequently explain a rise in feeding in colder seasons. However, in this study, temperature has not been proved to have any significant effect on panda behaviour. Furthermore, this feeding duration is lower than the one found in other activity budget, usually around 30% (Liu et al., 2003; Owen et al., 2005; Powell et al., 2006). Though ethograms in these studies are not the same than the one used here, this difference might be meaningful.

The foraging behaviour does not seem to be much impacted by the season. Statistical analysis revealed a non-significant reduction of 0.66% of the foraging mean duration in summer compared to winter. Nonetheless, activity budgets divulge an opposite trend between the two animals, which could be the reason for this result. The female nearly quadrupled her time devoted to foraging from summer to winter (0.9% against 3.2%) whereas the male reduced this time (from 4.4% to 2.9%). The rise in foraging of the female is probably again explained by her pseudopregnancy decreased activity. The presumed decreased time in foraging of the male may have different explanations. First of all, since this difference between the two seasons has not been tested specifically for the male, it is impossible to draw a conclusion about the significance of this variation, especially since the variance is quite high. Despite this fact, if there is a difference, this decrease in foraging in winter could be the consequence of the action of the keepers. They regularly threw some carrots, apples, pieces of cake and even bamboos shoots into the panda enclosure, triggering foraging and feeding. It might be possible that these actions took place more frequently in summer to distract the pandas, which could have promoted foraging, but no systematic data were collected.

The effect of season on the drinking behaviour does not tend to be spectacular. A non-significant decrease of 0.22% in summer has been calculated. No relevant distinction appears even if activity budgets of both animal are separately analysed, with the exception that the male seems to drink much more than the female (around 1.5% of his daily time compared to around 0.2% of hers). The observation of this behaviour is possibly biased by the fact that a water trough was placed outside the field of observation. The female drank presumably more often from this one.

According to the statistical tests, stereotypies appear to be lower in summer than in winter. This effect is yet not significant, although, this does not appear to be verified by the female activity budget. She seems to have spent 7.5% of her winter time in stereotypies against only 0.5% in her summer time. This considerable difference might be explained by her lethargy in summer. It is therefore difficult to
make a valuable comparison for these stereotypies. This time is, in any case, lower than the time the male devoted to stereotypies, which was 20% in summer and 12% in winter. This difference is noticeable. It is known that male pandas are more prone to develop stereotypies than females (Swaisgood et al., 2006b), as a possible consequence of the males' larger home range in the wild, making them more affected than females (Liu et al., 2006). Furthermore, the male was peer-reared, which could be of importance to his behaviour. Snyder et al. (2003) suggest that subtle behavioural deficiencies are the consequence of peer-rearing because of lower social stimulation and interaction with the mother. His stereotypies rate was anyway higher than in most other studies (Liu et al., 2003; Powell et al., 2006). Only Liu et al. (2006) detected an even high duration of stereotypies bus their study focused on stereotypic behaviour.

Performing stereotypies can be an indicator of diminished well-being hence the importance of taking it into account (Liu et al., 2003; Liu et al., 2006; Swaisgood et al., 2006b). Restricted and monotonous environment is thought to be the cause of this abnormal panda's behaviour, although other causes are not excluded. The semi-natural enclosures in Pairi Daiza should thus be a favourable factor against stereotypies (Liu et al., 2003; Swaisgood et al., 2003; Swaisgood et al., 2006b). However, location budgets of both pandas show a limited use of these enclosures, pandas staying most of the time at specific locations (in the cave and in the yard area 1, thus nearest the cave, where they usually performed stereotypies). Another explanation for stereotypies could be a frustrated motivation. Pandas mostly performed stereotypies in the yard when they did not have access to the cave and generally stopped inside. Consequently, their motivation was probably to go inside, because of low noise pressure, a controlled atmosphere, less public pressure, etc. However, the female has been observed, in winter, going back into the yard in the afternoon, then starting to perform stereotypies. Since she had access to her cave, her motivation had to be another one. This might also be the evidence of emancipation (Mason, 1991; Vickery and Mason, 2004). Food anticipation is also believed to be a cause of repetitive behaviours. As these pandas received most of their food in the cave and little in the yard, they may be therefore more prone to go in the cave (Swaisgood et al., 2003; Liu et al., 2006; Swaisgood et al., 2006b). Furthermore, pandas are known to select with great care the bamboo they consume. The pandas of this study were regularly observed showing interest in the bamboo given but could quick turn away of it, neglecting this food. Moreover, their feeding time has been found quite low. It might be possible that the quality of the bamboo may play a role in the behaviour of these pandas, as they show a high selectivity for their food (Rybiski Tarou et al., 2005; Hansen et al., 2010).

Notwithstanding, the behaviour of the pandas in winter was considerably disturbed by another element. They actually succeeded in opening the door between their caves, leading to a fight which can obviously have impacted their behaviour. Their door-directed behaviour has been shown to be significantly higher in winter than in summer, during which the duration was comparable to the one shown by Powell et al. (2006) The male switched from 0.6% of his time devoted to this behaviour in summer to 3.9% in winter, and the female switched from 0.1% to 3%. They could spend a lot of time and energy getting excited in front of this door, which was not observed in summer, when they only
smelled and investigated it. Thus, according to the substantial impact it could have had on the door-directed behaviour, this episode might also be the cause of general behaviour modification, which could consequently not be representative of the behaviour they would express in winter in general. Furthermore, season has also been confounded with the effect of construction and demolition works. Seeing that all works were performed almost entirely in winter and none in summer, it is impossible to make a distinction between these effects.

The door event could for example be the reason of the increased mean duration time of being vigilant in winter. Thanks to the statistical analysis, a significant difference of 0.56% has been revealed between summer and winter. In view of the relative low duration of this daily behaviour this difference is possibly considered as relevant. This duration actually nearly tripled for the female (from 0.6% of her daily time to 1.7%) whereas it increased only slightly for the male (from 0.8% to 1.2%). The significant increase of this behaviour could be the consequence, as for many of the behaviours, of the lethargy of the female in summer. Nonetheless, the male’s mean daily duration of being vigilant also exhibits an increase, even if this was less pronounced. It is difficult to establish the cause of this increase, seeing that many distinctions may be made between summer and winter (climatic conditions, visitors presence, works,...). Further analysis, below, of some of these factors may give some clues to the explanation of the behaviour modification.

The effect of season was tested on a last behaviour in the statistical tests. Locomotion appears to be significantly reduced in summer (mean relative duration in summer 4.3% smaller than mean relative duration in winter). This is, again, probably mainly due to the increased activity of the female when she was not in pseudopregnancy. In spite of this, the male also seems to perform more locomotion in winter (10% against 7%), which remains in the range shown by Powell et al. (2006). This increased activity has not been tested separately for the male, preventing any conclusion about the significance of this difference. If this difference is real, an increased activity could be explained by the more suitable climatic conditions of winter for the pandas, according to Owen et al. (2005). Nevertheless, as it is the case for feeding, temperature does not appear, in this study, to have a significant influence on panda behaviour.

The effect of the different factors was not tested on the other behaviours of the ethogram, which were performed during less than 1% of the day by the pandas. They also exhibit a large standard deviation. Statistically, they were therefore less relevant, since it would probably have led to many non-significant results in any case. Furthermore, these behaviours do not match with the literature. Some behaviours may still be important such as marking behaviours. Ano-genital marking soar for both animals in winter. The male also exhibit a considerable increase in urine marking in this period. This massive augmentation in marking could be the consequences of the “door event”. In the wild, direct encounters are relatively rare (Swaisgood et al., 2006b). Furthermore, after breaking the door, the pandas exchanged their enclosures, a period characterized by an elevated rate of investigating and marking. Even if pandas are believed to be solitary, they presumably communicate through a complex and sophisticated chemical system (Swaisgood et al., 2003; Swaisgood et al., 2006b; Wei et al., 2015).
Scent markings, via their urine and ano-genital glands, allow for the persistence of their signals in the environment. Counterparts are thought to be able to extract much information from these. By exchanging their enclosures, the pandas occupied the restricted home range of their analogous and left many signals. This may thus be the cause of an increase in marking behaviour in winter.

The use of the duration was also not relevant for some behaviours like vocalising and startling. These really short behaviours are probably best characterized by the frequency of their bouts (Powell et al., 2006; Swaisgood et al., 2006a). Additionally, recording vocalising was difficult during the observation. As pandas mostly do not express vocalising, a record is only possible when the sound they produce is audible. This was not possible when pandas were in the insulated caves or when the observer was surrounded by a crowd. Moreover, pandas vocalised simultaneously with other behaviours (probably mainly locomotion), preventing recording vocalising as the behaviours were mutually exclusive. In light of this, the vocalising record is probably not reliable.

Effect of season was also visible in location budgets, with the female much more inside in summer than in winter and standard variations much higher in winter. These elements can be explained by the schedule followed by the pandas. In summer, the pandas generally went immediately inside when they could (at the opening of the doors, around noon) and remained there whereas in winter they would go back outside in the afternoon. During the colder period (in December), they occasionally stayed in the yard until the keepers called them back inside for the night. The female in particular could stay outside longer in winter, leading to a long mean time outside in this season. As well, since she was in pseudopregnancy during some days of the summer observations, she also spent a lot of time resting and therefore sometimes staying in the cave all day, leading to a considerable shorter time spent outside in summer. This happened equally during the winter on a few occasions. The male’s schedule inside and outside was more constant, leading to a similar time outside in summer as well as in winter.

2 INFLUENCE OF CLIMATIC CONDITIONS ON PANDA BEHAVIOUR

The statistical tests did not reveal any significant influence of temperature on panda behaviour. However, with an increase in temperature pandas possibly decrease their activity (locomotion, being vigilant, foraging and self-grooming are susceptible to be inhibited) whereas they presumably perform more stereotypies. This appears to agree with the fact that pandas are believed to prefer colder temperatures, allowing them to achieve a higher activity and feeding rate (Owen et al., 2005; Ellis et al., 2006). However, specific studies on effect of temperature on panda behaviour are lacking. Although high temperatures are shown to provoke a lower activity in bears in some studies (Garshelis and Pelton, 1980; Schwartz et al., 2010), McLellan and McLellan (2015) similarly found that grizzly bears are probably not affected by the temperature. They underlined the resistance of these bears to heat, despite their poor adaptation to it. Likewise, even though the pandas of this study experienced quite high temperatures (up to 30°C), there is no evidence of a relevant and significant impact of temperature.
On the contrary, relative humidity seems to have a significant influence on the mean relative duration of, at least, four behaviours: locomotion, resting, performing door-directed behaviour and being vigilant. Moreover, this influence can be biologically relevant. An increase in relative humidity could be the reason for a decline in locomotion, door-directed behaviour and being vigilant, as well as an increase in resting. This may be logical, as a high relative humidity prevents water evaporation, which is the basis for heat dissipation (Brody, 1956). Furthermore, heat dissipation is necessary if body temperature increases, for example, during animal activity. A higher relative humidity might thus be the reason for a decrease in animal activity, thus an augmentation of resting. It also appears that performing stereotypies and foraging could drop with a rise in relative humidity, which might strengthen this hypothesis. Drinking and feeding tend to increase with the relative humidity but this effect is not considered to be biologically relevant.

Even though some influences have been proven, climatic conditions do not seem to have a spectacular effect on panda behaviour. Only temperature and relative humidity were measured although many other factors could be taken into account such as solar radiation and air movements. For example, the animals, especially the male, are believed to be sensitive to storms. They seemed to be more nervous at the approach of and during a storm. This can though not be verified, due to lack of data. Measures were also not taken with professional equipment, as well the thermometer could have been located in a suboptimal position (not in the enclosure of the pandas). These factors may possibly have led to error in the data.

3 INFLUENCE OF SOUND LEVEL AND VISITORS PRESENCE ON PANDA BEHAVIOUR

Our study indicated that the influence of sound is significant and biologically relevant for most behaviours. High sound levels seem to largely inhibit stereotypies, door-directed behaviour and vigilance. In other words, these abnormal behaviours (door-directed and stereotypies) increase if the noise level is lower. Noise is shown to encourages feeding, drinking and self-grooming, which should not be detrimental. The effect on other behaviours might be neglected. Noise is thus not believed to be associated with poor well-being. This is the opposite to what is found in other studies, where noise is associated with stress-related behaviours (Owen et al., 2004; Powell et al., 2006).

However, animals might show behavioural habituation to sound but still be stressed (Quadros et al., 2014). Besides, noise measurements in this study are censurable. Chiefly, sound was measured at the visitor side and not in the panda enclosure. The decibel pressure can thus be very different between these two places, markedly if the measure was taken beside a loud visitor. Also, music was played at the visitor walkway, which could similarly create interference. Above all, the quality of the decibel meter employed is questionable. The observer noted, the penultimate day of observation, that pushing on the button to measure the maximum decibel level created on its own a sound in the apparatus. Thus, if the button was not pushed in a very soft way, the level indicated was inevitably above 60dB. Similarly, the instrument was never calibrated, as performed in other studies (Quadros et al., 2014). The quality of the apparatus used has thus probably created an error in the data.
Moreover, noise measurements were based on only one characteristic of the sound: its amplitude. Yet, sound is also characterized by its frequency and its duration. Indeed, Owen et al. (2004) found different responses as a function of the sound’s frequency. On the other hand, this study’s decibel meter was intended for human use which probably means that it monitored the A-weighted frequency spectrum. Other studies on giant pandas preferred instruments monitoring the broad-spectrum frequency (L-weighted) (Owen et al., 2004; Powell et al., 2006). As the perceivable auditory frequency spectrum is not described in ursids, pandas could also possibly hear lower and higher sounds than humans (Owen et al., 2004). The choice of the instrument could have compromised any conclusion.

The effect of visitors on panda behaviour is not easy to define. However, it probably does not increase abnormal behaviours as it could be beneficial, by leading to a moderate increase in foraging, drinking and self-grooming. At first sight, visitor presence does not seem to be prejudicial to animal well-being. Only the rise in locomotion and the fall in resting time could suggest a more nervous status of the animals. Even though visitor presence is believed to become detrimental above a specific threshold for every species (Fernandez et al., 2009), literature on giant pandas is missing. However, one might argue that, here, visitor presence was only assessed according to its number. Visitor population may, though, be defined by many characteristics: number, density, agitation, noise, etc. (Davey, 2007). Visitor presence actually differed between inside and outside. In the cave, a smaller number of visitors could lead to much higher percentage of space occupied, since the space was much smaller. Moreover, visitors were much closer to the animals in the cave than in the yard. According to Fernandez et al. (2009), the negative effect of a crowd is predominantly determined by its type of interaction rather than its number. Another possible meaningful factor of stress is the occurrence of camera flashes. It has been observed that the female frequently hid her eyes when inside, where the darkness promoted the occurrence of this possible stressor. Nevertheless, these effects have not been tested in this study in assessing the effect of number of visitors.

4 ADDITIONAL COMMENTS

In this study, activity budgets were used in order to evaluate the behaviour of two pandas at Pairi Daiza. However, interpretation of activity budgets may be complex, as they do not reveal everything. For instance, modification in welfare is not always translated by a change in the animal activity budget. It is thus of crucial importance to pay attention to how the animal looks and behaves (Swaisgood et al., 2006a).

Many other factors may influence an animal’s behaviour. The effect of solar radiation and light, animal keepers and odours could as well be determinant in animal well-being. The decisions of keepers are believed to have a meaningful impact on the animals such as determining the schedule for the animals (opening and closing of the doors), provisions of enrichment and deciding what food is furnished. For example, one keeper tended to give much more cake to the male than the others, leading to a possible decrease of bamboo consumption, allowing more time for stereotypies. Zhang et al. (2004) underlined the importance of husbandry practices. Gentle management styles were apparently
associated with increased reproduction success in timid females. According to the same author, temperament of the animals may, as well, be of great importance. The male of Pairi Daiza was considered more nervous and fearful, which may be reflected in his activity budget (higher rate of stereotypies, door-directed behaviour, locomotion, etc.).

Another criticism of activity budgets is that they only take into account the duration of the behaviours. In this light, short behaviours will not appear important, although their only occurrence may have a signification (vocalising for instance).

5 CONCLUSION

This study provides knowledge about the two animal’s time budgets within the Pairi Daiza context. This individual information may be used to evaluate the pandas behaviour and form a basis for further research. Indeed effects of management changes may be assessed by making a comparison to this baseline. It may help husbandry and management decisions for these two animals, such as the implementation of an environmental enrichment. This could include some efforts to increase behavioural variability and reduce the occurrence of stereotypic behaviour.

Furthermore, a considerable effect of season on panda behaviour has been found, as well as an influence of sound and visitors, even so none of these factors are believed to be prejudicial for animal well-being. However, two notable biases (the female’s pseudopregnancy, the fight before the second month observation) have compromised the interpretation of these results.
LIST OF REFERENCES


Wei, F., Y. Hu, L. Yan, Y. Nie, Q. Wu, Z. Zhang (2015). Giant pandas are not an evolutionary cul-de-sac: evidence from multidisciplinary research. Molecular Biology and Evolution 32, 4-12.


APPENDIX

*Illustrations of the pandas enclosure*

<table>
<thead>
<tr>
<th>Male enclosure</th>
<th>Female enclosure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The yard</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image1" alt="Male enclosure" /></td>
<td><img src="image2" alt="Female enclosure" /></td>
</tr>
</tbody>
</table>

*Figure 12*

*Figure 13*

*Figure 14*

*Figure 15*
The cave

Figure 16

Figure 17

The male cave. The visible window is the separation between the male and female panda caves

Figure 18

The female panda cave. The right window allows visitors to look at the cave, the left window is the separation between the male and female panda caves

Figure 19