Linköping University | Department of Physics, Chemistry and Biology
Type of thesis, 60 hp | Educational Program: Physics, Chemistry and Biology
Spring or Autumn term 2019 | LITH-IFM-x-EX—19/3610 --SE

Effect of sensory enrichments on the behaviour of captive Northern lynx (*Lynx lynx lynx*) and assessment of automated behaviour monitoring technologies

Uranie JEAN-LOUIS

Examinator, Per Jensen
Tutor, Mats AMUNDIN

Avdelning, institution
Division, Department

Department of Physics, Chemistry and Biology
Linköping University

Datum
Date
02/06/2019

Språk
Language

☐ Svenska/Swedish
☒ Engelska/English

☐ _______________

Rapporttyp
Report category

☐ Licentiatavhandling
☒ Examensarbete
☐ C-uppsats
☐ D-uppsats
☐ Övrig rapport

☐ ____________

ISBN

ISRN: LITH-IFM-x-EX--19/3610--SE

Serietitel och serienummer Title of series, numbering

ISSN ___________________________

URL för elektronisk version

Titel
Title

Effect of sensory enrichments on the behaviour of captive Northern lynx (*Lynx lynx lynx*) and assessment of automated behaviour monitoring technologies.

Författare
Author

Uranie JEAN-LOUIS

Sammanfattning
Abstract

Captive environments like zoo exhibits offer limited space, lacking many of the environmental stimuli that are present in the wild. This may reduce animal welfare and potentially lead to the development of stereotypic behaviour like pacing. Environmental enrichment is used to prevent and reduce pacing and enhance animal well-being. The aim of this project was to evaluate sensory enrichments, and the effect of such enrichment on pacing in a group of Northern lynx by means of new, automated monitoring technologies in combination with traditional visual observations. The lynxes were exposed to valerian, catnip and cinnamon as olfactory enrichment. The acoustic enrichments were play-backed mouse squeals, roe deer barking and lynx vocalizations, and live crickets. The responses of the lynx were recorded by logging their subcutaneous HDX pit tags, and Bluetooth Low Energy (BLE) tags mounted on collars and using a wildlife camera. The results showed that catnip elicited clear "catnip responses" i.e sniffing, rubbing, biting and licking. The sounds were found to attract the lynxes and increase their arousal. One of the sounds, the lynx calls, elicited social behaviour. However, none of the sensory treatments reduced pacing. The combination of these automated technologies with visual observation was powerful to evaluate the effect of enrichment on captive lynxes and to monitor their activity patterns and stereotypic behaviours. Sensory enrichment could also be used in the wild as lures to attract lynxes to BLE or HDX PIT tag logging stations and to wildlife cameras, as part of monitoring a lynx population.

Nyckelord
Keyword

Automatic monitoring, Environmental enrichment, Northern Lynx, Pacing, Odour, Sound

Contents

1 Abstract

Captive environments like zoo exhibits offer limited space, lacking many of the environmental stimuli that are present in the wild. This may reduce animal welfare and potentially lead to the development of stereotypic behaviour like pacing. Environmental enrichment is used to prevent and reduce pacing and enhance animal well-being. The aim of this project was to evaluate sensory enrichments, and the effect of such enrichment on pacing in a group of Northern lynx by means of new, automated monitoring technologies in combination with traditional visual observations. The lynxes were exposed to valerian, catnip and cinnamon as olfactory enrichment. The acoustic enrichments were play-backed mouse squeals, roe deer barking and lynx vocalizations, and live crickets. The responses of the lynx were recorded by logging their subcutaneous HDX pit tags, and Bluetooth Low Energy (BLE) tags mounted on collars and using a wildlife camera. The results showed that catnip elicited clear "catnip responses" i.e *sniffing, rubbing, biting* and *licking*. The sounds were found to attract the lynxes and increase their arousal. One of the sounds, the lynx calls, elicited social behaviour. However, none of the sensory treatments reduced pacing. The combination of these automated technologies with visual observation was powerful to evaluate the effect of enrichment on captive lynxes and to monitor their activity patterns and stereotypic behaviours. Sensory enrichment could also be used in the wild as lures to attract lynxes to BLE or HDX PIT tag logging stations and to wildlife cameras, as part of monitoring a lynx population.

Keywords : Automatic monitoring, Environmental enrichment, Northern Lynx, Odours, Pacing, Sounds.

2 Introduction

2.1 Stereotypic behaviour

Captive environment like zoo exhibits offers limited space which lack a lot of the environmental stimuli that can be found in the wild, like predators, prey and unpredictable events (Skibiel et al., 2007). In some cases, too static and barren captive environment can lead to reduced animal welfare, eventually potentially leading to the development of stereotypic behaviour (Chester Zoo, 2009; Swaisgood and Shepherdson, 2005; Watters, 2009). Stereotypic behaviour is defined as repetitive, fixed behaviour without a function and indicate poor welfare (Mason, 1991) . Stereotypic behaviour tends to be more present in captive/zoo carnivores, especially locomotory stereotypies. Usually, this form of stereotypic behaviour, called pacing, involves walking back and forth, walking in circles or in figure eights (Clubb and Vickery, 2006; Damasceno et al., 2017). A potential explanation for such locomotory stereotypies in carnivores is frustration due to inability to perform species-specific behaviours or because they were prevented from obtaining resources. For example foraging: searching for prey, hunting and capturing can be difficult to provide in captivity (Clubb and Vickery, 2006; Mason et al., 2007). Indeed, the inability to perform these specific behaviours could induce pacing in captive carnivores. Likewise, as suggested by Hughes and Duncan (1988), cited by Swaisgood and Shepherdson, (2006): "Animals may suffer, and develop stereotypies, in situations where they are motivated to perform behaviours but are frustrated from performing them." In addition, lack of stimulation and the incapacity to perform some desired behaviour could also lead to stereotypies. An example of a desired behaviour would be to be in contact with a conspecific/mate (Clubb and Vickery, 2006; Swaisgood and Shepherdson, 2006). However, Clubb and Mason (2007) suggested that pacing is associated with the natural home range of the species. They found that species with a natural large home range will develop higher frequency of pacing. Moreover, pacing might also occur when the animal encounters aversive stimuli or during a stressful situation extended in time and is prevented form avoiding it by the exhibit limitations. The causes for such stress could be the presence of too many visitors, or visitors coming too close, a noisy environment or being confined with an undesired conspecific (Swaisgood and Shepherdson, 2006). Consequently, in such situations the animal tries to avoid the aversive stimuli by moving as far away from the stressful situation as possible, and this escape reaction may be shaped into pacing by e.g. a fence or a wall and become fixed over a period of time (Clubb and Vickery, 2006; Swaisgood and Shepherdson, 2006). Furthermore, stereotypic behaviour could also be due to a malfunctioning of the Central Nervous System (Mason et al., 2007)

Pacing has been documented for different species of carnivores, and more specifically among felids, including tiger (*Panthera tigris*), snow leopard (*Panthera uncia*) and ocelot (*Leopardus pardalis*) (Macri and Patterson-Kane, 2011; Miller et al., 2008; Rose et al., 2017; Weller and Bennett, 2001a).

Environmental enrichment is used to manage these stereotypies or prevent them from developing. Swaisgood and Shepherdson (2006) define some strategies for environmental enrichment which will prevent or reduce pacing : 1) Imitate the natural environment by reproducing environmental factors found in the wild that will enhance natural behaviour; 2)

Provide a more complex environment by adding physical elements that will stimulate exploratory behaviour; 3) Boost the sensory stimulation; 4) Target specific frustrated behaviour like foraging; 5) Exclude stressful situations and 6) Implement enrichment which can be managed and controlled by the animal like a food delivery system.

2.2 Environmental enrichment

Environmental enrichment can be defined as changes in an animal's captive environment by providing environmental features that stimulate species-specific behaviour, and hence enhance its well-being (Maple and Perdue, 2013; Swaisgood and Shepherdson, 2006, 2005; Young, 2003). Young (2003) describes five main goals for environmental enrichment: 1) increasing behavioural diversity, 2) reducing the frequencies of abnormal behaviour, 3) increasing the range or number of natural, "wild" behaviour patterns, 4) increasing the positive utilisation of the environment and 5) increasing the ability to cope with challenges in a more normal way. Stimulating species-specific behaviour such as hunting, may be difficult to provide for captive felids, since it involves patrolling large distance to search a prey, and attacking it with the characteristic "stalk-rush-kill" . For ethical reasons live prey cannot be offered, and mechanical alternatives are difficult to realize, even if it is not impossible. For example Markowitz (1982) constructed an automated apparatus delivering artificial prey (rabbit and squirrel) for tigers when they indicated their desire to hunt by scratching trees which were provided with a sensing device. After the catch the tigers were rewarded with fresh meat. Moreover, finding a mate and communicating with conspecifics by scent marking and vocalization may also be complicated to provide due to the limited size of the enclosure and the low number of individuals in captivity compared to the wild (Shepherdson et al., 1998).

Environmental enrichment for captive animals can be classified into seven types: tactile, structural, cognitive, social, sensory, nutritional and human-animal interactions (Chester Zoo, 2009; Hoy et al., 2010; Maple and Perdue, 2013; Young, 2003). Tactile enrichment involves physical stimulation provided when e.g. manipulating objects. Structural enrichment focuses on the modification of the enclosure by adding new structures like platforms, visual barriers, hides etc. Cognitive enrichment stimulates the memory, decision-making and learning abilities of the animals by introducing problems that can be solved by the animal. Social enrichment refers to changes in the social composition by adding or removing individuals, conspecifics or other species. Mixed species exhibits are examples of captive environment offering social enrichment. Sensory enrichment involves stimulation of the animal's senses with the introduction of sound, scent or visual stimuli. Nutritional or feeding enrichment refers to food manipulation or searching. The food can be hidden in the enclosure, different types of food, and in carnivores, whole carcasses instead of pieces of meat, can be offered. Finally, human-animal interactions refer to the interaction between the keeper and the animal which can be performed during training sessions (Chester Zoo, 2009; Hoy et al., 2010; Maple and Perdue, 2013; Young, 2003).

2.3 Lynx ecology

The Eurasian lynx or Northern lynx (*Lynx lynx lynx*) is widely distributed in Europe and Asia, inhabiting mainly forests and/or mountain areas. The Eurasian lynx is classified as of least concern by International Union for Conservation of Nature (IUCN) (von Arx, 2018) and threatened mainly by conflicts with humans, such as poaching, or being killed by car or train collision (Breitenmoser, 2017; Krelekamp, 2004). The lynx is a solitary and crepuscular animal (more active during dusk and dawn) (Boulat, 2010; Krelekamp, 2004; Podolski et al., 2013). It is carnivorous and hunt mainly ungulates including roe deer, musk deer, red deer and reindeer. When ungulates are rare it favors small mammals such as hares, rodents, foxes, birds but also domestic animals like goats and sheep. They are effective hunters, stalking and attacking their prey after a short, fast rush. They can bring down larger prey by biting their throat, thus suffocating them (Boulat, 2010; Breitenmoser, 2017; Krelekamp, 2004; Podolski et al., 2013; SCANDLYNX).

2.4 Lynx monitoring in the wild

In northern Europe, the Scandinavian lynx research project SCANDLYNX (https://scandlynx.nina.no/) conducts studies on lynx ecology, social organisation, reproduction, predation and survival by monitoring them, with the main aim to maintain a sustainable lynx population on the Scandinavian peninsula . To monitor the lynx, SCANDLYNX uses invasive techniques such as mark-recapture, using dog or box traps. However, non-invasive methods can also be used to monitor lynx, such as camera trapping (Brassine and Parker, 2015; Monterroso et al., 2014) which is used by SCANDLYNX . Camera trapping detects the presence of an animal by taking pictures of animals moving in front of the camera and thus triggering it and may allow identification using specific marks on its fur (Brassine and Parker, 2015; Kelly and Holub, 2008; Monterroso et al., 2014). To maximise the number of detections the camera may be placed together with lures like scent lures (du Preez et al., 2014). Scent lures attract the animal's interest and stimulate investigation of the camera site (Monterroso et al., 2011; Schlexer, 2008). Scent lures can include plants, animal-based scent like urine or artificial scents (Schlexer, 2008). Garrote et al., (2012) found an increase in camera captures of Iberian lynx (*Lynx pardinus*) in sites which had live lures such as rock pigeons (*Columba livia*). A similar study on Eurasian lynx found a higher visit rate at cameras with scent lures than at cameras without (Classon, 2017).

Nevertheless, the scent in such lures needs to be correctly chosen and evaluated. Scent lures can be tested in the wild by assessing the detection rate of the target animal (Schlexer, 2008). To test the effect of different scent lures, studying animals in captivity can be more efficient. Specific behaviours towards the lure can be recorded, such as sniffing, scent marking, rubbing, licking and biting. As a result, the most effective scent lure can be assessed through these behavioural responses (Schlexer, 2008).

Valerian has been suggested to be an effective attractant for feline species (Monterroso et al., 2011; Steyer et al., 2013). In captive European wildcat (*Felis silvestris*) valerian elicited exploratory behaviour including an intensive rubbing behaviour (Monterroso et al., 2011). Valerian was found to elicit such rubbing behaviour only in wildcats but not in six other captive carnivores (Monterroso et al., 2011). Moreover, valerian has been used in the field to collect hair from European wildcat for DNA analysis (Steyer et al., 2013). In a study performed in the National Park Mavrovo in Macedonia valerian was used as a scent lure for camera trapping to estimate the distribution and minimum abundance of Balkan lynx (*Lynx lynx balcanicus*). Camera traps baited with valerian resulted in 29 lynx pictures taken of 7 to 10 Balkan lynxes present in the park area (Melovski et al., 2008).

Sniffing, licking and chewing with head-shaking, chin- and cheek rubbing, head-over rolling and body rubbing are known to be the four behavioural groups responses induced by catnip (Todd, 1963 cited in Tucker and Tucker, 1988). This specific "catnip response" has been seen in bobcats (*Lynx rufus*) and Eurasian lynxes Todd (1963) cited by Tucker and Tucker (1988). Catnip combined with beaver (*Castor canadensis*) castoreum have been found in the field to elicit similar behavioural response (rubbing) as valerian in Canadian lynx (*Lynx canadensis*) and Eurasian lynx (McDaniel et al., 2000; Schmidt and Kowalczyk, 2006).

2.5 Lynx senses

Like all felids, lynxes use their senses for communication. They use scent marking in connection with reproduction, social competition and territoriality (Allen et al., 2015; Vogt, 2015; Vogt et al., 2014, 2016). Scent marking includes urine spraying, scraping and claw raking, head or cheek rubbing and feces deposits (Allen et al., 2015; Boulat, 2010; Mellen, 1993; Vogt, 2015; Vogt et al., 2014, 2016). The scent mark from urine spray on trees or on the ground can last for several weeks . Lynxes spray urine by standing with the tail erected or crouching. Claw raking on trees leaves a visual mark together with scent from their sudoriparous glands situated between the pads of their paws. Similarly, head and cheeks possess glands which leave scents by rubbing. During defecation, scent from the anal glands is secreted (Boulat, 2010). Lynx scent markings usually convey information on identity, sex, age and sexual receptivity (Boulat, 2010). Lynxes scent mark more often during the breeding season and males scent mark more frequently than females (Allen et al., 2015; Vogt et al., 2014).

Many felids including the lynx use hearing to locate prey. Lynxes have very sensitive hearing and can detect frequencies up to ~45kHz, which allows them to detect a mouse from 65 meters distance and a roe deer from 500 meters distance (Kitchener et al., 2010; Krelekamp, 2004).

Mew, gurgle, purr, snort, spit, hiss and growl are the most frequent communication sounds described for the lynx (Peters, 1987). Mew serves to bring male and female together during the mating season (Boulat, 2010; Krofel and Kos, 2009; Peters, 1987). Gurgle is frequently used by females with kittens and during courtship and mating. Purr corresponds to the “rrrrr” rolling sound, usually heard during close contact due to its low amplitude. Snort is a threat signal. Spit, hiss and growl are agonistic sounds, indicating both aggressive or defensive motivation (Peters, 1987).

2.6 Sensory enrichment

Olfactory enrichment involves the introduction of scents to the animal’s enclosure. Scents can include herbs, spices, animal-derived scent like faeces and urine or artificial scents (Clark and King, 2008). Among felids, like cheetah (*Acinonyx jubatus*), tiger (*Panthera tigris*), jaguar (*Panthera once*), lion (*Panthera leo*), ocelot (*Leopardus pardalis*), and cougar (*Puma concolor*), the introduction of olfaction stimuli such as spices (cinnamon, chili powder, and cumin) have been shown to increase exploratory behaviour and reduce pacing (Damasceno et al., 2017; Skibiel et al., 2007). In the oncilla cat, the presentation of cinnamon has also been found to reduce pacing (Resende et al., 2011). Herbs including nutmeg and catnip have been found to increase activity of the black-footed cat (*Felis nigripes*) (Wells and Egli, 2004). Domestic cats showed a positive response (sniffing, licking, chin/ cheek rubbing or rolling) to catnip, silver vine, Tatarian honeysuckle and valerian (Bol et al., 2017).

Sound enrichment refers to the addition of sounds to the animal's enclosure. Sounds can include natural environment sounds, prey sounds, predator sounds, conspecific sounds, and non-natural sounds like classical or rock music (Wells, 2009). Among felids, African leopards (*Panthera pardus pardus*) have been found to increase their activity level and decrease stereotypies when exposed to prey sound (Markowitz et al., 1995). In lions (*Panthera leo*), the playback of naturalistic male lion roar was found to elicit live roaring (Kelling et al., 2012). Auditory enrichment in felids is poorly studied.

2.7 Aims and Predictions

Aims

- To evaluate the effect of sensory enrichments in three captive Northern lynx (*Lynx lynx lynx*) through behavioural observation.
- To measure the effect of sensory enrichments in in three captive Northern lynx (*Lynx lynx lynx*) by means of new, automated monitoring technology
- To evaluate the pros and cons with monitoring the behaviour of captive lynxes using automatic monitoring technology and traditional visual observations.
- To evaluate the effect of sensory enrichments on pacing in three Northern lynx (*Lynx lynx lynx*).

Predictions

- The number or the mean duration of *treatment directed behaviour* is higher when the sensory treatments are applied compared to the control treatment.
- The results of the automated monitoring techniques should support and supplement the results of the visual observations.
- The automated monitoring techniques but not the visual observations will reveal diurnal patterns in behaviour and activity

- The number and the mean duration of visits at the scent station as measured by the automated monitoring techniques should be higher with sensory treatments compared to control treatment.
- The duration of pacing should be reduced during the sensory treatments compared to the control treatment.

3 Materials and Methods

3.1 Lynx

The study was performed on three adult lynxes, two males and one female, kept at the Kolmården Wildlife Park, Sweden. The male Bore was born in 2005 in the wild near Bergsjö (Sweden), was found as an orphan and brought for rehab and raising in a Swedish zoo. He was transferred to Kolmården Wildlife Park in January 24, 2017. Loger was born, and parent.raised in 2012 in Kolmården Wildlife Park and the female Lovika was born and likewise parent-raised in 2011 in Kolmården Wildlife Park (Figure1).

Figure 1: The lynxes Bore (left), Loger (middle), Lovika (right). Photos taken by Evelina Torvi

3.2 Lynx enclosure

The lynxes were housed in a 2168m^2 outdoor exhibit. It was divided into four enclosures by 4m high Gunnebo fences, topped by metal sheets, tilted ca 45 degrees inwards: the main public display enclosure of 1625m^2, two back enclosures of 216m^2 and 282m^2, respectively, and a staff corridor of 45m^2 in between. All three enclosures have a lot of trees, trunks, rocks, and in the big enclosures there is also a high vantage point on top of a rock. There were electrical wires along the fences, close to the ground to prevent digging and climbing the fence (Figure 2). The visitors could see the exhibits through windows from a building along the western perimeter of the main enclosure. There was also access for the visitors to the northern fence of the main exhibit.

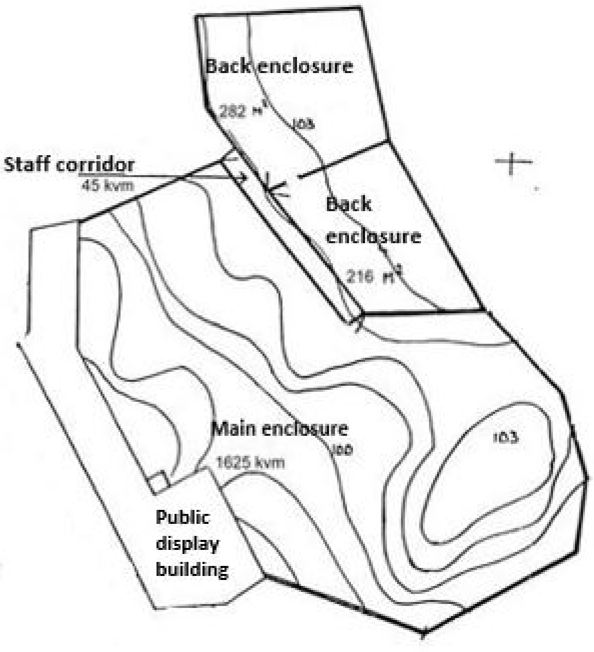

Figure 2: Schematic drawing of the outdoor exhibit of the lynxes

3.3 Daily routine /Husbandry

Between July and August, every morning around 8.30 am, the lynxes were gated into the back enclosures, allowing safe access to the main exhibit. This training was focussed on maintaining gating behaviour. The cats were fed small pieces of meat (mainly horse meat), thrown directly to each cat, as reinforcement to staying with the keeper. During this session, the various enrichments were introduced, the SD card in a wildlife camera was exchanged, and smartphone data was uploaded (see below). In the afternoon between 3 and 4 pm the enclosure fences were checked from the outside by the keepers.

In September and October, the lynxes were fed three time a week at different times of the day with several types of meat (horse ribs, whole mice, cow meat pieces) to be as unpredictable as possible, in addition to the training session mentioned above.

3.4 Experimental setup olfactory enrichment

3.4.1 Olfactory enrichment

In the present study, three odours were used as enrichment; valerian (*Valeriana officinalis*), catnip (*Nepeta cataria*) and cinnamon (*Cinnamomum verum?*). Catnip (F&T Fur Harvesters Trading Post, Catnip Oil, 30 ml) and valerian (Z-aim professional hunting equipment, Valerian extract oil, 30 ml) were oil solutions. Cinnamon was fine grinded (Kroken's). A control treatment containing only water was also tested. The odour treatments were applied on a branch (fir tree or spruce) using a paintbrush for catnip and valerian; for the control treatment the branch was washed with water and cinnamon powder was dusted on a wet branch. The branch was attached to a tree in the enclosure at a place called the scent station, inside a HDX PIT tag square loop antenna (see below) (Figure3). One odour was applied every morning, and it was replaced with another odour or control after 24 hours. A new branch was cut for each new odour. The odours were chosen by pseudo-randomization to avoid the same odour being applied on two consecutive days. No testing were performed on rainy days, since the rain might wash away the odour. Each odour treatment was presented a total of 9 days over a total of 36 observation days.

Figure 3: Scent station. 1 : HDX PIT tag antenna. 2 : Spruce branch, 3 : BLE tag logging smartphone inside a waterproof plastic box

3.4.2 Olfactory response monitoring

3.4.2.1 Behavioural observation

Due to the inactivity of the lynxes during the day, the observations were done between 8.30am and 10.30 am and 9.30pm and 11.30 pm, with a total of 4 hours per day in July and between 8.30am and 10.30 am and 8.00pm and 10.00 pm in August. The observations were shifted since it became darker in the evenings by the beginning of August.

Selected behaviours were recorded using one-zero sampling with 2 min interval (Altmann, 1974) according to an ethogram (Table 1). The observations were collected during July and August 2018.

Table 1: Ethogram

Behaviour	Description
	Olfactory enrichment
Licking	Lynx tongue touches the branch
Sniffing	Lynx holds its nose close to the branch, the antennas or inside the area.
Head rubbing	Lynx rubs its forehead or cheeks against the branch or the antenna
Body rubbing	Lynx rubs its body against the branch or the antenna
Rolling	Lynx is lying on the ground and pivots his body from one side to another
Biting	Lynx squeezes/grips the enrichment with its teeth
Scent marking	Lynx sprays urine on the scent station or on other objects/elements
Scratching	Lynx uses his claws to scratch a tree
	Olfactory and Auditory enrichment
Approach	Lynx moves toward the enrichment while listening for and/or looking at it

Pacing	Lynx performs stereotypic locomotory behaviour, i.e. walking, trotting or running back and forth along the public house or along the fence. Pacing was counted if there were more than 4 turns.
Pacing Event	Numbers of event with a series of pacing
Auditory enrichment	
Alert	Head and/or ears movement to try to locate the incoming sound of the enrichment
Retreat	Lynx back off from the enrichment while looking at or/and listening for it
Crouch	Lynx in alarm position, body close to the ground
Explore	Lynx moves around the enclosure, sniffing the ground
Freeze	Lynx suddenly stops moving while is walking/pacing/running
Grooming	Lynx licks the fur of its own body to clean it
Investigate	Lynx attracted by the enrichment and sniffs, paws, bites or turn around it
Lying	Horizontally resting position on the ground
Sitting	Vertically resting position on the ground
Sleeping	Lying with eyes close
Watching	Lynx observes and listens the enrichment. Head directed toward the enrichment
Social interaction	Physical contact with another lynx
Scared	Lynx approaches the enrichment and/or sniff around but abruptly stops and retreats from it
Out of sight	Lynx cannot be seen by the observer

3.4.2.2 Wildlife camera

To be able to record the behaviour of the lynxes visiting the scent station around the clock and especially during the night, a wildlife camera, Reconyx Hyperfire, was mounted on the visitors' house wall, 6 meters away from the scent station. The camera was protected from biting inside a plywood box (Figure 4). The camera can record for several months on its 12 AA NiMH batteries. It has IR LED lights, allowing it to take photos during night time. The number of photos were set to five per trig and the interval between them defined by the Rapidfire mode. Data were stored on a 16GB SD cared, and manually uploaded to a computer.

3.4.2.3 HDX PIT tag antenna

All lynxes were provided with 23mm long HDX PIT tags (https://www.oregonrfid.com/) placed subcutaneously between the shoulder blades. Loger erroneously was first provided with a 12mm PIT tag, which has a shorter read range. When this was corrected by injecting a 23mm PIT tag, also between the shoulders, it was found that they interfered with each other, making the detection of both unreliable. The scent station was integrated with an HDX PIT tag antenna fixed to a fur tree (Figure 3). The "scent" antenna was rectangular, with three wire loops. It was designed to detect the cat's PIT tag when it investigated the baited tree branch inside the loop. The antenna detected the PIT tag already 50-60cm in front of the loop, making sure that it was detected even though it was placed between the shoulder blades of the cats.

Later the "scent" antenna was moved to one of the upper, back enclosures to log pacing (see below).

The antenna was connected to an Oregon RFID HDX 4-channel long range reader (www.oregonrfid.com), placed in a room next to the enclosure and provided with external power. Data was saved in the reader's internal memory and was uploaded to a laptop via a serial USB connection, using a freeware called PuTTY (https://www.chiark.greenend.org.uk/~sgtatham/putty/). The data format is csv, which can be easily imported into Excel, where further analysis was carried out.

3.4.2.4 Blue Tooth Low Energy (BLE) tracking system

To supplement the detection of the lynxes at the scent station, a BLE tracking system was installed. It is based on BLE transmitters (https://senion.com) attached to a collar, which each lynx, after being immobilized by the veterinarians, was provided with. The signal from the BLE transmitters was recorded by a smartphone, placed in a waterproof box tied to the same tree as the scent tree branch. The smartphone, running a custom-made app (Goat Data Collect; courtesy

Gustaf Hendeby, LiU), stores the BLE tag ID code together with the signal strength measured in dB re. to 1V. The signal is transmitted every 100ms, around the clock, and the logging is provided with a timestamp with a resolution to 0.01s. Using the signal strength, it is possible to gauge the distance between the animal and the smartphone and determining the time of day when the animals showed interest to the scent. The data was automatically uploaded to a Google cloud drive when the smartphone was connected to internet, using another smartphone as a mobile hotspot.

3.4.3 Pacing

Pacing was also measured using HDX PIT antennas. One was placed perpendicular to the wall of the public display building and was considerably bigger than the "scent" antenna (fig 4). Later, when the scent experiment was finished, the "scent" antenna was moved to one of the back enclosures and placed perpendicular to the fence where the lynxes were also pacing.

It is also possible, using an array of smartphones placed strategically in the enclosure, to track the movements of the animals, like a mini-GPS system, by means of an algorithm developed by Fredrik Gustafsson, LiU (Figure 4). Three smartphones running the Goat Data Collect app (Courtesy Gustaf Hendeby, LiU) were placed inside the windows of the public display building, to provide supplementary data on pacing. Visual observations through the visitor house windows were also made to record pacing.

All the material was introduced into the enclosure in June 2018 before the beginning of the study to habituate the animals to the new technologies/objects.

Figure 4: Automated monitoring. 1 : HDX PIT tag "pacing" antenna, 2 : Scent station with HDX PIT tag "scent" antenna, 3 : Reconyx Hyperfire wildlife camera, 4 : BLE tag logging smartphone.

3.5 Experimental setup auditory enrichment

3.5.1 Auditory enrichment

In the present study, two conspecific sounds and two prey sounds were played as auditory enrichment: roe deer (*Capreolus capreolus*) barking, mouse (*Mus musculus*) squeal and lynx vocalizations including growl and call. The roe deer barking, and the lynx call were separated into male and female vocalizations to assess if the lynxes would respond differently toward them. The sounds were found via Internet in a sound library called Animal Sound Archive of the Museum für Naturkunde Berlin (© 2006 - 2016 Tierstimmenarchiv; http://www.tierstimmenarchiv.de/). A control sound was also played and arranged with the ambient sound coming from the lynx enclosure which was recorded with a Sony DAT tape recorder (Sony TCD D8 Walkman Portable Digital Audio Tape Recorder) and a microphone (Roland CS-15 Stereo microphone). The sounds were played through a Bluetooth speaker (JBL Flip 3) placed inside a plastic tube to protect it from rain and the lynxes. The speaker was controlled by a smartphone placed 30 meters away inside the public display building. The speaker was provided with rechargeable batteries. The animals were exposed to a total of 20 min of sound per day. One sound was played four times a day during 5 min, twice in the morning with 25 min interval and the same in the afternoon and was replaced with another sound the next day. Due to the speaker switching off automatically after 20 min if no sound was played, a weak background sound was played in a loop between the morning and afternoon session to ensure that the speaker did not switch off. This background sound was composed of 5 s of a "walking on dry leaves" noise and 15 min of silence.

In case the connection between the speaker and the smartphone was lost, the phone close to it (see below) was used as replacement and the sound was played through it. The speaker was placed 20 meters away from the windows in the public building to ensure a strong Bluetooth connection. The location of the speaker was changed each time the enclosure was accessible. The locations were chosen randomly. The choice of the sounds was pseudo-randomized to avoid the same sound being played on two consecutive days. Each sound was presented a total of 4 days resulting in 16 repetitions and 20 treatment days. Additionally, a sound recording station was installed next to fence outside the enclosure to record possible lynx vocalizations in response to the playbacks. The sound was recorded with the same Sony DAT recorder and microphone mentioned above.

To complete the auditory enrichment, live prey, in the form of crickets (*Grillus campestris*), was introduced into the enclosure through a 5m long PEM tube attached to the fence and provided with escape holes in its lower end (Figure 5). The crickets were collected from the "cricket factory" at Kolmarden Wildlife Park, where they are bred as live food for meercats, primates and some of the bird species. They were transported in a plastic bottle to the lynx enclosure. Then they were dropped through a funnel into the PEM tube. The cricket enrichment was introduced only when the lynxes were close to the plastic tube or else the crickets might escape in the enclosure without the lynxes noticing their presence. The time for adding the crickets was selected by a distinct pseudo-randomization than with the playback sounds with the possibility to offer crickets for two consecutive days. Each cricket enrichment was presented a total of 8 days resulting in 8 observations days.

Figure 5: Cricket enrichment setup

3.5.2 Auditory enrichment response monitoring

3.5.2.1 Behavioural observation

The visual behaviour observations were done between 7.30 and 10.00 am and 7.00 and 8.00 pm, with a total of 1 hour per day in the beginning of September and between 7.30 and 10.00 and 6.00 and 7.00 pm in the end of September. The observations were shifted since it became darker earlier in the evening by the end September. The main objective with choosing these periods was to cover the lynxes' pacing. During public opening days (Friday, Saturday and Sunday) the observations were made before 8.30 am to avoid possible disturbance from different sound sources in the vicinity of the lynx exhibit, including epic music played along the entrance and inside the escalator leading up to the exhibit, and the different test procedures with a roller coaster in a nearby children theme park. Half an hour of observations was carried out in the morning and the same amount of time during the late afternoon. The behaviour was recorded during 15 min including 5 min of pre-treatment, 5 min of sound treatment and 5 min of post-treatment. This procedure was repeated after a 15 min break (without any observation). Before the morning observations, when the batteries of the speaker needed to be replaced, the lynxes were called to a station in the back enclosures, and the gate to the main enclosure was closed, making it safe to enter. This was done every 3-4 days.

Cricket observations were performed during 20 min at any time of the day to be unpredictable. During the public opening days, the observations were made only before and after opening hours, i.e. before 10 am and after 5 pm. The same procedure as with the sounds was used, i.e. recording 5 min of pre-treatment, 10 min of treatment and 5 min of post-treatment.

Selected behaviours were recorded using continuous sampling and a video camera (Gopro4) according to an ethogram (Table 1). This ethogram was based on an ethogram for Felidae suggested by Stanton et al. (2015).These observations were collected during September and October 2018.

3.5.2.2 Wildlife camera

To have alternative behavioural observations of the lynxes, a wildlife camera, Reconyx Hyperfire 500, was mounted on the public house wall, ca.10 meters away from the speaker positions (see above for more details). The camera was always pointed at the new position of the speaker.

3.5.2.3 BLE tag detector

A smartphone was placed inside the protective tube for the speaker, to offer additional recording of the animals' responses to the sounds by means of the custom made app (Goat Data Collect; courtesy Gustaf Hendeby, LiU). The phone was powered by the same power bank as the speaker.

3.6 Statistical analysis

3.6.1 Visual observation

To assess the effectiveness of the olfactory treatments on the lynxes, the total number of each *treatment directed behaviour* (i.e *approach, biting, licking, rubbing, scent-marking, scratching* and *sniffing*) of all individuals combined were summed. A chi-square test was performed comparing the number of behaviours for each *treatment directed behaviour* observed in each scent treatments. To find out which scent was more attractive, a pairwise chi-square test was performed using a False Discovery Rate (FDR) correction.

A Spearman correlation test was used to assess possible habituation (i.e. decrease in number of behaviours over time) by comparing the number of behaviours for each scent treatment and the consecutive number of the sessions.

To determine the effectiveness of the sound treatments, a Kruskal-Wallis test was used to compare the duration (in seconds) that each lynx spent performing *treatment-directed behaviour* (*alert, approach, investigate, retreat, watching), social behaviour, exploratory behaviour* and *resting behaviour* (*sitting, standing, sleeping*) per session between the **three treatments phases** (pre-treatment, treatment, post-treatment). In order to determine if there were any differences between treatments phases, a post-hoc Dunn test was conducted using a False Discovery Rate (FDR) correction. A Kruskal-Wallis test was also used to compare the duration (in seconds) that each lynx spent performing *treatment-directed behaviour, social behaviour, exploratory behaviour* and *resting behaviour* during the **treatment phase**.

In order to determine if there were any differences between sounds, a post-hoc Dunn test was conducted using a False Discovery Rate (FDR) correction. A Spearman's correlation test was used to assess possible habituation (i.e. decrease of duration of *treatment directed behaviours* over time) by comparing the time spent during the treatment phase for the treatment selected behaviour with the consecutive number of sessions. In order to assess whether there was a difference in interaction time between male and female sounds, a Mann-Whitney U test was used.

To assess the effectiveness of the cricket treatment on the lynxes, a Kruskal-Wallis test was used to compare the duration (in seconds) that each lynx spent performing selected behaviours toward the treatment (*approach, investigate, watching*) between the three treatments phases (pre-treatment, treatment, post-treatment). In order to determine if there were any differences between treatments phases, a post-hoc Dunn test was conducted using a False Discovery Rate (FDR) correction.

3.6.2 Automatic logging

For the Reconyx Hyperfire photos, logging one behaviour per picture, a chi-square test was performed comparing the number of selected behaviours identified in each scent treatment.

For the HDX PIT tag detections by the "scent" antenna, a chi-square test was performed comparing the total number of visits i.e (50 seconds between consecutive detections was logged as a new visit) at the antenna for each scent treatment. To find out which scent was more attractive, a pairwise chi-square test was performed using a False Discovery Rate (FDR) correction. To test the association between the duration of visits at the scent station per session a Kruskal-Wallis test was used.

For the BLE tag data collected by the scent station smartphone, a chi-square test was performed comparing the number of visits i.e (50 seconds between consecutive detections was logged as a new visit) at the scent station for each scent treatment. To find out which scent was more attractive, a pairwise chi-square test was performed using a False Discovery Rate (FDR) correction. To test the association between the duration of visits at the scent station per session a Kruskal-Wallis test was used. In order to determine if there was any difference between scents, post-hoc Dunn test was conducted using a False Discovery Rate (FDR) correction.

3.6.3 Pacing

Olfactory treatment

To test the association between the duration of pacing per sessions and the olfactory treatments, based on the visual observations, a Kruskal-Wallis test was used.

To test the association between the duration of pacing per session, based on the HDX PIT tag detections by the visitor house "pacing" antenna, and the olfactory treatment a Kruskal-Wallis test was used. To compare the number of pacing events for each scent the number of "positive pacing hours" (PPH) were extracted and a chi-square test was performed. A pacing event was an uninterrupted series of ≥4 pacings back and forth, passing through the "pacing" antenna, with ≤50s between consecutive passings. A PPH was an hour where pacing occurred, irrespective of duration or number of pacing events. For example, if during the catnip treatment pacing occurred between 8am and 9am, 9am and 10am and 12am and 1pm, they counted as 3PPHs. If during the valerian treatment pacing occurred between 22 and 23 it counted as 1PPH.

Auditory treatment

To assess if pacing was interrupted by the sound treatments, the total number of times pacing was performed by the lynxes during pre-treatment was compared with number of "pacing interruptions" and "pacing continued" during treatment. Pacing interruption corresponds to the number of times pre-treatment pacing was interrupted by another behaviour during sound treatment. Pacing continued corresponds to the number of times pre-treatment pacing continued during sound treatment. A chi-square test was performed to determine the difference between pacing interruption and pacing continue across pre-treatment and treatment phases.

To determine the effectiveness of the sound treatments, a Kruskal-Wallis test was used to compare the duration of pacing per session between the three treatments phases (pre-treatment, treatment, post-treatment). In order to determine if there was any difference between treatments phases a post-hoc Dunn test was conducted using a False Discovery Rate (FDR) correction. The same procedure was used for the cricket treatment.

To test the association between the duration of pacing per session and the sound treatment a Kruskal-Wallis test was applied on the HDX PIT tag detections by the pacing antenna. To compare the number of PPH (see above), a chi-square test was performed for each sound.

To find out which sound was more attractive, a pairwise chi-square test was performed using a False Discovery Rate (FDR) correction.

A P-value of <0.05 was considered statistically significant and R studio (Version 1.1.456 – © 2009-2018 RStudio, Inc.) was used for the statistical analyses

4 Results

4.1 Visual observations

4.1.1 Olfactory treatment

During the 36 days of testing the odours, the lynxes displayed a total of 425 behaviours (see ethogram, Table 1 in Materials and Methods) directed toward the olfactory treatment with 107 *rubbings* and 60 *sniffings* in the catnip treatment being the most frequently displayed behaviours. (Table 2).

Table 2 : Total number of behaviours displayed by the lynxes

	Catnip	**Cinnamon**	**Valerian**	**Control**	**Total**
Approach	18	11	17	16	**62**
Biting	23	0	0	0	**23**
Licking	15	0	0	0	**15**
Rubbing	107	11	1	5	**124**
Scent marking	16	23	23	29	**91**
Scratching	4	1	0	0	**5**
Sniffing	60	15	12	18	**105**
Total	**243**	**61**	**68**	**53**	**425**

There was a significant difference in the number of *biting*, *licking*, *rubbing*, and *sniffing* displayed towards each scent (*biting*, $X^2_{(3)} = 69$, p-value <0.001 ; *licking*, $X^2_{(3)} = 45$, p-value < 0.001; *rubbing*, $X^2_{(3)} = 250.06$, p-value <0.001 ; *sniffing*, $X^2_{(3)} = 58.543$, p-value <0.001). there was a close to significant difference in the number of *scratchings* between the scents ($X^2_{(3)} = 8.6$, p-value = 0.03511).

There was no significant difference in the number of *approach behaviour*, *scent markings* and *scratchings* between the scents (*approach*, $X^2_{(3)} = 1.871$, p-value = 0.5996; *scent marking*, $X^2_{(3)} = 3.72$, p-value = 0.2927).

The catnip treatment was found to induce a significantly higher number of *biting, licking, rubbing,* and *sniffing* than the cinnamon, valerian and control treatments (Table 3). The lynxes interacted significantly more with the catnip treatment compared to the other two treatments and control. For the comparison of cinnamon, valerian and control no significant differences were found for the behaviours *biting, licking, rubbing, scratching* and *sniffing* (Table 23 and Figure 6). However, the cinnamon treatment was found to induce significantly higher number of *rubbing* than the control treatment (Table 3 and Figure 6)

Table 3 : Pairwise comparison of each treatment for the selected behaviours with their p-values

	Catnip/ Control	**Valerian/ Control**	**Cinnamon/ Control**	**Catnip/ Valerian**	**Catnip/ Cinnamon**	**Cinnamon/ Valerian**
Biting	**<0.001**	-	-	**<0.001**	**<0.001**	-
Licking	**<0.001**	-	-	**<0.001**	**<0.001**	-
Rubbing	**<0.001**	0.1230	**0.0058**	**<0.001**	**<0.001**	0.1336
Scratching	0.11375	-	0.3173	0.11375	0.2995	0.3173
Sniffing	**<0.001**	0.64	0.64	**<0.001**	**<0.001**	0.48

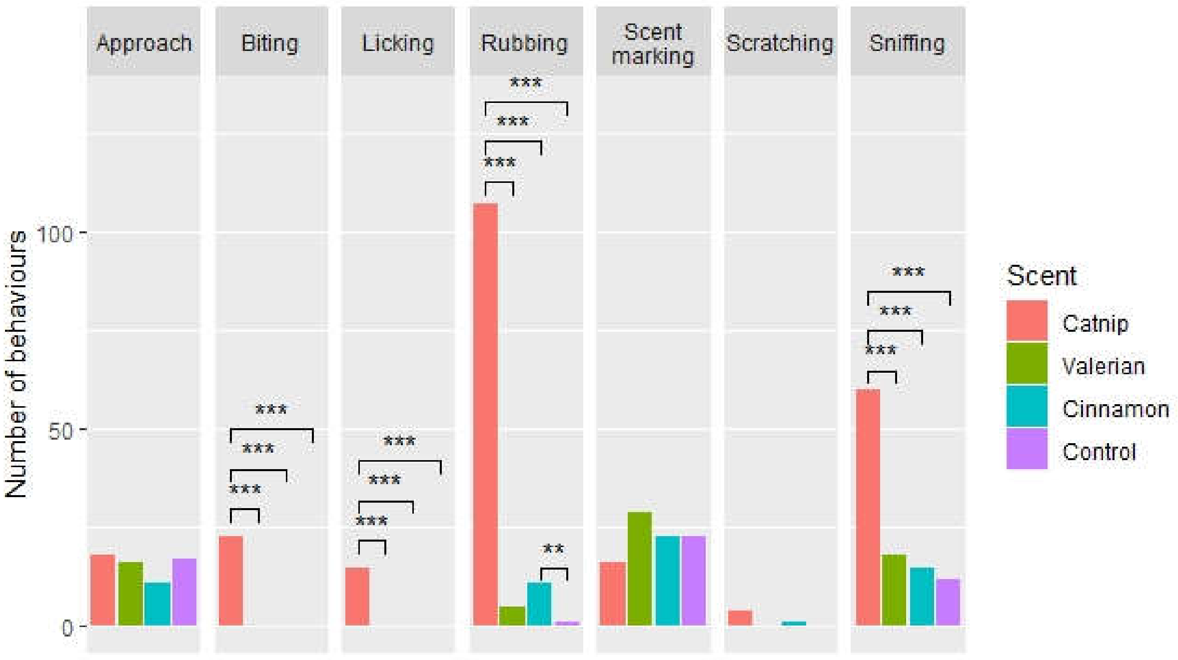

*Figure 6 : Number of the selected behaviours performed by the 3 lynxes combined in each treatment . * : p<0.05, ** : p<0.01, *** : p<0.001*

Considering each scent individually and with all behaviours compiled, no significant trend was found between the number of behaviours per session over the 9 sessions in any of the treatments (catnip, rho = 0.125, p-value = 0.7476; valerian, rho = -0.376, p-value = 0.3178; cinnamon, rho = 0.366, p-value = 0.3326; control, rho = -0.455, p-value = 0.2176) (Figure 7).

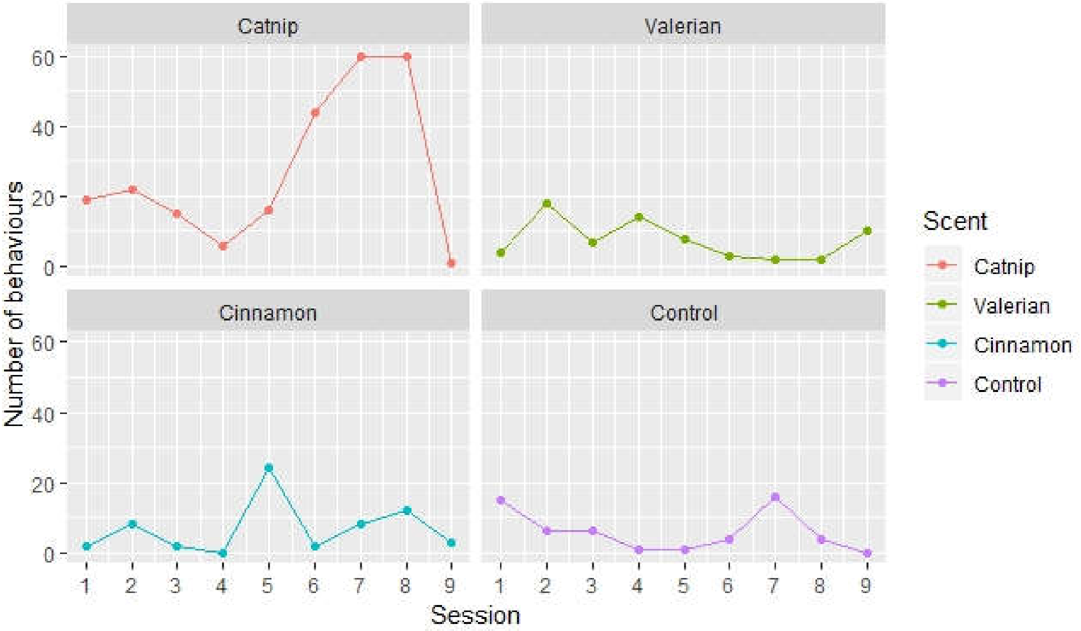

Figure 7: Number of behaviours performed by the 3 lynxes combined towards the different odour treatments over 9 sessions.

4.1.2 Auditory treatment

For *sound-directed behaviours* there was a significant difference in duration per session between the treatment and pre- and post-treatments (Kruskal-Wallis: $H_{(2)} = 75.81$, p-value < 0.001) (Figure 23). The sound treatments were found to induce a significantly higher duration of sound-directed behaviours compared to the pre-treatment and post-treatment phase (post-Hoc Dunn test : treatment vs pre-treatment, p-value < 0.001; treatment vs post-treatment, p-value <0.001) (Figure 8).

For *social behaviour* there was a significant difference in duration between the treatment and pre- and post-treatment ($H_{(2)} = 8.3696$, p-value = 0.01523). The sound treatments were found to induce a significantly higher duration of *social behaviour* compared to the pre-treatment and post-treatment phase (treatment vs pre-treatment, p-value = 0.019; treatment vs post-treatment, p-value = 0.043) (Figure 8).

For *exploratory behaviour* there was no significant difference in duration between the three treatment phases ($H_{(2)} = 2.072$, p-value = 0.3549) (Figure 8).

For *resting behaviour* there was no significant difference in duration between the three treatment phases ($H_{(2)} = 0.49864$, p-value = 0.7793) (Figure 8).

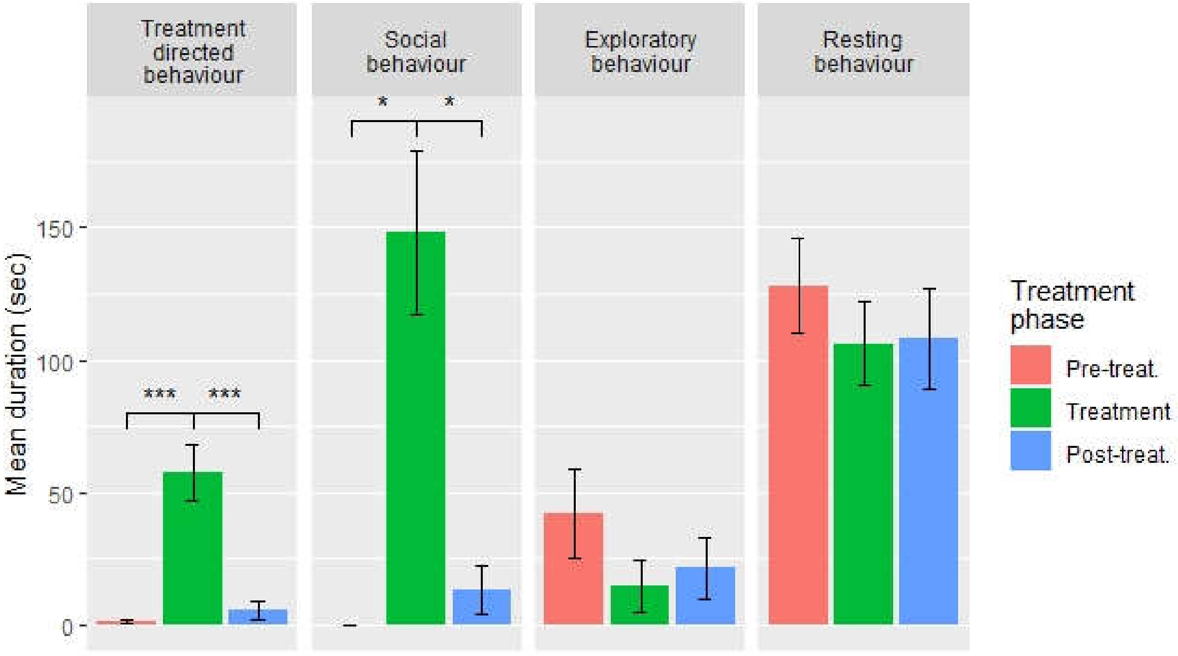

*Figure 8 : Sound treatment: Mean duration of selected behaviours per treatment phase for the 3 lynxes combined. * : p<0.05, ** : p<0.01, *** : p<0.001. Coloured bars show mean and vertical lines show ± SE.*

There was a significant difference between the different sounds in the duration of lynxes' *sound-directed behaviours* ($H_{(4)}$ = 18.913, p-value < 0.001). The lynxes spent more time directed towards the treatment when roe deer barks, lynx calls and lynx growls were played compared to the control sounds (roe deer bark vs control, p-value < 0.001; lynx call vs control, p-value = 0.02033; lynx growl vs control, p-value = 0.02033) (Figure 9). Since no significant difference was found between the three treatments phases (see above) for *social behaviour*, *exploratory behaviour* and *resting behaviour* no test was done.

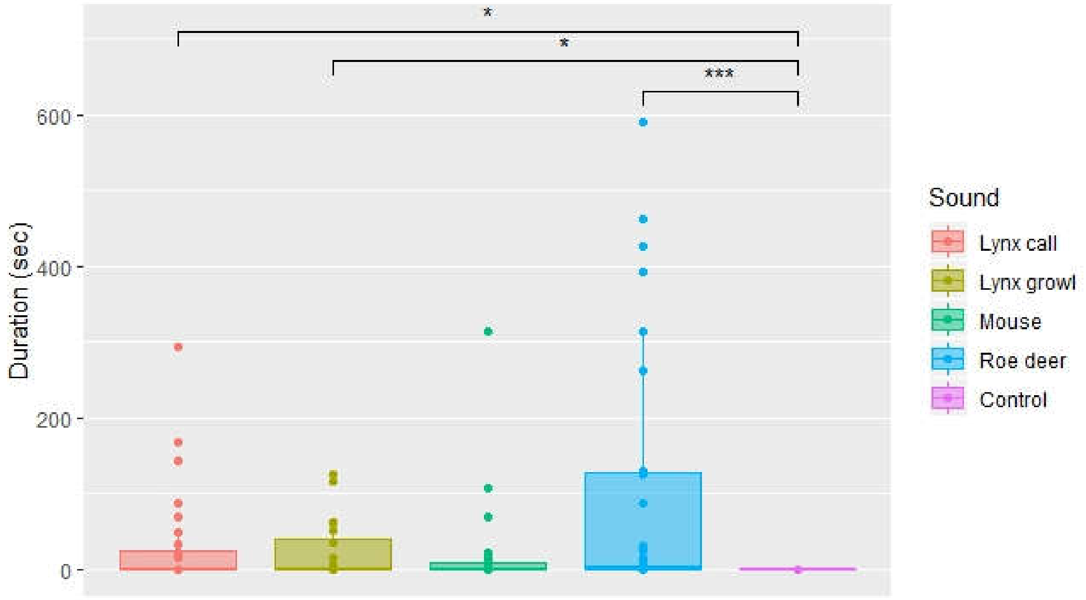

*Figure 9 : Duration of treatment directed behaviours per sound treatments for the 3 lynx combined. * : p<0.05, ** : p<0.01, *** : p<0.001. Bars show average and dots all values.*

There was also a significant difference between the sounds in time spent on *social behaviour* ($H_{(4)}$ = 18.708, p-value <0.001). The duration of *social behaviour* was higher when the lynx calls were played compared to the control, lynx growls, roe deer barks and mouse sounds (lynx call vs control, p-value = 0.0016 ; lynx call vs lynx growl, p-value = 0.0016; lynx growl vs roe deer, p-value = 0.0016; lynx call vs mouse, p-value = 0.0016) .

Since no significant difference was found between the three treatments phases for *exploratory behaviour, resting behaviour* (see above) no test was done.

Response to gender-specific sounds

There was no significant difference in the duration of *sound-directed behaviour* directed towards male and female lynx calls and male and female roe deer vocalizations (lynx call, W = 41.5, p-value = 0.921; roe deer, W = 50, p-value = 0.3166).

Habituation

Considering each sound individually no significant association that would indicate habituation was found between the time spent on *sound-directed behaviours* over the 4 sessions.

However, there was a tendency of a decreasing duration of *sound directed behaviour* over time in the mouse treatments (lynx call, rho = 0.4, p-value = 0.75; lynx growl, rho = -0.8, p-value = 0.333; roe deer, rho = 0.4, p-value = 0.75; mouse, rho = -1, p-value = 0.08333) (Figure 10).

The Spearman's correlation was not calculated for the control treatment since the lynxes did not perform any *sound-directed behaviour* during control sessions.

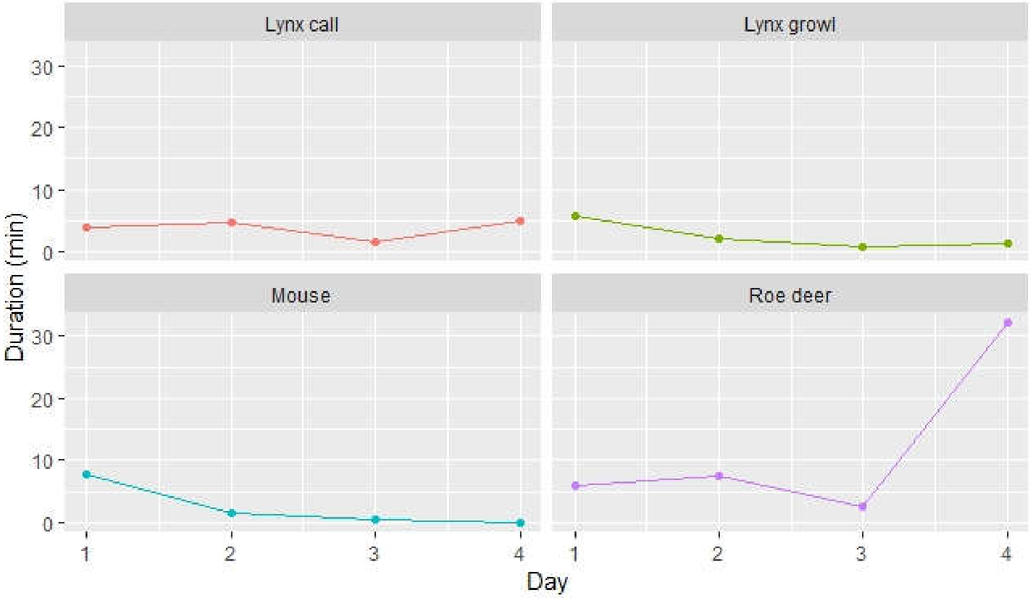

Figure 10 : Duration of sound directed behaviours performed by the 3 lynxes combined over 4 sessions

Crickets

There was a significant difference for *approach behaviour* between the cricket treatment and the pre- and post-treatment ($H_{(2)}$ = 7.6235, p-value = 0.02211).

The cricket treatments were found to induce a significantly higher duration of *approach behaviours* compared to the pre-treatment and post-treatment phases (treatment vs pre-treatment, p-value = 0.025; treatment vs post-treatment, p-value = 0.025). However no significant differences were found in duration between the three treatments phases for *investigate* and *watching* behaviours (*investigate*, $H_{(2)} = 4.8$, p-value = 0.09072; *watching*, $H_{(2)} = 2.9032$, p-value = 0.2342).

4.2 Automatic logging

4.2.1 Olfactory treatment

Reconyx hyperfire photos

The Reconyx photos were counted for the whole 24hours starting at 8:30 AM each test day corresponding to the application of each odour treatments. The total number of photos taken was 93, including all 9 treatments for all animal. For each photo only one behaviour (*approach*, *investigate*, *rubbing* or *scent marking*) was recorded. *Approach* was seen in 51 photos, *investigate* in 17 photos, *rubbing* in 2 photos and *scent-marking* in 23 photos (Figure11).

There was no significant differences in the number of the selected behaviours between the scents (*approach*, $X^2_{(3)} = 2.7255$, p-value = 0.4359, *investigate*, $X^2_{(3)} = 2.5294$, p-value = 0.47, *scent-marking*, $X^2_{(3)} = 7.4348$, p-value = 0.05926, *rubbing* , $X^2_{(1)} = 0$, p-value = 1) (Figure 11).

Figure 11 : Number of selected behaviours identified for the three lynxes in the wildlife camera photos in the different scent treatments

Concerning the timing of the wildlife camera detections, the lynxes visited the scent station during the night between 3.30pm-4.30am. They were also at the scent station in the morning between 7.30-10.30 am in connection with the application of the treatment (Figure12).

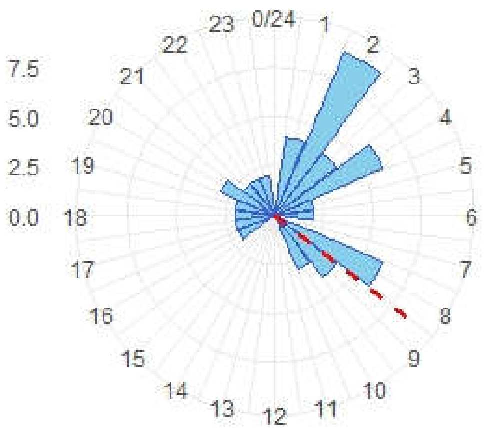

Figure 12 : Timing of wildlife camera visits for the three lynxes combined. The red line represents the time when the scent treatment was applied. The circles show the number of visits within the time scope of the sector, with the inner-most one at 2.5 detections

.

HDX PIT tag

Due to a technical malfunction, the HDX PIT tag data from the scent station was collected during only 20 days. The lynxes were detected at the scent station a total of 49 times.

There was a significant association between the total number of visits and each odour treatment ($X^2_{(3)} = 14.102$, p-value = 0.002769). The lynxes visited the scent station more often with the catnip treatment compared to the control (p-value <0.001). There were also significantly more visits with the valerian treatment compared to the control treatment (p-value = 0.00485), and in the cinnamon treatment compared to the control treatment (p-value = 0.01505) (Figure13A).

There was no significant difference between the odour treatments and the mean duration of visits per session ($H_{(3)}= 5.6934$, p-value = 0.1275). (Figure13B)

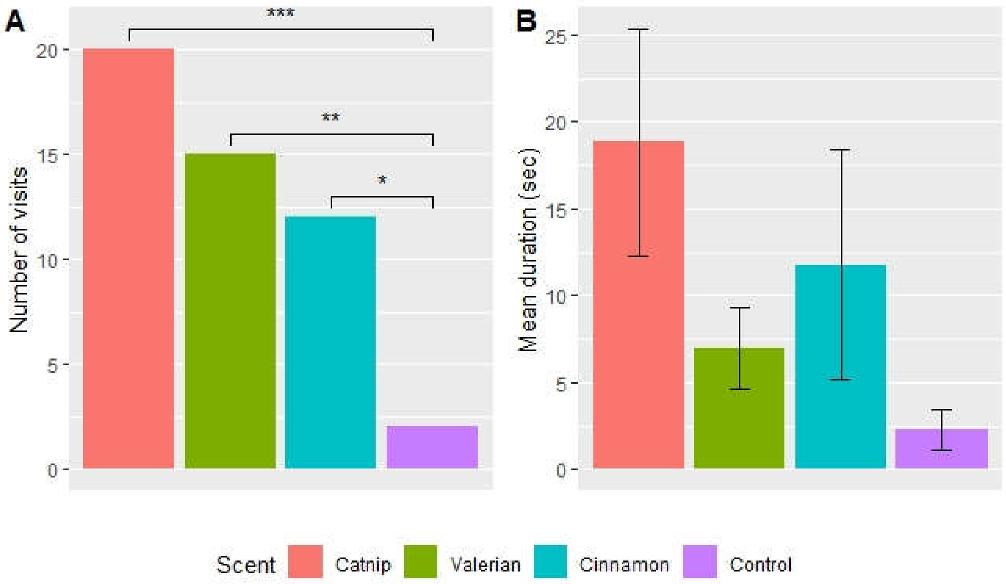

*Figure 13 : HDX PIT tag detections. A: Total number of visits at the scent station for each odour treatment with all three lynxes combined. * : p<0.05, ** : p<0.01, *** : p<0.001. B: Mean duration per session? at the scent station for the three lynxes combined. Coloured bars show mean and vertical lines show ± SE.*

The lynxes visited the scent station antenna mostly during the evening around 7.00pm and during the night between 10.30pm and 2.30am. The majority of the detections was in the morning between 7:30 and 9:30 am, in connection with the application of the odours (Figure14).

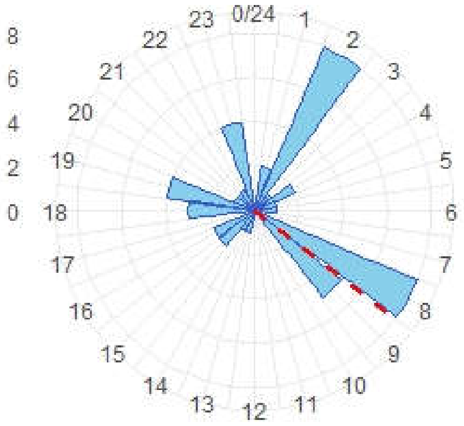

Figure 14 : Timing of HDX PIT tag visits for the three lynxes combined. The red line represents the time when the treatment was applied (8.30 am). The circles show the number of visits within the time scope of the sector with the inner-most circle at 2 visits.

BLE tag

The BLE tags transmitted, with 0.5s intervals, an individual ID number, allowing for each lynx to be analyses separately for each scent treatment. This number, together with the signal strength, was recorded by the app in the scent station smartphone.

The signal strength, which depended on the distance to the smartphone, could be between -100 and -20 dB re. 1 V, but for this analysis signal strengths between -50 and -20 dB re. 1V were selected to focus on close inspections of the scent station. Due to a technical malfunction, the BLE tag data from the scent station was collected for only 25 days. The lynxes visited the scent station a total of 45 times during these 25 days.

There was no significant association between the total number of visits and each odour treatment ($X^2_{(3)} = 2.3778$, p-value = 0.4978) (Figure 15A).

There was a was a close to significant difference between the odour treatments and the mean duration of visits per session for the lynxes ($H_{(3)} = 9.0842$, p-value = 0.02819). The cinnamon treatment was close to be significant with a higher visiting duration at the scent station compared to valerian treatment (p-value = 0.057) (Figure 15B).

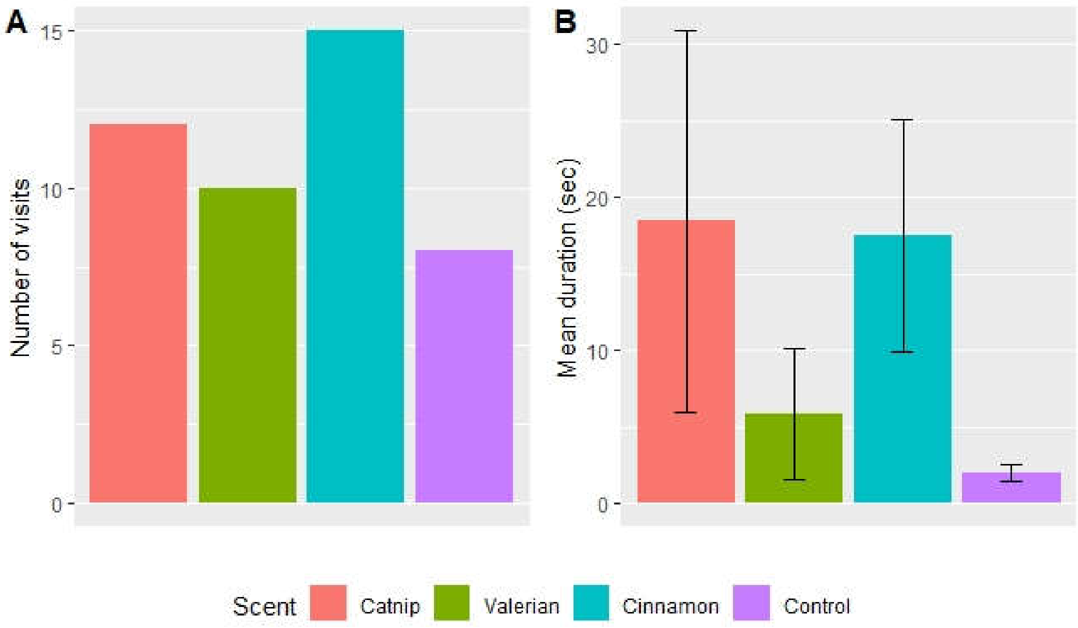

*Figure 15 : BLE tag detections, A: Total number of visits at the scent station for each odour treatment with all three lynxes combined. * : p<0.05, ** : p<0.01, *** : p<0.001. B: Mean duration at the scent station all three lynxes combined. Coloured bars show mean and vertical lines show ± SE.*

Concerning the timing of BLE tag detection, the lynxes visited the scent station mostly during the late vening between 9:30 and 10.30pm and during the night between 00.30 -02.30 am, but also with detections in the morning between 6.30-9.30am in connection with the application of the scent treatment (Figure 16).

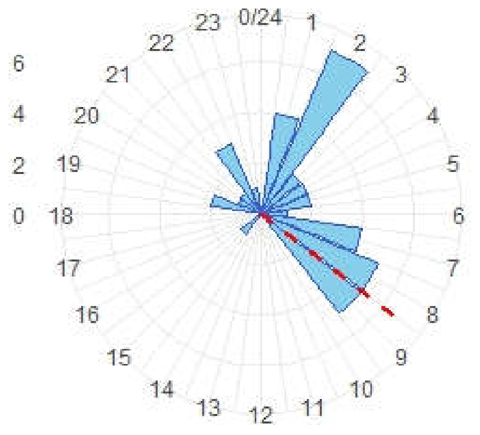

Figure 16 : Timing of BLE tag visits for the three lynxes combined. The red dashed line represents the time when the scent treatment was applied (8.30 am). The circles show the number of visits within the time scope of the sector with the inner-most circle at 2 visits.

4.2.2 Comparisons between the Reconyx wildlife camera, HDX PIT tag antenna and BLE tag detection smartphone app data collected at the scent station.

The HDX PIT tag and the BLE tag were working at different periods of time only with 3 days of matching data where the visits were overlapping at 7 moments at the same time and date (Figure 17). The HDX PIT tag was working between July 5th – July 19th , July 22nd -July 31st and August 15th- August 18th. The BLE tag was working between July 8th – July 24th and August 4th- August 18th . For the Reconyx camera the lynxes were triggered visiting the scent station mostly in August. (Figure 17 and Figure 18). Overall, the three systems were overlapping only during 3 days, in July 23th at 4.00am, July 24th at 2.00 am and August 18th at 7.00pm.

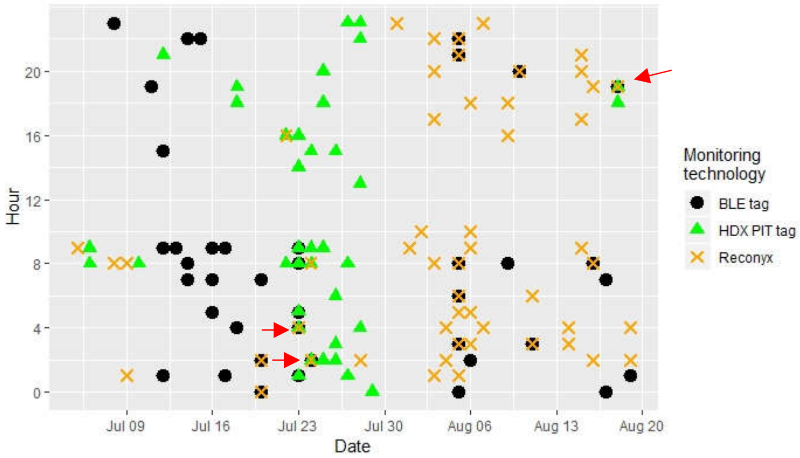

Figure 17 : Comparison of timing of the lynxes' visits at the scent station as logged by the three monitoring technologies. Red arrow show overlap of the three technologies at the same time and date.

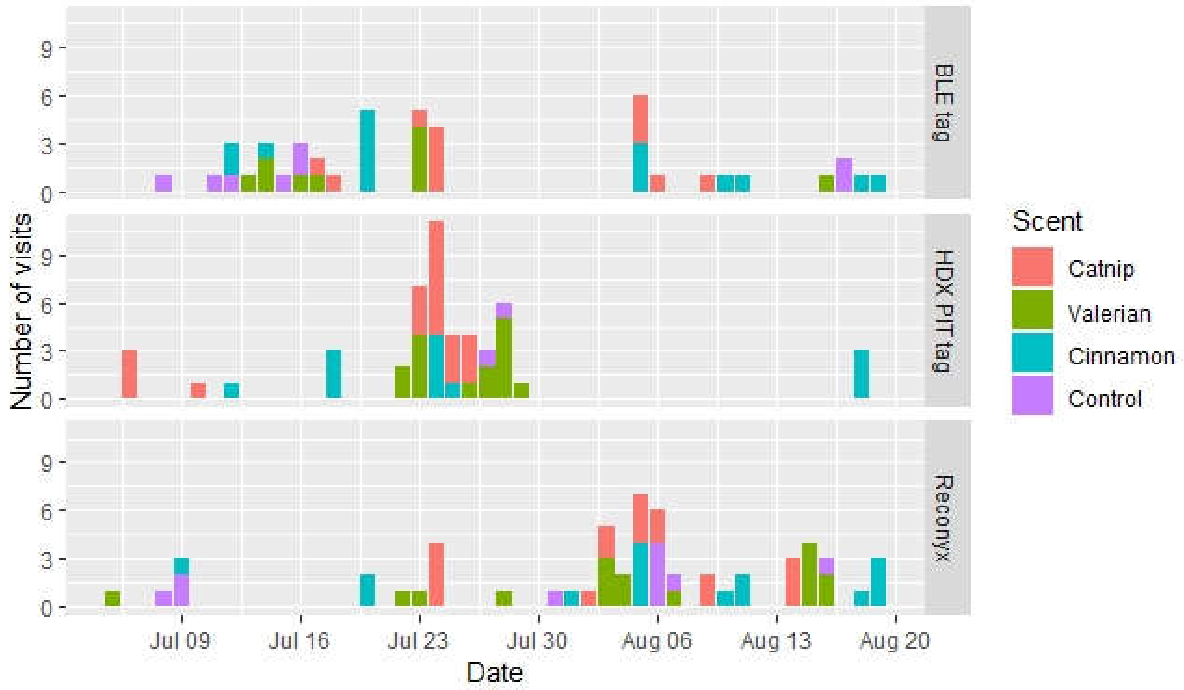

Figure 18 : : Timing of BLE tag, HDXPIT tag and Reconyx hyperfire camera visits.

4.2.3 Auditory treatment

Reconyx Hyperfire photos

There were not enough data from all the sound treatment sessions to do statistical analyses. Moreover, depending on the position of the speaker the camera was sometimes too far away to be triggered by the lynxes. The visual observations showed that the camera captured the same behaviour as the visual observations but since it was not trigged frequently enough it was not possible to follow the cats' interactions with the treatments with enough detail from the photos.

BLE tag

Due to a technical malfunction, there was not enough data to do statistical analysis. Only one week of good data was obtained, otherwise the files only contained GPS data or were empty.

4.3 Pacing

4.3.1 Olfactory treatment

Visual observation

There was no significant difference between the odour treatments and the mean duration of pacing per session for the lynxes ($H_{(3)}$= 0.476, p-value = 0.9239) (Figure 19).

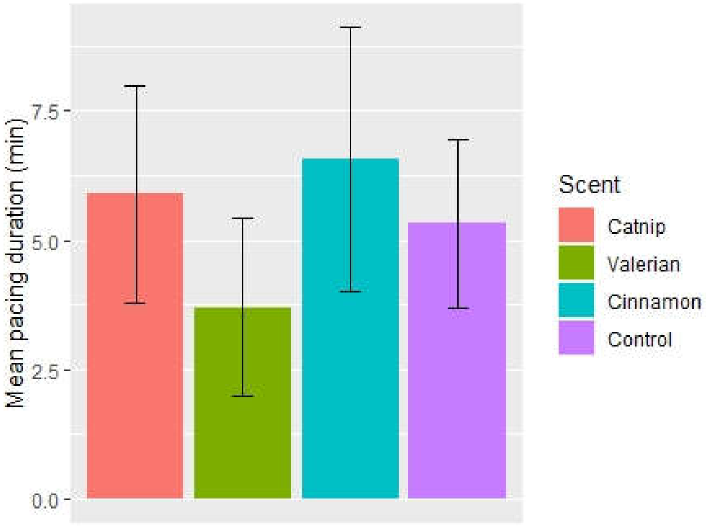

Figure 19 : Mean pacing duration per treatment session for the three lynxes combined for each odour treatments. Coloured bars show mean and vertical lines show ± SE.

HDX PIT tag

The lynxes had a total of 9 pacing events, with a total of 91 passes through the pacing antenna at the visitor house wall, 9 times during the catnip treatments, 55 times during the valerian treatments, 10 times during the cinnamon treatments and 17 times during the control treatments. Pacing data were available and analysed for 1 day with the catnip treatment, 3 days for the valerian treatment, 1 day for the cinnamon treatment and 2 days for the control treatment.

There was no significant difference between the odour treatments and the total duration of pacing ($H_{(3)}$= 6.7, p-value = 0.0821). (Figure 20A). There was no significant association between the number of positive pacing hours (PPH) and each odour treatment ($X^2_{(3)}$ = 2.11, p-value = 0.5497). (Figure 20B).

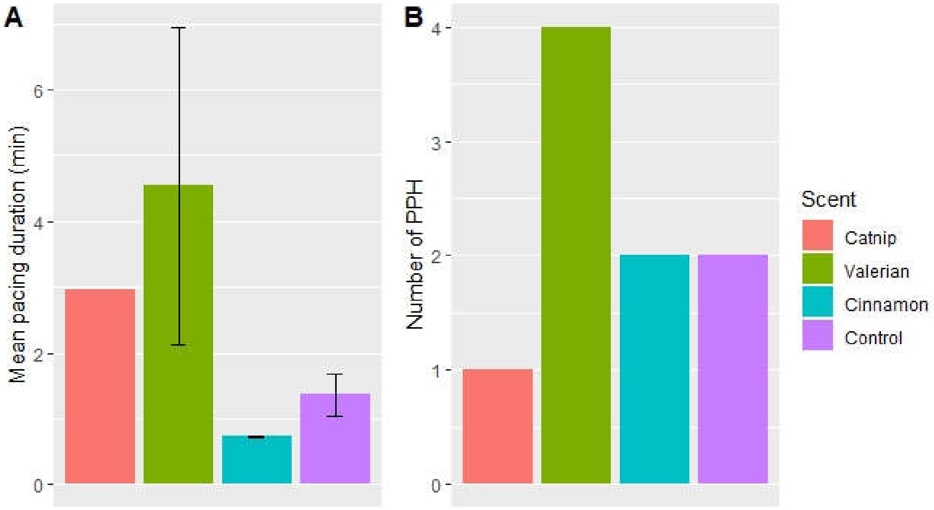

Figure 20 : A : Mean pacing duration per treatment session for the three lynxes combined. Coloured bars show mean and vertical lines show ± SE. B : Total number of positive pacing hours (PPH) per day (24hrs) for each scent treatment for the three lynxes combined

4.3.2 Auditory treatment

Visual observation

The lynxes paced 30 times during the 80 pre-treatment sessions, of which 63 % was interrupted by the sound treatments, making p*acing interruption* not significantly different from *pacing continue* ($X^2_{(1)} = 2.133$, p-value = 0.1441).

With the sound treatments, there was no significant difference in pacing duration per session between the three treatment phases ($H_{(2)} = 2.2375$, p-value = 0.3267) (Figure 21).

With the cricket treatment, there were no significant differences in pacing duration per session between the three treatments phases ($H_{(2)}= 3.5484$, p-value 0.1696) (Figure 21).

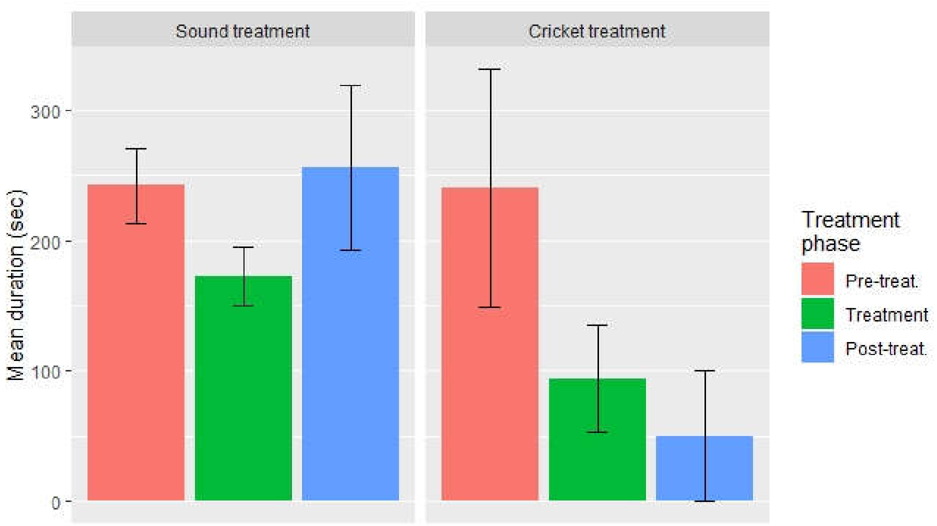

Figure 21 : Mean pacing duration per treatment phase for the three lynxes combined. Coloured bars show mean and vertical lines show ± SE.

HDX PIT tag

There was no significant difference between the sound treatment and the duration of pacing per session ($H_{(4)}$= 0.195, p-value = 0.9955) (Figure 22A). There was a close to significant association between the number of positive pacing hours (PPH) and sound treatments ($X^2_{(4)}$ = 11.875, p-value = 0.01831). The lynx calls, lynx growls and mouse squeal treatments were close to be significant with higher number of PPH compared to the control sounds (lynx call vs control, p-value = 0.057; lynx growl vs control, p-value = 0.057; mouse squeal vs control, p-value = 0.057) (Figure 21B). The lynx calls, lynx growls and mouse squeal treatments were also close to be significant with higher number of PPH compared to the roe deer bark sounds (lynx call vs roe deer, p-value = 0.057; lynx growl vs roe deer, p-value = 0.068; mouse squeal vs roe deer, p-value = 0.057) (Figure 22B).

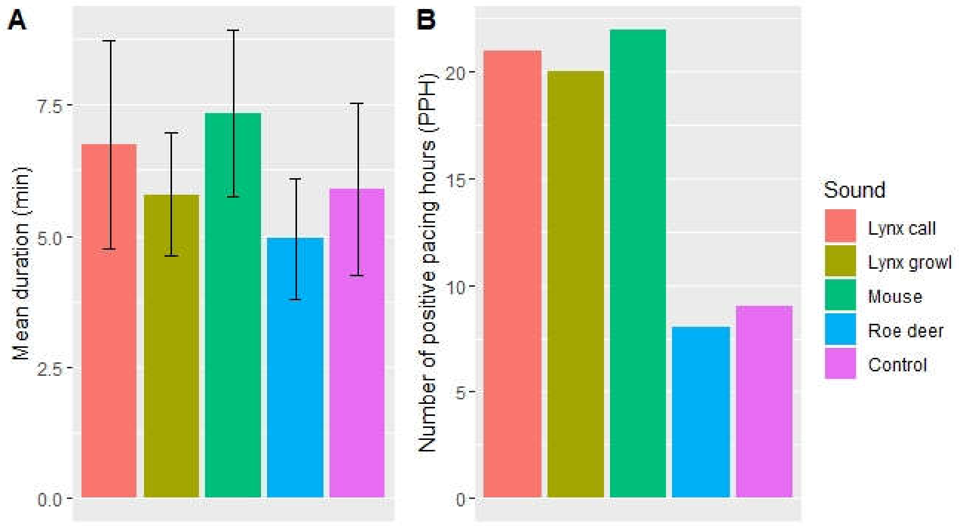

Figure 22 : A : Pacing duration per sound treatment. Coloured bars show mean and vertical lines show ± SE; B: Number positive pacing hours (PPH) per day (24hrs) for each sound treatment

4.3.3 Individual pacing habits

Between June and November, pacing was recorded by the HDX PIT tag antenna at the visitor house, which provided information about pacing habits for Bore and Lovika. Loger’s pacing was not analysed since his HDX chip could not be well detected by the antenna.

Bore

Pacing duration in minutes was summed by hour for the overall 6 months. Bore was pacing along the house wall during the morning between 8.30-12.30 am and during the afternoon between 4.00-5.30pm (Figure A1). Pacing duration was also quantified by month to see if there were any changes in hourly pacing habits over time. Bore’s pacing was rather low during the summer months June-August, increased slightly in September, and peaked during day time in October, to be reduced again in November (Figure A2). From June to August, Bore had 1 or 2 positives pacing hours (PPH) per day which increased to around 3 PPH per day in September and 5 pacing events per day in October, with a maximum of 8 PPH per day on the 10th of October. In November pacing events decreased to 2 PPH per day (Figure A3).

Lovika

Pacing duration in minutes for Lovika was summed by hour for 1 month and a half, with pacing data available for the end of September, full October and early November.

Lovika was not pacing through the pacing antenna at the visitor building, so no pacing data was available until a second antenna was placed next to the fence in the upper enclosure in the end of September. Lovika was pacing through this second antenna, mostly during the night between 5:30pm-3.30 am, peaking between 3:30 and 5:30 am. She also paced to a lesser degree during the morning between 6.00-9.30 and during the afternoon between 3.00-4.30pm (Figure A4). During September Lovika was pacing from 5pm throughout the night until 6.00am with some pacing also around 8.00 am. In October Lovika was pacing every hour with higher pacing duration between 0.00 am – 5.00 pm and around 6.00 pm. In November, Lovika was pacing less than the previous months, during the morning between 05.00 am- 8.00am and during the night between 10.00 – 11.00pm. She was predominantly pacing at night, and rather little during daytime. She paced more in October than September and November, although one should bear in mind that the two last mentioned months are based on only ca 1 week of data each (Figure A5). In end of September, Lovika had around 8 positives pacing hours (PPH) per day. The number of PPH/day decreased markedly in mid-October but then returned to approximately the same levels as in late September and early October. (Figure A6).

5 Discussion

5.1 Olfactory treatment

The aim of this study was to evaluate the behavioural effect of olfactory and acoustic treatments on the behaviour of captive lynxes by the means of new automated monitoring technology. When exposed to the odour treatments, the lynxes were found to interact differently with each odour. Based on the visual observations, they interacted more often with the catnip treatment than with cinnamon, valerian and control treatments. *Sniffing, rubbing, biting and licking* were the most significant displayed behaviour during the catnip treatments. These treatment-direct behaviours indicated a stronger response to catnip than to cinnamon, valerian and control. The lynxes were also found to perform the behaviour *rubbing* more often with cinnamon treatment compare to the control. The results from the HDX PIT tag antenna and BLE tag smartphone were slightly different. The HDX PIT tag antenna showed a higher number of visits in the catnip, cinnamon and valerian treatment compared to the control treatment. In the BLE tag data, the lynxes did not interact significantly more with any of the odour treatments compared to control. The overall results still suggested more interaction with the catnip treatment, in agreement with the visual observations.

The different results between the monitoring technologies and the visual observations may be explained by the different type of data. It is actually difficult to compare the data from the visual observations with those of the HDX antenna and BLE smartphones. The visual observations are a measure of the quality of the interaction, where some behaviour scored very high (rubbing and sniffing), which would not be reflected by the HDX and BLE data . Also, with the HDX PIT tag and the BLE tag data are generated 24 hours a day whereas the visual observations only give a maximum of 4 hours of data in the morning and evening of each day. Hence, this difference may be explained by the timing of visit. The lynxes visited the scent station most of the time during the night between 10.00pm and 2.00am and early in the morning between 7.00am and 8.00am whereas the visual observations were carried out between 8.00pm and 11.30 pm and 8.30 and 10.30 am. This stresses the fact that results from behaviour observations may be very much affected by diurnal patterns and that automated around-the-clock data collection using techniques like HDX PIT tags and BLE tags may give a better picture of the responses to e.g. different odour treatments.

Furthermore, the HDX PIT tag antenna and the BLE tag smartphones registered only the cats being close to the treatment station and not their behaviour. Some visits, especially as logged by the BLE smartphone, could have been the lynxes just passing by the scent station without displaying any behaviour directed towards the treatment. This, however, may be revealed by the duration of the visit. With the HDX PIT tag data, the number of visits during the valerian treatment was higher than during the cinnamon treatment, but the duration during valerian treatment was shorter (Figure 13). During the catnip treatment both number of visits and duration were the highest. The low duration during the valerian treatment may be explained by the PIT tag being just at the max range for being detected, and that the cats while interacting with the valerian was moving the tag in and out of reading range, while they were staying inside reading range for longer periods of time with the other odours. According to the visual observations the cats performed a lot of rubbing (107; Table 2) with catnip, which would require the head being in close contact with the branch, and hence the PIT tag inside the antenna read range, whereas they did only 11 rubbings with cinnamon and one with the valerian.

A somewhat different picture is shown with the BLE tag data (Figure 15). Here too the number of visits during the valerian treatment is correlated with short duration, whereas the number of visits as well as duration during the cinnamon treatment was high than both catnip and valerian treatments. The control treatment is characterized by rather high number of visits but with a very short duration, indicating that the cats were passing by the scent station rather than approaching it to investigate. The duration of the visits as logged by both HDX PIT tag antenna and the BLE tag smartphone during the scent treatments was similar (Figure 13 and 15), indicating that the cats were approaching the scent station directly and purposefully. If the duration logged by the BLE tag smartphone would have been considerably longer, then this would have indicated that the cats spent more time away from the station, out of range by the HDX PIT tag antenna. This was obviously not the case.

The Reconyx Hyperfire camera was placed to verify that the detections matched with some treatment directed behaviour but unfortunately, due to the extremely hot weather during the summer of 2018, the camera's trig function did not work as expected and therefore only very few photos were obtained, showing no significant interactions with the odour treatments for any of the lynxes.

The differences between the three monitoring technologies could be explained first by the different period of time when the data were recorded. The BLE tag data covered the beginning and middle of July, the HDX PIT tag data covered the end of July and the Reconyx camera pictures covered the month of August. The three technologies monitored the same visit only on 3 occasions at the same time and date. Since the schedule for each odour treatment was chosen by pseudo-randomization, the monitoring technologies detected visits with a different number of odour treatment sessions. For example, the HDX PIT tag recorded during 3-4 odours treatments sessions compared to one control treatment session. For the BLE tags and Reconyx camera the number of sessions per odour treatments were equally much the same. Moreover, the distance of detections at the scent station could also explain the discrepancies between the monitoring technologies. The Reconyx camera covered a wide scene so the lynxes should be easily detected in the vicinity of the scent station, whereas the HDX antenna required the cats to get very close to the scent station and the BLE tag required the cats to be at 1m from the scent station to be detected.

The overall results from the present study indicate an influence of catnip on the behavioural response of the lynxes, with an increase of treatment-directed behaviour, including *sniffing, rubbing, biting, licking*. The effect of catnip on lynxes has been poorly investigated and the present result that lynxes respond to catnip agrees with the results of only one previous study. Todd (1963) cited by Tucker and Tucker (1988) reported the specific "catnip response" in one Eurasian lynx, i.e. (1) sniffing, licking and chewing with head-shaking, (2) chin- and cheek rubbing, and (3) head-over rolling and (4) body rubbing, i.e. very similar to the behaviours seen in Loger. However, other species of the genus Lynx, i.e. bobcats, have also been found to respond positively towards catnip (Todd (1963) cited by Tucker and Tucker (1988); Bol et al., (2017). In contrast, Hill et al. (1976) found only a weak response to catnip in bobcats. In domestic cats (*Felis silvestris catus*) exposure to cloth impregnated with catnip increased playing behaviour, including pawing, wrestling and rolling (Ellis and Wells, 2010), which are similar to the behaviours included in *treatment-directed* behaviour in the present study. Moreover, catnip was also found to increase active behaviour, which includes moving and exploring, in black-footed cats (*Felis nigripes*) (Wells and Egli, 2004). In the wild, to detect and monitor the lynx population, the mixture of catnip oil and beaver castoreum was used to promote rubbing behaviour in order to collect hair samples and as an olfactory attractant for camera trapping (McDaniel et al., 2000; McKelvey et al., 1999; Nielsen and McCollough, 2009).

In the present study, the lynxes showed an increase in treatment-directed behaviour towards catnip, by displaying the typical "catnip response". Catnip contains nepetalactone which is the active molecule responsible for the "catnip response" (Bol et al., 2017; Palen and Goddard, 1966; Tucker and Tucker, 1988). The intraspecific variation in behavioural responses has been suggested to be explained by genetics: Todd (1962) observed that only 70 % of domesticated cats reacted positively to catnip during a controlled breeding study. He concluded that the catnip response is heritable through an autosomal dominant gene, making these cats specifically responsive to nepetalactone. There may be a similar process in lynxes with a proportion of lynx hereditably predisposed to the catnip response.

No evidence of habituation was found in the odour responses. This finding suggests that these olfactory treatments may be offered frequently to the lynxes without them losing their interest. However, a study conducted on black-footed cats (*Felis nigripes*) by Wells and Egli (2004), found contradictory results and habituation was observed over five days of exposure of catnip, nutmeg and prey scent. Overall, catnip elicits consistent treatment-directed behaviour in the three lynxes in the present study, which seems to fulfil the requirement of an effective olfactory enrichment for captive lynxes.

Curiously, the valerian treatment did not have the expected effects on the behaviour of the lynxes. Valerian oil is extracted from the valerian root and contains a chemical compound called actinidine. This has a similar structure as nepetalactone which causes the "catnip response" in Felids (Bol et al., 2017; Tucker and Tucker, 1988). Actinidine is supposed to be responsible for the attractiveness of valerian in domestic cats (Bol et al., 2017; Tucker and Tucker, 1988), and Bol et al.(2017) found that valerian root could be an alternative olfactory enrichment for domestic cats not attracted by catnip. Furthermore, in captivity, valerian has been found to attract and induce rubbing behaviour in European wild cats (Monterroso et al., 2011). Nevertheless, no significant behavioural response was found with the valerian treatment in the lynxes in this study. This may be due to the concentration of the valerian oil extract used in this study, although unfortunately this could not be determined. The oil smelled strongly to the human nose and is intended for use in lures for canids and cats, hence expected to be strong enough to be clearly detected by the lynxes.

Interestingly, the result that the lynxes were affected to a lesser degree by cinnamon by only displaying the behaviour *rubbing* significantly more often than during control also contradicted results of previous studies on other felids. Odorous herbs (here catnip and valerian) are not considered as biologically meaningful compared to other scents, like body odours or urine from predators or the lynxes' prey species (Wells, 2009). However, spices have proved to be effective olfactory enrichment. For example, Skibiel et al (2007) tested several spices including cinnamon as olfactory enrichment for six species of captive Felids, including tigers, ocelots, jaguars, cougars, cheetahs and lions. They found a significant increase of active behaviours in these cats, compared to baseline, which included locomotion, social behaviour, feeding, rolling, scent marking, and vocalizing. The same results were also obtained with snow leopards (*Panthera uncia*) which interacted most often with cinnamon (Rosandher, 2009).

Concerning stereotypic behaviour, no difference was found in average duration of pacing between odour treatments and control, and pacing was not reduced by the olfactory treatments. Resende et al. (2011) tested the effect of olfactory enrichment (cinnamon and catnip) on the stereotypical behaviour in oncilla cats (*Leopardus tigrinus*). In contrast with the findings in the present study, they found a decrease in time spent pacing with cinnamon treatment, whereas no significant responses were found for the catnip treatment. Similarly, Skibiel et al. (2007) found a decrease of the frequency of pacing after exposure to cinnamon, cumin and chilli powder in five species of felids (tigers, ocelots, jaguars, cougars and lions). The difference in these finding may be explained by the fact that the HDX PIT tag antenna detected the pacing of only one of the cats, Bore, but did so automatically 24/7. During the scent treatments only one antenna was placed in the pacing track used by Bore and Loger, the latter of which was not properly logged due to the interference between the 12mm and 23mm PIT tags. Lovika was pacing close to this antenna but without going through it, so she was not logged either. In addition, due to a close-by stroke of lightning the HDX PIT tag reader was damaged, interrupting scent treatment data collection for 12 days while it was repaired. To summarize, a lot of pacing data were lost and only a part of the cats' pacing could be analysed. For the following enrichments, the scent station antenna was moved in September to the upper enclosure, where Lovika did most of her pacing. Bore changed his pacing track in September and did most of his pacing in the upper enclosure as well, where he was logged by this antenna.

5.2 Auditory treatment

One of the aims of this study was to evaluate the effect of auditory treatments on the behaviour of captive lynxes by the means of new automated monitoring technology. Unfortunately, the BLE tag detections and the Reconyx hyperfire wildlife camera did not provide enough data during these experiments to perform the statistical analysis, so only the results of the visual observations will be discussed here. The lynxes were found to spend significantly more time on behaviours directed toward the speaker playing the sounds compared to pre- and post-treatment. The mean duration of sound-directed behaviours was higher towards lynx calls, lynx growls and roe deer barks, compared to control. These results suggest that the type of sound treatments influenced the responses of the lynxes. The lynxes tended to be attracted by either conspecific sounds (i.e. lynx growls and lynx calls) and/or prey sounds (i.e. roe deer barks) which are both ecologically relevant sounds that lynxes may encounter in the wild (Wells, 2009). Ecologically relevant sounds have been used in other felids studies as auditory enrichment. Prey sounds corresponding to flying birds were found to increase activity level of a female African leopard (*Panthera pardus*) (Markowitz et al., 1995). The study was designed with four speakers arranged around the indoor enclosure with the first one provided with a motion detector which made it possible to switch the sound to the next speaker until the last one. The sound was automatically controlled by a computer and could be played randomly (Markowitz et al., 1995).The last speaker was provided with a sensor, which when triggered by the cat approaching it activated a food delivery system. A critique to this study claimed that this food treat might have interfered with the direct effect of the sound treatment, with the animal only following the sound in order to collect the food (Wells, 2009). Moreover, another study on lions found that playback of conspecific sounds, i.e. male lion roars, increased roaring in the study lions. In contrast, the lynxes in the present study did not respond by vocalizing after being exposed to conspecific sounds. Other studies on ecologically relevant sounds have also reported contradictory results: natural habitat sounds such as ambient sounds from the rain forest was found to increase activity in two captive adult lowland gorillas (*Gorilla gorilla gorilla*) compared to control treatment (i.e. no auditory stimuli) and likewise to increase normal flying in three African birds, Lady Ross's Turacos (*Musophaga rossae*), Speckled Mousebirds, (*Colius striatus*), and Superb Starlings (*Lamprotornis superbus*) (Ogden et al., 1994; Robbins and Margulis, 2016). Similarly, after exposure to conspecific sounds, Lar gibbons (*Hylobates lar*) increased their general activity level but did not respond by vocalizing (SHEPHERDSON et al., 1989). On the contrary, no significant effects of rain forest sounds were found on the behaviours of twelve other captive lowland gorillas (Brooker, 2016; Wells et al., 2006).

Ecologically non-relevant sounds are sounds that an animal would not be exposed to in its natural environment, such as classical, rock, country, or pop music (Wells, 2009). Some studies have investigated the influence of such ecologically non-relevant sounds on captive animals. Unfortunately, none of these studies include lynx or other felids species. However, it is still interesting to determine the effect of such ecologically non-relevant sounds on animals. In comparison to control treatments, classical music was found to increase resting behaviours and decrease barking in kennelled dog (Bowman et al., 2015; Kogan et al., 2012; Wells et al., 2002 cited by Wells (2009)), and tended to decrease agonistic behaviours in lowland gorillas (Wells et al., 2006).

Furthermore, the other major finding from one of the sound treatments, the lynx call, was that the lynxes were found to spend significantly more time on social interactions with each other during the treatment phase compared to pre- and post-treatment. They did not exhibit social behaviour to any of the other sound treatments. This result suggests that lynx calls might induce social behaviour. Similar results, with increased social behaviours, were obtained with laboratory-housed chimpanzees (*Pan troglodytes*) exposed to radio music (Fritz et al., 2003; Videan et al., 2007). Likewise, Brooker (2016) found that classical music increased social behaviour in captive lowland gorillas. However, sound treatments must be used carefully to ensure not to induce stress, anxiety or maybe even stereotypic behaviour in the animals. Habituation also has to be taken into consideration, indicated by the finding that lynx's attraction to mouse sounds tended to decrease over time. This decrease of attraction may be explained by the motivation of response to mouse sounds, i.e. the potential chance to catch a prey, and if this prey is never found, the response is doomed to fade out. Normal hunting is often failed, but if occasionally successful, then the behaviour is maintained. Further research is needed to determine what type of sounds may be enriching for the lynxes and to conclude if and how auditory enrichment should be used.

The cricket treatment (presence of crickets and their sounds) was found to significantly increase *approach* behaviours, compared to pre- and post-treatment. This suggests that the lynxes were attracted by the sound emitted by the crickets. However, there was no clear evidence that it was the cricket sounds that made the lynxes approached, it may also have been due to the human presence when the crickets were poured into the PE plastic hose. Moreover, the cricket treatment did not have the excepted effects on the lynxes. They were expected to chase, catch and play with the crickets when they were falling out through the holes drilled in the tube. Live crickets have been used as food enrichment, stimulating hunting behaviour in sand cats (*Felis margarita*) and black-footed cats (*Felis nigripes*) (MELLEN and SHEPHERDSON, 1997). However, the lynxes were never seen to interact at all with the crickets.

From the visual observations of pacing, no difference was found in average time pacing between the sound treatments and pre- and post-treatments and pacing was not significantly interrupted by the sound treatments. These findings suggest that pacing was not influenced by the sound treatments. Markowitz et al. (1995) found a decrease of pacing during prey sound treatment compared to baseline in African leopards (*Panthera pardus*). The same result was found in Asian elephants (*Elephas maximus*) where pacing was reduced during playback of classical music compared to the control treatment (Wells and Irwin, 2008). In contrast, Hanbury et al (2009) found no decrease in pacing during classical music treatment compared to baseline in a prosimian primate, Garnett's bushbaby (*Otolemur garnetti*i). The results from the visual observations were supported by those from the HDX PIT tag antenna, which showed no differences between treatments in average time pacing. even though the number of pacing events was found to be weakly associated with some of the sound treatments. The lynxes tended to have more pacing events during playback of lynx calls, lynx growls and mouse squeals compared to the control and roe deer bark. However, the HDX PIT tag pacing data matched with only eight days out of 20 with the sound treatments, with lynx growl and control available two days each. Since HDX PIT tag data were missing for more than half of the sound treatment period, other factors such as feeding time, enclosure size or individuals in the group, which were found to have influenced stereotypical behaviour in cheetahs, this might also explain the difference in number of pacing events between lynx growls and control treatments (Quirke et al., 2012).

5.3 Time budget

In the wild, lynxes are active during night and dusk (Boulat, 2010; Krelekamp, 2004; Podolski et al., 2013). Based on this activity pattern the enrichments were applied in the morning and/or late in the afternoon, and the visual observations were synchronized with this.

Lynx time budgets in captivity have been poorly investigated. However, Resende et al. (2014) found that oncilla cats (*Leopardus tigrinus*) in captivity spent 66 % of their time resting. Likewise, ocelots spent 69.3 % of their time resting (Weller and Bennett, 2001b). Tigers in captivity spend also most of their time resting (76%) (Pitsko, 2003). Based on these results, the effect of enrichments on the lynxes, as measured through the visual observations, in this study may have been influenced by their inactivity during the day, especially during the warm summer months, which makes lynxes reduce their activity even more (Beltrán and Delibes, 1994). This was exaggerated in 2018 since the summer months offered extremely hightemperatures. Even if the observations were adjusted to when the lynxes were the most active, Mellen et al. (1998) argued that inactivity during the day is instinctive in both wild and captive small felids and trying to change their nocturnal patterns by stimulating activity during the day would be unnatural and not beneficial. Hence the time when enrichments are introduced should be adapted to the lynxes' most active phase, i.e. during the night, even though it may be more difficult to manage for the keepers. Further studies on enrichment offered by night and observed at the same time would be interesting to do.

Furthermore, Lovika did a total of 68% of her pacing in September and November during the night and Bore did a total of 80.3% of his pacing in June and August during the night. So, offering the enrichment at night might have a better effect. However, Bore changed his pacing in September and November to pacing a total of 90.6% during the day. This difference between summer and autumn may be explained by the cooler temperature during daytime. Vlaming (2013) found a decrease of pacing with an increase of temperature in South China tigers (*Panthera tigris tigris*). However, further research is needed to confirm or reject a possible temperature effect on the pacing of the Kolmarden lynxes and to find out what other factors may influence their pacing.

5.4 Improvement of the study

The monitoring technology used in this study helped to support and supplement the results of the visual observations by adding more data and cover 24hours per day. However, some adjustments need to be done to optimize the data collection.

First, the Reconyx camera could not be relied upon in the summer time when the temperatures were high; the camera required a clear difference between the animal's body surface temperature and the ambient temperature. Maybe this limits the use of this camera to only during colder seasons. Furthermore, a pilot study to find the location of main pacing tracks should be carried out, to make it possible to determine the best positions for and the number of necessary pacing HDX PIT tag antennas. Only the large (23mm) PIT tag should be used in big animals like lynx, in order to get the necessary read range. If an animal has already been provided with a small tag, and longer read range is required, the bigger tag should not be placed too close to the small one, since they will interfere with each other and reduce the detections.

The smartphones used to detect the BLE tags were first placed rather high up on the house wall and on top of the fence, to prevent any damage by the cats. It turned out that this reduced the read range too much; the total read range is only ca. 50 m. Therefore some smartphones were suspended with a line inside the fence, approximately 2m above the ground. Other phones were placed in the viewing windows inside the visitor house, in the same level as the cats. For the latter to work, it is important to test if and how much the glass attenuates the BLE signal. Some glasses are provided with a low emission coating to improve the U-value or to get a sun protection effect, which may completely attenuate the BLE signal. Also metal fences will prevent the BLE signals to pass.

The HDX PIT tags and the BLE tags can only track the animals' movements, and different behaviours cannot be distinguished. Stationary behaviours can be implied by a fixed signal strength logged by the BLE tag smartphone, but a fixed signal strength may also be produced if the cat moves along the same radius from the smartphone. The HDX PIT tag cannot detect a stationary cat, if it is not staying inside the antenna frame.

The wildlife camera can be set to take short video sequences, but still the scope of behaviours captured by these will be limited. An alternative to manual, visual observations would be continuous video, but this requires lengthy analysis, which may not be feasible due to limited man power. Here a combination with the automatic techniques may be possible: the BLE tag and/or HDX PIT tag log can give the time stamp of a visit to e.g. a scent station, which make it possible to zoom in on the interesting video sequence from which detailed behaviours can be studied.

5.5 Conclusion

The results of the present study show that catnip was effective in eliciting a clear "catnip response" i.e *sniffing, rubbing, biting, licking*. Catnip was shown to be a suitable olfactory enrichment for increasing *treatment-directed* behaviour for these lynxes. In contrast, valerian treatment had no significant effects on the lynxes and cinnamon had only a partial effect with an increase of *rubbing* behaviour. None of the olfactory treatments was found to decrease pacing

In the present study, conspecific and prey sounds were found to attract the lynxes by increasing their arousal. In addition, one sound treatment, the lynx call, provoked social behaviour. Further research is needed to determine the effect of such replayed calls on the social behaviour of the lynxes.

Furthermore, olfactory and sound treatments could be used together as a new form of enrichment for captive lynxes but could also be used in the wild as lures to attract lynxes to BLE or HDX PIT tag logging stations, as part of monitoring a lynx population. For this to work properly, the individual differences must be further explored, in order to assess to what extent the responding animals' visits are representative to the population.

Concerning the new automated technology, the combination of the HDX PIT tags, BLE tags and Reconyx Hyperfire camera are powerful tools for evaluating the effect of enrichment on captive lynxes. However, although these technologies as applied in this study covered the whole 24 hours per day, they only logged limited aspects of the cats' behaviour. The visual observations, on the other hand, produced detailed data on behaviour, but was much more limited in time. Hence a combination of these techniques is recommended.

6 Societal and ethical considerations

During gating, the lynxes were called by the keepers to a station in one of the upper back enclosures. This procedure was based on cooperation with the lynxes. If they did not want to participate gating was stopped to ensure that no harm and stress was done. The next gating was not attempted until the following day. This gating procedure was part of routine management training which were only carried out by the keepers. It was based on operant conditioning, where the primary reinforcement was small pieces of meat. The lynxes were never over fed or food deprived. Collars were applied after immobilisation, done by the zoo vets, according to an ethical permit issued by the Linköping Board for Research Ethics. The collars were carefully adjusted to the size of each lynx' neck. The cats showed no sign of irritation or being negatively affected by the collars. They were continuously assessed by the keepers and the veterinarians to ensure no harm. The HDX PIT tags were injected subcutaneously between the shoulder blades while the cats were immobilized. This was done by the zoo veterinarians.

Norther lynx is classified at least concern and conservation plan are still needed. According to the IUCN monitoring is part of the research plan needed to evaluate the current trend of the lynx population. This thesis project could serve as a part of this research plan where the monitoring technology ie BLE tag, HDX PIT and Reconyx hyperfire camera could be used as a trapping station. This station could be baited with scent and sound lures to attract the lynxes. Since one of the odour treatment was found to be suitable as environmental enrichment in captivity, it could be advised to used it as a scent lure in the wild paired with the logging station to help monitor the lynx population. A better understanding of which enrichment or which combination of enrichment provoked behavioural response in lynxes is useful for increasing their welfare. And finding the most suitable enrichment could also help to improve lynx welfare in captivity.

7 Acknowledgements

I would like to thank my supervisor Mats Amundin for his help and support throughout the study which includes building and installing the materials into the enclosure, helping with planning the enrichments and also feedback to the written report. I would like also to thank the keepers at the carnivore department for their patience, help, advice, friendship and for carrying out amazingly the gating sessions. The study would not have been possible without them. I'm also grateful to the other staff members of Kolmården Wildlife Park to allow me and making it possible to realize the study. I would like to thank Gustaf Hendeby and Fredrik Gustafsson for letting me use their BLE tag technology and providing their assistance with it.

8 References

Allen, M.L., Wallace, C.F., and Wilmers, C.C. (2015). Patterns in bobcat (Lynx rufus) scent marking and communication behaviors. Journal of Ethology *33*, 9–14.

Altmann, J. (1974). Observational study of behavior: sampling methods. Behaviour *49*, 227–266.

von Arx, M. 2018. Lynx lynx (errata version published in 2019). The IUCN Red List of Threatened Species 2018: e.T12519A145266191. https://www.iucnredlist.org/species/12519/145266191 (Acessed 5 May 2019).

Beltrán, J.F., and Delibes, M. (1994). Environmental determinants of circadian activity of free-ranging Iberian lynxes. Journal of Mammalogy *75*, 382–393.

Bol, S., Caspers, J., Buckingham, L., Anderson-Shelton, G.D., Ridgway, C., Buffington, C.A.T., Schulz, S., and Bunnik, E.M. (2017). Responsiveness of cats (Felidae) to silver vine (Actinidia polygama), Tatarian honeysuckle (Lonicera tatarica), valerian (Valeriana officinalis) and catnip (Nepeta cataria). BMC Veterinary Research *13*, 70.

Boulat, C. (2010). Elevage de jeunes lynx (Lynx Lynx) en captivité en vue de leur relâcher: Protocoles réalisés en France jusqu'en 2008.

Bowman, A., Scottish, S., Dowell, F.J., and Evans, N.P. (2015). 'Four Seasons' in an animal rescue centre; classical music reduces environmental stress in kennelled dogs. Physiology & Behavior *143*, 70–82.

Brassine, E., and Parker, D. (2015). Trapping elusive cats: using intensive camera trapping to estimate the density of a rare African felid. PloS One *10*, e0142508.

Breitenmoser, U., Breitenmoser-Würsten, C., Lanz, T., von Arx, M., Antonevich, A., Bao, W. & Avgan, B. (2015). Lynx lynx (errata version published in 2017). The IUCN Red List of Threatened Species 2015: e.T12519A121707666.(Accessed on 30 March 2018).

Brooker, J.S. (2016). An investigation of the auditory perception of western lowland gorillas in an enrichment study. Zoo Biology *35*, 398–408.

Chester Zoo (2009). Environmental Enrichment.

Clark, F., and King, A.J. (2008). A critical review of zoo-based olfactory enrichment. In Chemical Signals in Vertebrates 11, (Springer), pp. 391–398.

Classon, I. (2017). Inventering av lodjur (Lynx lynx), och vilka faktorer som kan påverka lodjursbesök vid kamerastationer.

Clubb, R., and Mason, G.J. (2007). Natural behavioural biology as a risk factor in carnivore welfare: How analysing species differences could help zoos improve enclosures. Applied Animal Behaviour Science *102*, 303–328.

Clubb, R., and Vickery, S. (2006). Locomotory stereotypies in carnivores: does pacing stem from hunting, ranging, or frustrated escape. Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare *2*, 58–79.

Damasceno, J., Genaro, G., Quirke, T., McCarthy, S., McKeown, S., and O'Riordan, R. (2017). The effects of intrinsic enrichment on captive felids. Zoo Biology *36*, 186–192.

Ellis, S.L.H., and Wells, D.L. (2010). The influence of olfactory stimulation on the behaviour of cats housed in a rescue shelter. Applied Animal Behaviour Science *123*, 56–62.

Fritz, J., Roeder, E., and Nelson, C. (2003). A stereo music system as environmental enrichment for captive chimpanzees. Lab Animal *32*, 31.

Garrote, G., Gil-Sánchez, J.M., McCain, E.B., de Lillo, S., Tellería, J.L., and Simón, M.Á. (2012). The effect of attractant lures in camera trapping: a case study of population estimates for the Iberian lynx (Lynx pardinus). European Journal of Wildlife Research *58*, 881–884.

Hanbury, D.B., Fontenot, M.B., Highfill, L.E., Bingham, W., Bunch, D., and Watson, S.L. (2009). Efficacy of auditory enrichment in a prosimian primate (Otolemur garnettii). Lab Animal *38*, 122.

Hill, J.O., Pavlik, E.J., Smith, G.L., Burghardt, G.M., and Coulson, P.B. (1976). Species-characteristic responses to catnip by undomesticated felids. Journal of Chemical Ecology *2*, 239–253.

Hoy, J.M., Murray, P.J., and Tribe, A. (2010). Thirty years later: enrichment practices for captive mammals. Zoo Biology *29*, 303–316.

Hughes, B.O., and Duncan, I.J.H. (1988). The notion of ethological 'need', models of motivation and animal welfare. Animal Behaviour *36*, 1696–1707.

Kelling, A.S., Allard, S.M., Kelling, N.J., Sandhaus, E.A., and Maple, T.L. (2012). Lion, ungulate, and visitor reactions to playbacks of lion roars at Zoo Atlanta. Journal of Applied

Animal Welfare Science *15*, 313–328.

Kelly, M.J., and Holub, E.L. (2008). Camera trapping of carnivores: trap success among camera types and across species, and habitat selection by species, on Salt Pond Mountain, Giles County, Virginia. Northeastern Naturalist *15*, 249–262.

Kitchener, A.C., Van Valkenburgh, B., and Yamaguchi, N. (2010). Felid form and function. Biology and Conservation of Wild Felids 83–106.

Kogan, L.R., Schoenfeld-Tacher, R., and Simon, A.A. (2012). Behavioral effects of auditory stimulation on kenneled dogs. Journal of Veterinary Behavior: Clinical Applications and Research *7*, 268–275.

Krelekamp, C.J. (2004). HUSBANDRY GUIDELINES Eurasian lynx (Lynx lynx sspp.). European Association of Zoos and Aquaria (EAZA). Amsterdam, Netherlands.

Krofel, M., and Kos, I. (2009). Recording the Eurasian lynx (Lynx lynx) vocalization sequences on Snežnik plateau, Slovenia. Natura Sloveniae.

Macri, A.M., and Patterson-Kane, E. (2011). Behavioural analysis of solitary versus socially housed snow leopards (Panthera uncia), with the provision of simulated social contact. Applied Animal Behaviour Science *130*, 115–123.

Maple, T.L., and Perdue, B.M. (2013). Zoo animal welfare (Springer).

Markowitz, H. (1982). Behavioral enrichment in the zoo (Van Nostrand Reinhold).

Markowitz, H., Aday, C., and Gavazzi, A. (1995). Effectiveness of acoustic “prey”: Environmental enrichment for a captive African leopard (Panthera pardus). Zoo Biology *14*, 371–379.

Mason, G.J. (1991). Stereotypies: a critical review. Animal Behaviour *41*, 1015–1037.

Mason, G., Clubb, R., Latham, N., and Vickery, S. (2007). Why and how should we use environmental enrichment to tackle stereotypic behaviour? Applied Animal Behaviour Science *102*, 163–188.

McDaniel, G.W., McKelvey, K.S., Squires, J.R., and Ruggiero, L.F. (2000). Efficacy of lures and hair snares to detect lynx. Wildlife Society Bulletin 119–123.

McKelvey, K.S., Claar, J.J., McDaniel, G.W., and Hanvey, G. (1999). National lynx detection protocol. US Forest Service, Rocky Mountain Research Station, Missoula, MT.

Mellen, J.D. (1993). A comparative analysis of scent-marking, social and reproductive behavior in 20 species of small cats (Felis). American Zoologist *33*, 151–166.

Mellen, J.D., Hayes, M.P., and Shepherdson, D.J. (1998). Captive environments for small felids. Second Nature: Environmental Enrichment for Captive Animals. Smithsonian Institution Press, Washington 184–201.

MELLEN, J.D., and SHEPHERDSON, D.J. (1997). Environmental enrichment for felids: an integrated approach. International Zoo Yearbook *35*, 191–197.

Melovski, D., Ivanov, G., Stojanov, A., TRAJÇE, A., Zimmermann, F., and VON ARX, M. (2008). First camera-trap survey in the National Park Mavrovo, Macedonia. In Proceedings of the International Conference on Biological and Environmental Sciences, p.

Miller, L.J., Bettinger, T., and Mellen, J. (2008). The reduction of stereotypic pacing in tigers (Panthera tigris) by obstructing the view of neighbouring individuals. Animal Welfare *17*, 255–258.

Monterroso, P., Alves, P.C., and Ferreras, P. (2011). Evaluation of attractants for non-invasive studies of Iberian carnivore communities. Wildlife Research *38*, 446–454.

Monterroso, P., Rich, L.N., Serronha, A., Ferreras, P., and Alves, P.C. (2014). Efficiency of hair snares and camera traps to survey mesocarnivore populations. European Journal of Wildlife Research *60*, 279–289.

Nielsen, C.K., and McCollough, M.A. (2009). Considerations on the use of remote cameras to detect Canada lynx in northern Maine. Northeastern Naturalist *16*, 153–157.

Ogden, J.J., Lindburg, D.G., and Maple, T.L. (1994). A preliminary study of the effects of ecologically relevant sounds on the behaviour of captive lowland gorillas. Applied Animal Behaviour Science *39*, 163–176.

Palen, G.F., and Goddard, G. V (1966). Catnip and oestrous behaviour in the cat. Animal Behaviour *14*, 372–377.

Peters, G. (1987). Acoustic communication in the genus Lynx (Mammalia: Felidae)-comparative survey and phylogenetic interpretation. Bonner Zoologische Beitrage *38*, 315–330.

Pitsko, L.E. (2003). Wild tigers in captivity: a study of the effects of the captive environment on tiger behavior.

Podolski, I., Belotti, E., Bufka, L., Reulen, H., and Heurich, M. (2013). Seasonal and daily activity patterns of free-living Eurasian lynx Lynx lynx in relation to availability of kills. Wildlife Biology *19*, 69–77.

du Preez, B.D., Loveridge, A.J., and Macdonald, D.W. (2014). To bait or not to bait: a comparison of camera-trapping methods for estimating leopard Panthera pardus density. Biological Conservation *176*, 153–161.

Quirke, T., O'Riordan, R.M., and Zuur, A. (2012). Factors influencing the prevalence of stereotypical behaviour in captive cheetahs (Acinonyx jubatus). Applied Animal Behaviour Science *142*, 189–197.

Resende, L. de S., Pedretti Gomes, K.C., Andriolo, A., Genaro, G., Remy, G.L., and Almeida Ramos, V. de (2011). Influence of cinnamon and catnip on the stereotypical pacing of Oncilla Cats (Leopardus tigrinus) in captivity. Journal of Applied Animal Welfare Science *14*, 247–254.

Resende, L. de S., Neto, G.L. e, Carvalho, P.G.D., Landau-Remy, G., Ramos-Júnior, V. de A., Andriolo, A., and Genaro, G. (2014). Time budget and activity patterns of oncilla cats (Leopardus tigrinus) in captivity. Journal of Applied Animal Welfare Science *17*, 73–81.

Robbins, L., and Margulis, S.W. (2016). Music for the birds: effects of auditory enrichment on captive bird species. Zoo Biology *35*, 29–34.

Rosandher, Å. (2009). Olfactory enrichment for captive snow leopards (Uncia uncia).

Rose, P.E., Nash, S.M., and Riley, L.M. (2017). To pace or not to pace? A review of what abnormal repetitive behavior tells us about zoo animal management. Journal of Veterinary Behavior: Clinical Applications and Research *20*, 11–21.

SCANDLYNX, the Scandinavian lynx project http://scandlynx.nina.no/scandlynxeng/About-SCANDLYNX (Accessed March 30 2018)

Schlexer, F. V (2008). Attracting animals to detection devices. Noninvasive Survey Methods for Carnivores 263–292.

Schmidt, K., and Kowalczyk, R. (2006). Using scent-marking stations to collect hair samples to monitor Eurasian lynx populations. Wildlife Society Bulletin *34*, 462–466.

Shepherdson, D., Mellen, J.D., and Hutchins, M. (1998). Second nature : environmental enrichment for captive animals. (Washington ; London : Smithsonian Institution Press, cop. 1998).

SHEPHERDSON, D., BEMMENT, N., CARMAN, M., and REYNOLDS, S. (1989). Auditory enrichment for Lar gibbons Hylobates lar at London Zoo. International Zoo Yearbook *28*, 256–260.

Skibiel, A.L., Trevino, H.S., and Naugher, K. (2007). Comparison of Several Types of Enrichment for Captive Felids. Zoo Biology *26*, 371–381.

Stanton, L.A., Sullivan, M.S., and Fazio, J.M. (2015). A standardized ethogram for the felidae: A tool for behavioral researchers. Applied Animal Behaviour Science *173*, 3–16.

Steyer, K., Simon, O., Kraus, R.H.S., Haase, P., and Nowak, C. (2013). Hair trapping with valerian-treated lure sticks as a tool for genetic wildcat monitoring in low-density habitats. European Journal of Wildlife Research *59*, 39–46.

Swaisgood, R., and Shepherdson, D. (2006). Environmental enrichment as a strategy for mitigating stereotypies in zoo animals: a literature review and meta-analysis. Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare *2*, 256–285.

Swaisgood, R.R., and Shepherdson, D.J. (2005). Scientific approaches to enrichment and stereotypies in zoo animals: what’s been done and where should we go next? Zoo Biology *24*, 499–518.

Todd, N.B. (1962). Inheritance of the catnip response in domestic cats. Journal of Heredity *53*, 54–56.

Todd, N.B. (1963). The Catnip Response. Cambridge: Harvard University 45,46.

Tucker, A.O., and Tucker, S.S. (1988). Catnip and the catnip response. Economic Botany *42*, 214–231.

Videan, E.N., Fritz, J., Howell, S., and Murphy, J. (2007). Effects of two types and two genre of music on social behavior in captive chimpanzees (Pan troglodytes). Journal of the American Association for Laboratory Animal Science *46*, 66–70.

Vlaming, Z. (2013). Factors influencing pacing in the South China tiger (Pt amoyensis).

Vogt, K. (2015). Olfactory communication and hunting behaviour of Eurasian lynx Lynx lynx in the Northwestern Swiss Alps.

Vogt, K., Zimmermann, F., Kölliker, M., and Breitenmoser, U. (2014). Scent-marking behaviour and social dynamics in a wild population of Eurasian lynx Lynx lynx. Behavioural Processes *106*, 98–106.

Vogt, K., Hofer, E., Ryser, A., Kölliker, M., and Breitenmoser, U. (2016). Is there a trade-off between scent marking and hunting behaviour in a stalking predator, the Eurasian lynx, Lynx lynx? Animal Behaviour *117*, 59–68.

Watters, J. V. (2009). Toward a predictive theory for environmental enrichment. Zoo Biology *28*, n/a-n/a.

Weller, S.H., and Bennett, C.L. (2001a). Twenty-four hour activity budgets and patterns of behavior in captive ocelots (Leopardus pardalis). Applied Animal Behaviour Science *71*, 67–79.

Weller, S.H., and Bennett, C.L. (2001b). Twenty-four hour activity budgets and patterns of behavior in captive ocelots (Leopardus pardalis). Applied Animal Behaviour Science *71*, 67–79.

Wells, D.L. (2009). Sensory stimulation as environmental enrichment for captive animals: a review. Applied Animal Behaviour Science *118*, 1–11.

Wells, D.L., and Egli, J.M. (2004). The influence of olfactory enrichment on the behaviour of captive black-footed cats, Felis nigripes. Applied Animal Behaviour Science *85*, 107–119.

Wells, D.L., and Irwin, R.M. (2008). Auditory stimulation as enrichment for zoo-housed Asian elephants (Elephas maximus). Animal Welfare *17*, 335–340.

Wells, D.L., Graham, L., and Hepper, P.G. (2002). The influence of auditory stimulation on the behaviour of dogs housed in a rescue shelter. Animal Welfare *11*, 385–393.

Wells, D.L., Coleman, D., and Challis, M.G. (2006). A note on the effect of auditory stimulation on the behaviour and welfare of zoo-housed gorillas. Applied Animal Behaviour Science *100*, 327–332.

Young, R.J. (Robert J. (2003). Environmental enrichment for captive animals (Blackwell Science).

9 Appendix

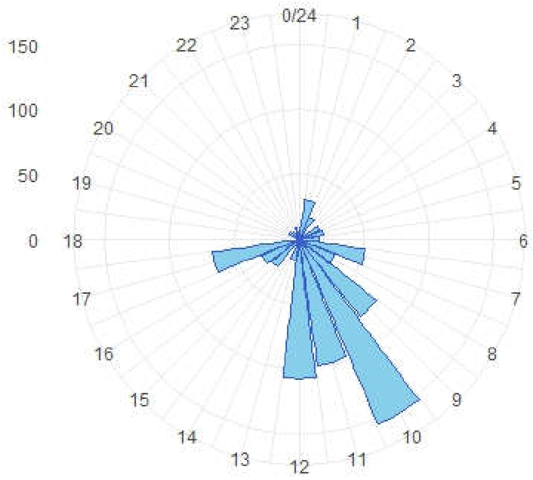

Figure A1 : Pacing habits for Bore summed during 6 months. The circles show pacing duration in minutes within the time scope of the sector with the inner-most circle at 50 min.

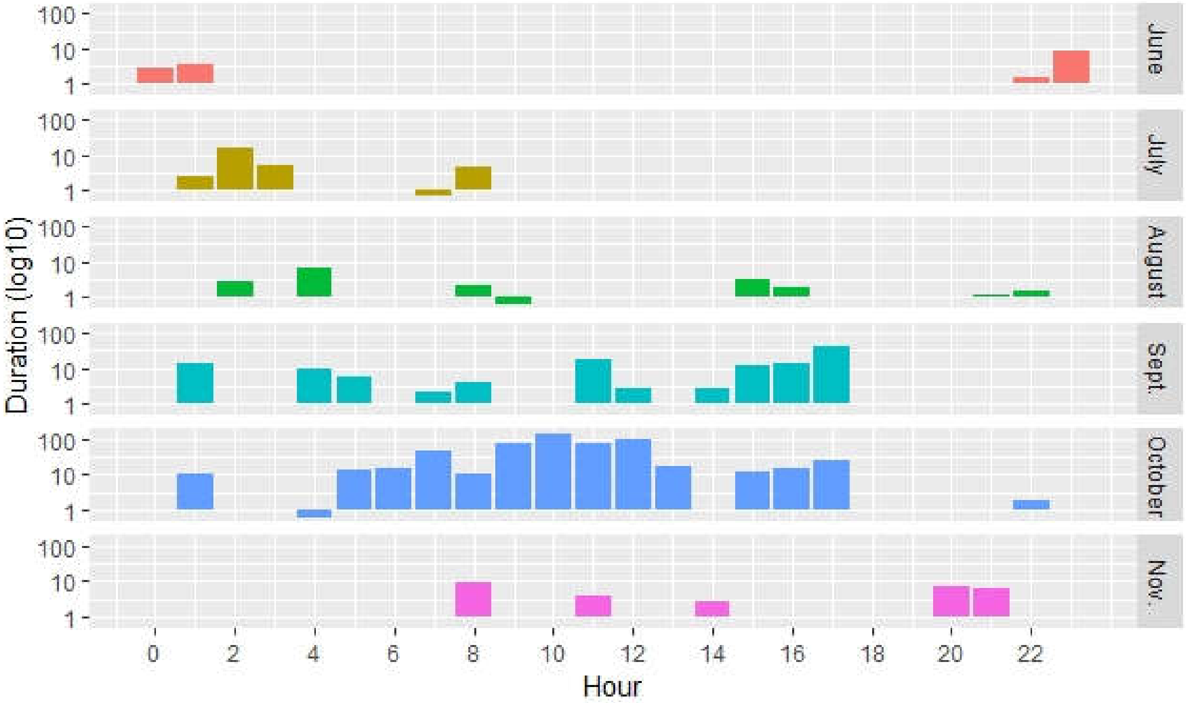

Figure A2 : Total pacing duration per hour by month for Bore

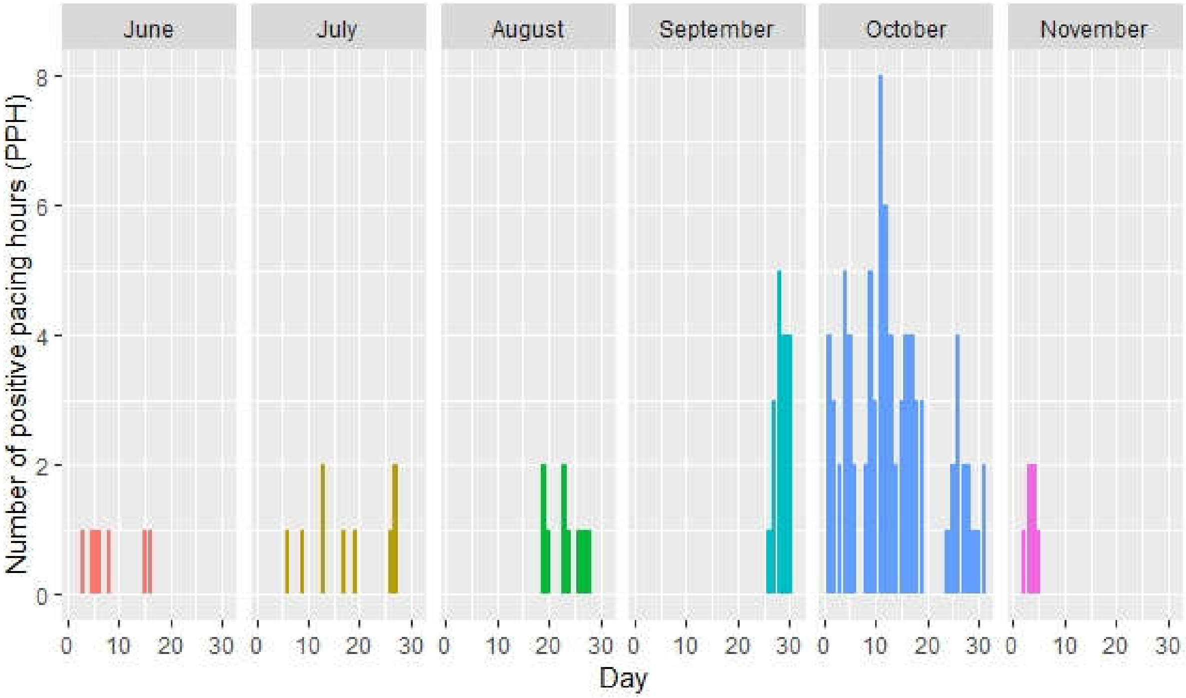

Figure A3 : Daily positive pacing hours (PPH) recorded by the HDX antenna during 6 months for Bore

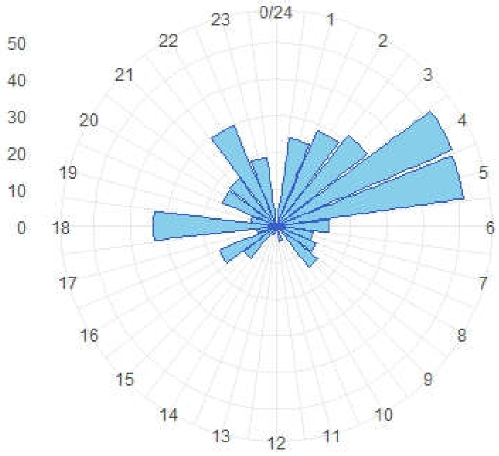

Figure A4 : Pacing habits for Lovika summed from end of September until beginning of November. The circles show pacing duration in minutes within the time scope of the sector with the first circle at 10 min.

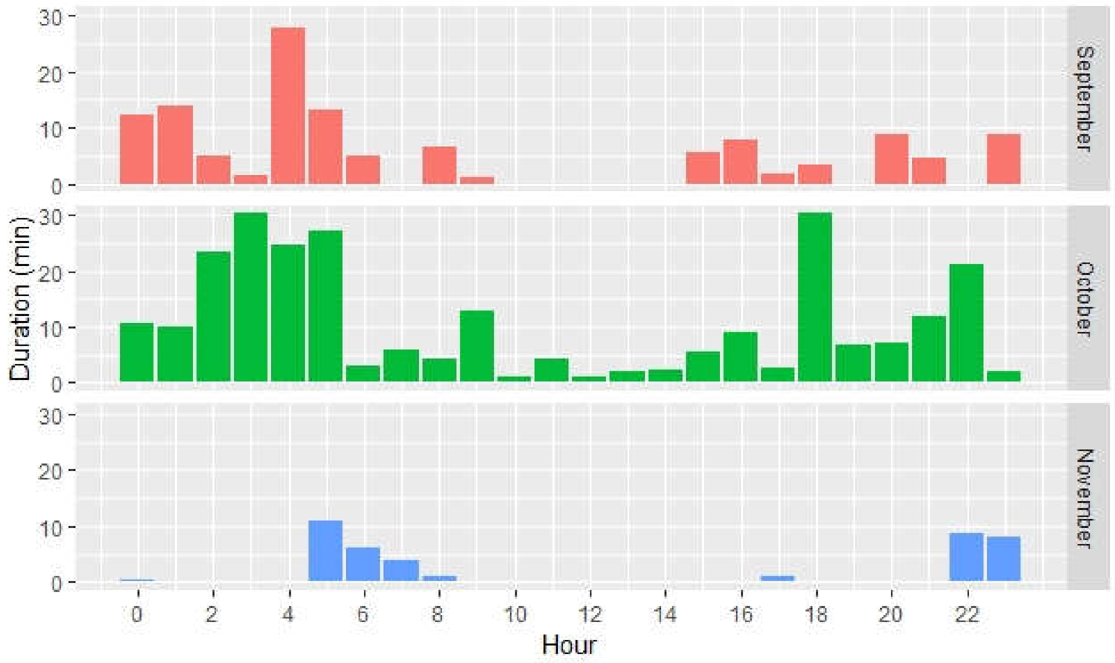

Figure A5 : Total pacing duration per hour by month for Lovika. Data collection started on September 1st and ended on November 6.

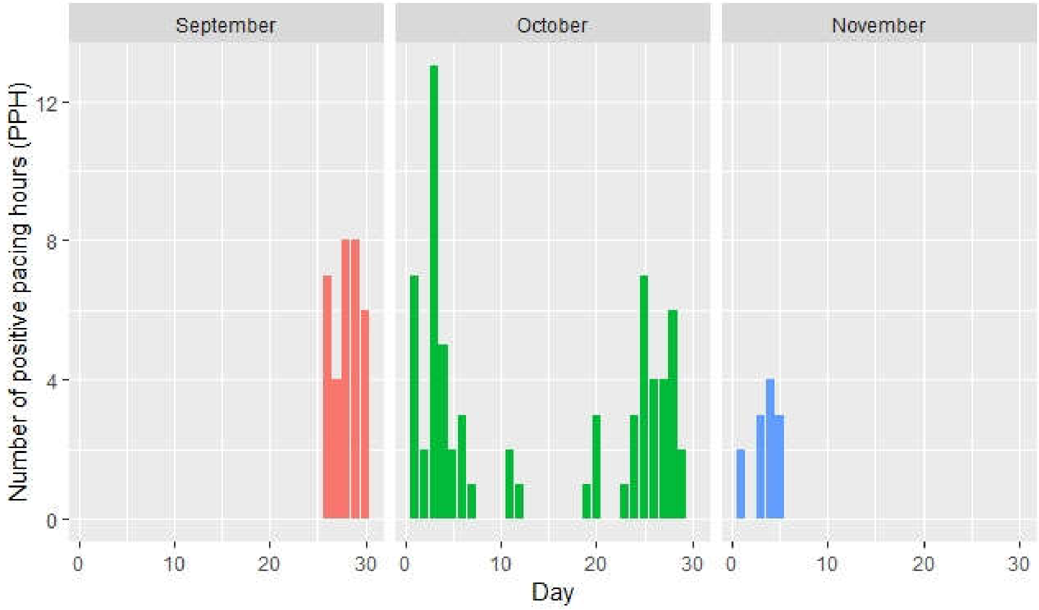

Figure A6 : Daily positive pacing hours (PPH) in September-October for Lovika. Data collection started on September 26th and ended on November 6.