Linköping University | Department of Physics, Chemistry and Biology
Type of thesis, 60 hp | Educational Program: Physics, Chemistry and Biology
Spring or Autumn term 2018 | LITH-IFM-A-EX—18/3448--SE

Behavioural responses of Amur tigers (*Panthera tigris altaica*) and African lions (*Panthera leo*) to conspecific urine and to a component of tiger marking fluid.

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Datum
Date 2018-05-29

Språk
Language

☐ Svenska/Swedish
☒ Engelska/English
☐ ______________

Rapporttyp
Report category

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

ISBN

ISRN: LITH-IFM-A-EX--18/3448--SE

Serietitel och serienummer
Title of series, numbering

ISSN

URL för elektronisk version

Titel
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Sammanfattning
Abstract

Olfactory signals are an important means of social communication among felids. However, not much is known about how individual volatiles of body-borne odours influence behavioural responses. 2-acetyl-1pyrroline has recently been identified as a characteristic component of tiger marking fluid, while being absent from lion marking fluid. One pride each of captive Amur tigers (Panthera tigris altaica) and African lions (Panthera leo) were presented with wooden logs impregnated with four different odours and their behaviour was observed. The tigers displayed significantly more interactions towards the marking fluid component (2-acetyl-1-pyrroline), the conspecific urine odour, and the fruity odour (iso-pentyl acetate) than towards the near odourless control (diethyl phthalate). The lions displayed significantly more behaviours towards conspecific urine than towards any of the other odours. In general all lions interacted more with the logs than tigers. Hence, these results support the notion that 2-acetyl-1-pyrroline is a species-specific odorant for tiger olfactory communication. Furthermore, the results show that a single compound (2-acetyl-1pyrroline) can elicit behavioural responses to the same degree as a complex chemical mixture (tiger urine). The high number of interactions performed by both species towards the wooden logs impregnated with conspecific urine suggests that conspecific odours are suitable to use as olfactory enrichment for captive felids.

Nyckelord
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Contents

1 Abstract

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Keyword: Tiger, Lion, Olfaction, Communication, Enrichment, Behaviour

2 Introduction

Felids are known to strongly rely on olfactory cues for social communication (Stoddart 1980; Gorman and Trowbridge 1989; Wyatt 2014). In addition to faeces and urine some felids, such as the Amur tiger (*Panthera tigris altaica*), use the secretions from specialized scent glands located within the urinary tract, for this purpose (Smith et al 1989; Asa 1993; Burger et al 2008). Behavioural studies suggest that these scent-marks may convey information about an individual's, sex, age, health, reproductive condition, identity and genetic status (van den Brink 1977; Bininda-Emonds et al. 2001; Barja and Javier de Miguel 2010). This variety of information provided through odour signals can be used for a variety of purposes, for example, establishing territorial boundaries, attracting non-related conspecifics as mates, and to avoid inbreeding (Stoddart 1980; Wyatt 2014). Despite the assumed importance of urine and marking fluid odours for behaviours displayed by felids not much is known about the volatiles that make up these complex odour mixtures (Soso et al 2014), and even less is known about if individual chemicals that are components of these odour mixtures may be sufficient to affect intraspecific behaviour (Whittle 1981).

Gas chromatography-mass spectrometry and gas chromatography-olfactometry are methods used to identify which chemicals are the active odour components in a mixture of volatiles.

These two methods were recently used to analyse the chemical composition of tiger marking fluid. Two substances were identified as the characteristic odour components of tiger marking fluid: 2-acetyl-1-pyrroline and furfural (Soso and Koziel 2016). Interestingly, 2-acetyl-1-pyrroline is not found in lion (*Panthera leo*) marking fluid (Poddar-Sarkar and Brahmachary 2014). However, it is found in the head- and cheek-rubbing markings of both tigers and lions (Soini et al. 2012). Humans have a high olfactory sensitivity for 2-acetyl-1-pyrroline (van Gemert 2011) with detection thresholds below 10 ppt (parts per trillion) and describe it as smelling like popcorn (Schieberle 1991). The second characteristic odour component, furfural, has also been found in the urine of wolf (*Canis lupus lupus*), dog (*Canis lupus familiaris*) (Wolfram 2013), and African wild dogs (*Lycaon pictus*) (Apps et al 2012), and even in the urine of humans (Wishart et al. 2013). Due to being present in several different species of mammals, furfural is likley to be less species-specific compared to 2-acetyl-1-pyrroline.

Tigers are solitary animals that roam in densely forested areas, where olfactory cues are essential in upholding the territorial boundaries. Tigers have been reported to discern the presence of conspecifics in dense vegetation through the use of olfactory cues (Shaller 1967). Additionally, tigers also use scent markings to covey information such as reproductive status of females (Soso et al. 2014) and male tigers scent mark at a lower frequency when no sexually receptive female is near (Poddar-Sarkar et al. 1994). Lions are group living animals that together defend their territory or mates from rival same-sex groups (Shaller 1972). One way lions socially discriminate between conspecifics is through olfactory cues and they have been shown to be able to discriminate between both sex and familiarity through urine markings (Gilfillian et al. 2017). Lions are less dependent on olfactory cues to signal their presence as the open landscape of the savannah favours visual and audio cues, and it have been found that lions urinate at a lower frequency and at a higher duration than tigers (Barja and de Miguel 2010).

Olfactory enrichment is already widely used with captive felids and has been found to be effective in preventing or reducing stereotypical behaviours. Previous studies reported that Amur tigers displayed high interest towards wooden logs that were impregnated with mammalian blood or a blood odour component (Sjöberg 2013; Nilsson et al. 2015). The results from these studies suggest that wooden logs impregnated with behaviourally relevant odours are an effective form of olfactory environmental enrichment for captive tigers. However, it is currently unknown whether the use of conspecific odours instead of prey-related odours is also effective as a form of environmental enrichment in this felid species (Skibiel et al 2007; Szokalski et al 2012).

Therefore, the aims of the present study were to: 1) assess the behavioural responses of captive Amur tigers *(Panthera tigris altaica)* and African lions *(Panthera leo)* towards conspecific urine odour and 2-acetyl-1-pyrroline, a volatile component found in tiger marking fluid. But also to 2) compare the behavioural responses towards the conspecific urine odour and the tiger marking fluid component, with the behavioural responses towards two control odours, one plant-derived and one near-odourless. An additional aim was to 3) assess whether conspecific odours presented on wooden logs are a useful form of environmental enrichment for captive tigers and lions.

3 Materials and methods

3.1 Animals

The experiments reported in the current study comply with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 86-23, revised 1996) and with current Swedish laws and regulations. They were performed according to a protocol approved by the regional ethical review board (Jordruksverket, dnr.5.2.19-5974/15).

Two feline species, Amur tigers (*Panthera tigris altaica*) and African lions (*Panthera leo*), held at Kolmården Wildlife Park were studied. The group of tigers consisted of two adult males and three adult females. The oldest individual was Timur, a sixteen-year-old male, also the possible father or uncle to the younger tigers. Second oldest was Tsar, a ten-year-old male. The females were the two nine-year-old sisters Olga and Kyra and their seven-year-old sister Kalinka. The animals were housed in an outdoor enclosure of 5,000 m^2 and a smaller indoor enclosure. The outdoor enclosure contained a small wooded area, mountainsides, a large open grass area and a man-made river dividing the area in two parts of roughly similar size. The tigers were fed portions of meat three times a week. The normal cleaning schedule for the enclosure was once a week, but this was temporarily increased to twice a week during the study.

The group of lions consisted of ten individuals. They comprised two adult males (a pair of seven-year-old brothers), four adult females (two eight-year-old and two three-year-olds), three juvenile males and one juvenile female (all four being siblings one and a half years of age). One of the three-year-old females was relocated to another zoo before the last two experimental days. The lions were kept in an outdoor enclosure of 20,000 m^2 and a smaller indoor enclosure. The outdoor enclosure contained a wooded area with several clearings as well as roofed shelters. The lions were fed half a carcass of horse or deer once every 5-7 days. At the end of the study, a new litter of cubs was born. However, they were kept separate from the pride due to their young age and did not take any part in the study. The animals of both species had no access to their indoor enclosures during the opening hours of the park.

3.2 Odour stimuli

The tiger and lion urine samples used in this study were collected at Borås zoo and then frozen for storage. The samples were gathered straight from the floor of the felines’ indoor enclosures, and then frozen in aliquots of 500 microliter. The samples were then thawed using a water bath to approximately room temperature immediately before they were used as an odour stimulus for the experiment. The conspecific urine samples were on purpose collected from individuals whom the experimental animals were unfamiliar with, in the hope that this would incite more interest and different behaviours than urine from familiar individuals, since urine is used to mark territory by both tigers and lions (Stoddart 1980; Wyatt 2014). 2-acetyl-1-pyrroline (CAS# 85213-22-5) is a characteristic volatile component identified in the marking fluid of tigers, while being absent in the marking fluid of lions (Poddar-Sarkar and Brachmachary 2014; Soso and Koziel 2016). The 2-acetyl-1-pyrroline used in the study was synthesised in a lab (aromaLAB, Freising, Germany) and diluted with the solvent diethyl phthalate to 1:100,000 concentration.

iso-pentyl acetate (CAS# 123-92-2) is an odour commonly found as a volatile component in different kinds of fruit, and described as a "Banana-like odour" in humans (Burdock 2005). The odorant was diluted to a 1:1,000 concentration with diethyl phthalate. *Diethyl phthalate* (CAS# 84-66-2) An organic solvent that was used as a control odour for its near-odourless properties. The solvent was also used to dilute both 2-acetyl-1-pyrroline and iso-pentyl acetate so that the odour would not be overwhelming yet still detectable for humans, if examined at a close range. The tiger and lion urine were presented undiluted.

3.3 Experimental procedure

The odours were presented to the animals using wooden (spruce) logs with the dimensions 48 x 4.5 x 4.5 cm. A hole with a diameter of 15 mm was drilled near the short end of each log for attaching a metal chain. For each trial, 500 µl of an odour was applied using a micropipette and impregnated upon each of five wooden logs, using ordinary paint brushes to distribute the odour evenly. Only a single odour was used for each individual trial. The logs were then placed inside the enclosure. Each day of data collection lasted for six hours of observation. Three hours of observations were performed in the morning and three hours of observations in the afternoon, usually between 8:00 am and 4:00 pm. An ethogram of log-directed behaviours was used during observation (Table 1). This ethogram was based on previous studies on carnivores using the same method of presenting odorized wooden logs, but using different odours for presentation (Nilsson et al., 2014; Norberg 2016). All the ten different behaviours (Table 1) that was performed by the animals that were directed towards the logs were recorded using continuous sampling, this was also the case for the recorded duration of the observed behaviours. Each of the four odours (conspecific urine, tiger marking fluid component, fruity odour, and solvent control) were presented five times to each feline species, giving a total number of 20 trials in total per species. All data collection was performed between May and September 2017.

The different odours were presented in a pseudorandom order with no odour being presented twice in a row to a given feline species. During the fifth and last trial with each odour, the amount of time spent performing behaviours directed towards the logs (table 1) was recorded as well. Due to the random placement and the ability to freely move the logs throughout the tiger enclosure I opted not to use cameras, though for future studies cameras may be used in static positions such as the chained logs in the lion enclosure. At the end of each experimental day, the logs were collected, removed from the enclosure and reused for subsequent trials, taking care to ensure that the same odour was presented on a given log as in previous trials. Due to the cleaning schedule, the logs used during the tiger experimental days were collected on the morning after a trial. The logs remained in use if they maintained good structural condition and cleanliness, or until they went missing due to an animal hiding them. To ensure that each log was used with the same odour and species, it was marked at the short end with a corresponding letter and colour, red for urine, blue for 2-acetyl-1-pyrroline, green for iso-pentyl acetate, and black for diethyl phthalate, as well as the letter T for tiger and L for lion. This colour coding of the logs was done to eliminate any chance that the wrong log was used for the wrong odour, and it is unlikely to have interfered with the experimentation, despite the possibility of colour vision in felines (Loop et al 1987).

For the tigers, the logs were placed freely within the enclosure allowing the animals to carry them around and move them at will. However, due to the size of the lion enclosure freely placed wooden logs would not have allowed for reliable observation and retrieval. Therefore, the logs used for the lions were chained to the observation area for keeping the logs within observation distance and to allow for easy retrieval. The tigers' logs were not chained in place for the additional reason that no good spot to fasten them to was present. Thus, the lions did not have the same freedom to move the logs around as the tigers.

Due to the soluble nature of the odours used all testing was performed during dry weather. In the rare case that rain started during an observation day, the trial was aborted. Similarly, testing was only performed on non-feeding days so that the animals did not lack or lose interest due to the presence of food. Additionally, care was taken that there was at least one day of intermission between consecutive testing of the same species.

Table 1. Ethogram of the behaviours considered in the present study

Behaviour	Description
Sniffing	The nose within a body length from the log.
Licking	Tongue in contact with wooden log
Biting	Teeth in contact with wooden log
Pawing	Using the paw or claws to scratch a wooden log.
Flehmen	Curling of the upper lip and ‘‘grimacing’’ without vocalization
Self-impregnation	Rubbing the face or other body part at a wooden log.
Scent-marking	Urinating or defecating or spraying onto a wooden log.
Orientating	Turning head (or ears or eyes) after an interaction (sniffing/licking/piting/pawing) with a wooden log.
Vocalizing	Producing sounds during or after an interaction (sniffing/licking/piting/pawing) with a wooden log.
Guarding	Sitting or lying within one head length to, or on top of, a wooden log.

3.4 Statistical analysis

To compare the behaviours displayed by the two species towards the odours the chi-square test was used. The Wilcoxon rank-sum test was used to compare the durations of the behaviours recorded during the last trials. Due to the differing group sizes of the tiger and lion groups, all comparisons between species were conducted using normalized data. These were calculated by dividing the total number of interactions for each odour by the number of individuals present in the corresponding group of feline species. The Spearman rank-order correlation test was used to assess the correlation between the number of behaviours displayed towards the logs across the five sessions for each odour. Minitab version 18.1 was used for all statistical analysis.

4 Results

4.1 Tigers

The tigers performed a total of 111 interactions with the odorized wooden logs (Table 2). Among the behaviours recorded, sniffing was clearly the most frequently displayed one, accounting for 53% of all observed behaviours, followed by orientating with 23%, biting 9%, flehmen 8%, licking 2%, pawing 2%, scent marking 1%, vocalising 1% and guarding 1%. Scent-marking, vocalising and guarding behaviours were only displayed by the tigers when exposed to the marking fluid component.

Table 2 The total number of behaviours displayed by the tigers.

Behaviour	Conspecific Urine	Marking fluid component[A]	Fruity Odour[B]	Solvent control[C]	**Total**
Sniffing	19	16	19	5	**59**
Licking	2	0	0	0	**2**
Biting	2	4	3	1	**10**
Pawing	1	1	0	0	**2**
Flehmen	7	2	0	0	**9**
Self-impregnation	0	0	0	0	**0**
Scent-marking	0	1	0	0	**1**
Orientating	3	17	6	0	**26**
Vocalising	0	1	0	0	**1**
Guarding	0	1	0	0	**1**
Morning	30	29	24	6	**89**
Afternoon	4	14	4	0	**22**
Total	**34**	**43**	**28**	**6**	**111**

[A]2-acetyl-1-pyrroline, [B] iso-pentyl acetate [C.] Diethyl phthalate

There was a significantly higher number of interactions directed towards the conspecific urine (χ^2 = 11.168, P = 0.001), tiger marking fluid component (χ^2 = 16.179, P < 0.001) and fruity odours (χ^2 = 16.179, P < 0.001) compared to the solvent control odour. However, there was no significant difference in the number of interactions displayed by the Amur tigers towards the odorised logs when comparing between the conspecific urine odour, the tiger marking fluid component and the fruity odour, respectively (conspecific urine *versus* tiger marking fluid component: χ^2 = 0.524, P = 0.469; conspecific urine versus fruity odour: χ^2 = 0.291, P = 0.590; tiger marking fluid component versus fruity odour: χ^2 = 1.591, P = 0.207).

The tigers performed the majority of their interactions with the logs during the morning (Table 2). The total number of behaviours occurring in the morning (89) was significantly higher than the total number of interactions recorded in the afternoons (22) (χ^2 = 23.655, P < 0.001). This was also true when looking at all four odour stimuli separately, as they all elicited more interactions with the wooden logs in the morning than in the afternoon (Conspecific urine, χ^2 = 11.643, P = 0.001, Tiger marking fluid component, χ^2 =2.326, P = 0.127, Fruity odour, χ^2 = 8.187, P = 0.004, Solvent control, χ^2 = 4, P = 0.046).

When considering the four odour stimuli combined, the tigers spent a mean of 2.2 ± 1.7 seconds for each interaction with the odour-impregnated logs. The time spent on logs with the tiger marking fluid component odour (2.6 ± 2.1) and those impregnated with the fruity odour (2.0 ± 1.5) was not significantly different (W = 36, P = 0.6). The tigers did not interact with the solvent odour logs during the final session, leaving this without any data regarding interaction time. The urine odour was interacted with only once for 1 second during the final session leaving it with too few data points for statistical analysis.

When considering all four odour stimuli combined, there was a non-significant trend for a decreasing number of interactions directed towards the wooden logs across all sessions considering all odour stimulus combined (r = -0.872, P = 0.054). However, no significant decrease was found when considering each odour stimulus separately (conspecific urine, r - 0,500, P = 0.39, tiger marking fluid component, r = -0,335, P = 0.581, fruity odour, r = -0.359, P = 0.553 and the solvent control, r = -0.335, P = 0.581). There was a significant difference between the number of interactions towards the urine impregnated logs between the first and last day of study (χ^2 = 5.042, P = 0.025) There were no significant difference between the number of interactions towards the logs between the first and last day for the remaining odours (tiger marking fluid component: χ^2 =1.544, P = 0.214; fruit: χ^2 = 1.504, P = 0.220; solvent control: P = 1).

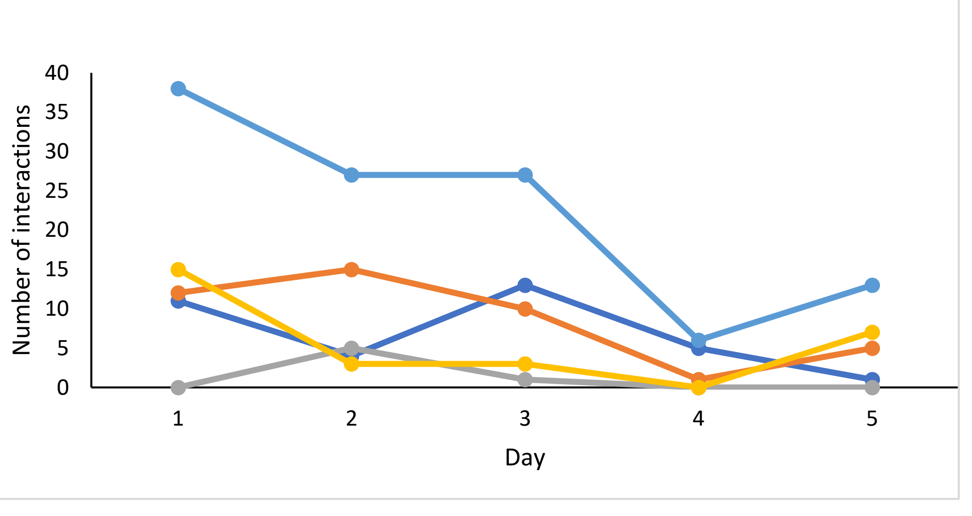

Figure:1 The number of interactions performed by the tigers towards the different odorized logs. Blue: conspecific urine, orange: tiger marking fluid component, grey: solvent control, yellow: fruity odour, red: the four stimuli combined.

4.2 Lions

The lions performed a total of 977 interactions towards the odour-impregnated wooden logs (Table 3). The three most frequently observed behaviours were pawing (35%), biting (29%) and sniffing (20%). Guarding, licking, orientating behaviours followed with 9%, 5% and 2%, respectively. Self-impregnation, flehmen, scent-marking and vocalising together made up less than 1% of recorded behaviours.

Table 3. The total number of behaviours performed by the lions.

Behaviour	Conspecific Urine	Marking fluid component[A]	Fruity odour[B]	Solvent control[C]	**Total**
Sniffing	84	40	63	9	**196**
Licking	33	0	10	1	**44**
Biting	191	6	77	7	**281**
Pawing	178	14	139	8	**339**
Flehmen	7	0	0	0	**7**
Self-impregnation	9	0	0	0	**9**
Scent-marking	2	0	0	0	**2**
Orientating	24	0	0	0	**24**
Vocalising	0	1	0	0	**1**
Guarding	51	0	23	0	**74**
Morning	30	29	24	6	**89**
Afternoon	4	14	4	0	**22**
Total	**579**	**61**	**312**	**25**	**977**

[A]2-acetyl-1-pyrroline, [B] iso-pentyl acetate [C.] Diethyl phthalate

The lions displayed a significantly higher number of behavioural responses towards the wooden logs impregnated with conspecific urine odour than for the tiger marking fluid component (χ^2 = 250.683, P < 0.001), the fruity odour (χ^2 = 40.902, P < 0.001) and the solvent control (χ^2 = 321.738, P < 0.001). The wooden logs impregnated with the fruity odour elicited a significantly higher number of interactions in comparison to the tiger marking fluid component (χ^2 = 95.509, P < 0.001) and the solvent control (χ^2 = 149.152, P < 0.001). Finally, the tiger marking fluid component elicited a significantly higher number of interactions from the lions in comparison with the solvent control (χ^2 = 7.88, P = 0.005).

There was a significantly higher total number of interactions with the logs performed during the mornings in comparison to the number of interactions towards the logs during the afternoons when looking at the combined number of interactions across all four odour stimuli (χ^2 = 91.595, P < 0.001). This was also found with the conspecific urine (χ^2 = 86.257, P < 0.001), marking fluid component (χ^2 = 8.361, P = 0.004), fruity odour (χ^2 = 15.780, P < 0.001) and solvent control impregnated (χ^2 = 4.864, P = 0.027).

In total the lions spent a mean of 24.78 ± 79.2 seconds interacting with the different odourised logs. The lions' interaction time with the logs impregnated with conspecific urine odour (30.5 ± 88.6) was significantly higher than that of the tiger marking fluid component (3.7± 2.6 seconds) odour (W = 4150, P = 0.0001). However, no significant difference was found when comparing the duration of interactions between the urine impregnated logs and the fruity odour (2.5 ± 0.7 seconds) impregnated logs (W = 3135.5, P = 0.0861) Additionally the solvent control odour did not elicit any interactions with the wooden logs during the final session and has thus no interaction time recorded.

There was no significant decrease in the number of interactions towards the odour impregnated wooden logs across the five sessions. This was true both when looking at the four odour stimuli combined (r = -0.700, P = 0.188), and when looking at the four odour stimuli separately, (conspecific urine: r = -0.500, P = 0.391, tiger marking fluid component: r = -0.100, P = 0.873, fruity odour: r = -0.872, P = 0.054 and solvent control: r = 0,112, P = 0.858. However, there was a significant difference in the number of observed behaviours when comparing the first and last day for the urine (χ^2 = 45.097, P < 0.001) and fruit (χ^2 = 95.766, P < 0.001). The number of behaviours directed odours tiger marking fluid component (χ^2 = 1.339 P = 0.247) did not significantly change between first and last day, and the number of behaviours directed towards the solvent control remained unchanged between the first and last day.

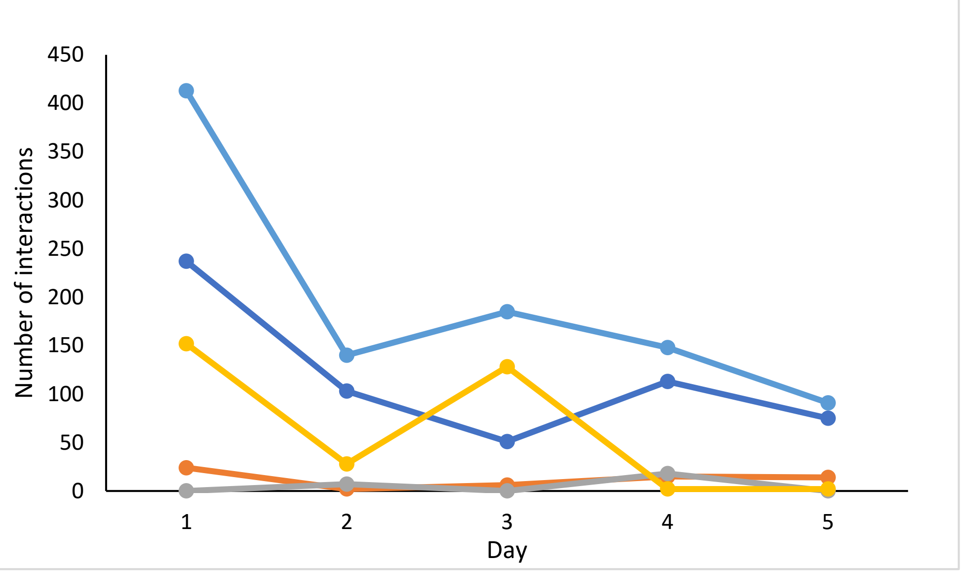

Figure 2. The number of interactions performed by the lions towards the different odourised logs. Blue: conspecific urine, orange: tiger marking fluid component, grey: solvent control, yellow: fruity odour, red: the four stimuli combined.

4.3 Comparison between species

When considering all four odour stimuli combined, the lions performed a significantly higher number of behaviours per individual directed towards the odour-impregnated wooden logs than the tigers (χ^2 = 26.749, P < 0.001. This was also true when comparing the conspecific urine (χ^2 = 23.530, P < 0.001 and the fruity odour separately (χ^2 = 9.437, P = 0.002). In contrast, there was no significant difference between the two species when comparing the response rate per animal between the tiger marking fluid component (χ^2 = 0.293, P = 0.588) and to the solvent control (χ^2 = 0.533, P = 0.465), respectively.

Species	Conspecific urine	Marking fluid component	Fruity odour	Solvent control	Total
Tiger[A]	6.8 ± 2.2	8.6 ± 2.5	5.6 ± 2.6	1.2 ± 1	22.2 ± 5.7
Lion[A]	57.9 ± 22.8	6.1 ± 2.7	31.2 ± 22.8	2.5 ± 2.5	97.6 ± 40

Tiger^{B}	1	2.6 ± 2.1	2.0 ± 1.5	0	2.2 ±1.7
Lion^{B}	30.5 ± 88.6	3.7± 2.6	2.5 ± 0.7	0	24.78 ± 79.2

Table. 4 Interaction with the different odourised wooden logs.

A = Total number of interactions per individual ± SE. B = *Mean duration in seconds and SD of interaction per species.*

Considering all four odour stimuli combined, the lions performed significantly longer interactions compared to the tigers ($W = 5927$, $P < 0.001$). This was, however, not the case when comparing between the lions and tigers for the tiger marking fluid component ($W = 48.50$, $P = 0.337$) and the logs impregnated with the fruity odour ($W = 32$, $P = 0.464$). Due to the Amur tigers only interacting once during the last session with conspecific urine odour, no statistical analysis could be carried out. No behaviours were recorded towards the solvent control from either of the two species during the final session.

5 Discussion

5.1 Differences

Amur tigers interacted more often with the tiger marking fluid component 2-acetyl-1-pyrroline than with urine from unfamiliar conspecifics. In contrast, the lions displayed significantly more interest toward urine from unfamiliar conspecifics than towards any of the other odours, including the tiger marking fluid component. Sniffing was the most commonly displayed behaviour in the tigers when presented with the odourised wooden logs, while the lions displayed pawing and biting on the wooden logs more often than sniffing.

An important difference in the behaviour of the tigers and lions, was found in their response towards the tiger marking fluid component: whereas the tigers devoted 39% of their interactions towards the tiger marking fluid component 2-acetyl-1-pyrroline, the lions only devoted 6% of their total interactions towards the same odour. This supports the idea that 2-acetyl-1-pyrroline may contain species-specific information and is therefore likely to play a role in the chemical communication of tigers, but not lions.

Sniffing was the most frequently displayed behaviour by the Amur tigers when investigating the odour-impregnated logs, suggesting that the sense of smell is important for them while investigating novel objects. Sniffing behaviour has been previously reported to be frequently displayed towards novel objects in several carnivore species, (South American bush dogs *(Speothos* venaticus): Sjöberg 2013; Nilsson 2015, African wild dogs (*Lycaon pictus*) and dholes (*Cuon alpinus*),: Nilsson et al 2014, Eurasian wolves (*Canis lupus lupus*): Norberg 2016, meerkats (*Suricata suricatta*): Pettersson 2017).These previous studies was carried out using the same method and all of the tested species; and all species displayed sniffing as the most frequently displayed behaviour (appendix 1).

The odour that sparked the most interest and thus elicited the highest number of interactions in the tigers was the tiger marking fluid component. However, they also displayed a similar degree of interest in the conspecific urine and the fruity odour. This suggests that perhaps any unfamiliar odour might initiate explorative behaviours. However, the tigers displayed flehmen behaviour only when interacting with the logs impregnated with conspecific urine and tiger marking fluid component, but not with the fruity odour and solvent control. This suggests that both odours may be involved in tiger social communication as flehmen behaviour facilitates the transport of an odour to the vomeronasal organ in order to decipher the chemical information found in these odours (Salazar and Sánchez-Quinteiro 2011). On average, the tigers investigated the logs only briefly and then lost interest in investigating further. This suggests that tigers either generally have a low interest in novel objects and odours or that they needed only these brief encounters to decipher the novel odours.

The lions mostly used their sense of touch to investigate the novel objects, with pawing followed by biting, being the most frequently observed behaviours when interacting with the odourised logs. Sniffing was the third most frequently performed behaviour, in a study by Skibel et al 2007 lions showed more activity when exposed to enrichment with bones and fish than when enriched with spices. This suggests that physical interaction, such as clawing or biting, is an important part of investigating novel odours and objects, in addition to the sense of smell. Since the lions' logs were fastened with chains, making it impossible for the lions to move them over a longer distance, this can be tone reason for the high number of pawing and biting behaviours. Flehmen behaviour was notably only observed during sessions using conspecific urine as an odour. This was expected since the tiger marking fluid component is not present within lion marking fluid (Poddar-Sarkar and Brahmachary 2014) and would thus not be of any informational use for the lions. This further indicates that 2-acetyl-1-pyrroline is part of the tigers' species-specific olfactory communication.

However, the number of interactions elicited by the tiger marking fluid component was by far outnumbered by those elicited from both the conspecific urine and fruity odour. This suggests that the odour of the tiger marking fluid component is not important for conveying social information between lions. This is interesting since while tiger marking fluid component is absent from lion marking fluid, it is present in their head- and cheek rubbing markings (Soini et al. 2012). The presence of the marking fluid component in lion cheek rubbings could be an evolutionary remnant as suggested by the lower numbers of interactions elicited by it.

Both the mean duration and number of interactions with the odorized logs displayed by the lions was significantly higher with the conspecific urine than with any of the other odours. This suggests that urine markings are an important way of intraspecific communication between lions, as a means to establish territorial boundaries and differentiate between individual conspecifics. Gilfillian et al (2017) determined that lions can differentiate between individuals through urine deposits. The high interest in urine odour during the present study further suggests that conspecific urine from unknown individuals would be useful as a form of olfactory enrichment for lions, and that 2-acetyl-1-pyrroline is unlikely to play a part in the olfactory communication in lions.

The lions displayed significantly more interactions per animal towards the odour-impregnated logs than the tigers (Table 8). Olfactory enrichment has been shown to increase the behavioural repertoire and to facilitate more social behaviours in lions (Baker 1997; Powell 1995). Lions have previously been reported to increase their activity and number of behaviours to a higher degree than tigers in response to olfactory enrichment (Van Metter et al. 2008). These previous results are in agreement with the difference in the number of interactions towards the odours by the lions and tigers found in the current study. The pride of lions studied here contained younger individuals than the tiger group, with four subadults present, three of these subadults being males in the typical dispersal age. The two adult males accounted for only a small part of the total behaviours observed, with most interactions elicited from the subadult individuals (both male and female) as well as from the adult females of the pride. Gilfillan et al (2017) suggest that subadult lions, specifically subadult males might be more interested in non-resident olfactory cues as these might lead to future mating opportunities after dispersal. Female lions are likely to respond to urine from unknown females, this is a defence mechanism to protect their territory from other prides. Adult male lions are more receptive to male urine as other lion males are a threat both towards the resident male's reproductive right and offspring (Gilfillan et al. 2017). Accordingly, many of the behaviours were performed by the adult females of the pride.

The youngest individual within the lion group was one and a half years old while the youngest tiger was eight years old. Additionally, the same tiger group has been used twice before in studies using the same method, but with different odour stimuli (Present study: tiger (1), Nilsson 2015: tiger (2), Sjöberg 2013: tiger (3)). When comparing the number of interactions performed by the tiger group during previous studies using the same method there was no significant difference in the number of interactions observed, (tiger (1) vs tiger (2): $\chi^2 = 2.332$, P = 0.127) however when comparing towards the first study performed four years prior there was a clear significant difference in number of behaviours tiger (1) vs tiger (3): $\chi^2 = 11.374$, P = 0.001 (Appendix)). When comparing the number of interactions for all odour stimuli combined obtained with the tigers in the present study with the data obtained with other carnivore species in earlier studies using the same method (Appendix 2) (Sjöber 2013; Nilsson et al 2014; Norberg 2016; Pettersson 2017), the following was found: Meerkats, Eurasian wolves, South American bush dogs, and African wild dogs all displayed a significantly higher number of interactions per animal than the Amur tigers (Tigers vs meerkats $\chi^2 = 78.963$ P = 0.0001: Tigers vs wolves χ^2 = 27.347 P = 0.0001; Tigers vs bush dogs χ^2 = 43.91 P = 0.0001; Tigers vs African wild dogs χ^2 = 7.049 P = 0.008). No significant difference was found between the tigers of the present study and results obtained previously with the Dholes (Tigers vs Dholes χ^2= 2.172 P= 0.14).

In contrast, the lions of the present study displayed a significantly higher number of interactions per animal compared to three of the previously studied species: Amur tigers, African wild dogs, and dholes (Lions vs Tigers χ^2 = 13.912, P = 0.0001; Lions vs African wild dogs χ^2 = 6.505, P = 0.011; lions vs dholes χ^2=13.912, P=0.0001). There was no significant difference in the number of interactions per animal between the lions studied here and all but one of the other species being compared. The only previously studied species that had a significantly higher number of interactions with the odourised wooden logs per animal compared to the lions were the meerkats (Lions vs meerkats χ^2 = 15.079, P = 0.0001). This demonstrates that the lions displayed a relatively high level of interest in the odorized wooden logs in comparison to other carnivores. The African wild dog, dhole and meerkat groups all contained both young and adult individuals'. Another explanation for the differing number of interactions per animal observed in each species might be attributed to differences in interest for novel objects or odours, which would suggest that lions are highly interested in novel objects and odours while tigers might have a low interest.

This suggests that the comparatively low interest displayed by the tigers towards the odours in the present study was probably not due to the nature of the odour stimuli used here, but rather is either a general feature of the tigers that comes with age or a specific response to the used method. Enclosure size does not explain this difference in interactions as it should be expected that a smaller enclosure size increases the probability of an animal finding an odourised wooden log. However, in this case the lions had an enclosure (20 000 m^2) that is four times the size of the tigers' enclosure (5,000 m^2).

The lions and tigers differed in their exposure to zoo-visitors which could have influenced the results by splitting the animals focus. The lion enclosure was part of a safari tour, with cable cars traveling above the enclosures. Thus, all visitors remained out of sight above the lions. The tiger enclosure was observable from a walkway, where the tigers could directly see the visitors. It is possible that tigers might be more inclined to hide due to this exposure. If that was the case, then the tigers would spend most of the time during opening hours within the forested area at the far side of the enclosure hiding among the bushes and rocks. However, a previous study on use of space by the Kolmården tigers showed that this was not the case (Nilsson 2012). Rather, the animals spent a relatively large proportion of time close to the visitor area. However it is still possible that this could split their focus from the logs. Another explanation for the differing number of interactions per animal observed in each species might be attributed to differences in interest for novel objects or odours, which would suggest that lions are highly interested in novel objects and odours while tigers might have a low interest.

5.2 Single odour components versus complex odour mixtures

During the current study, the tigers did not significantly differ in their interest towards the odour of urine from unfamiliar conspecifics and the odour of the tiger marking fluid component 2-acetyl-1-pyrroline. This lends support to the notion that a single volatile component can be as efficient in eliciting behavioural responses as a full odour mixture. In previous studies comparing behavioural responses of tigers, wolves, meerkats, dholes, South American bush dogs and African wild dogs, towards the odour of real blood and a single blood odour component (trans-4,5-epoxy-(E)-2decenal) there were also instances of equal interest in a complex odour mixture and a single component of the same mixture from tigers, meerkats, south American bush dogs, African wild dogs and wolves. (Sjöberg 2013; Nilsson et al 2014; Norberg 2016; Pettersson 2017). However, single components are not always effective at promoting the same response as the full chemical mixtures. 2,3,5-Trimethyl-3-thiazoline (TMT) is a component of fox faeces odour (McGregor et al 2002). It is often used to assess behavioural responses of prey species such as mice and rats to the scent of a predator. However, TMT has been shown to not be as effective at eliciting avoidance behaviour as the more complex odour mixture of genuine fox faeces (McGregor et al 2002; Blanchard et al 2003). Sievert and Laska (2016) reported that predator avoidance behaviour in mice was only observed when exposed to two out of six tested predator odour components. Additionally, frugivorous nonhuman primates have been found to be more attracted to the odour of real fruit than towards single characteristic compounds that are part of a complex mixture of volatiles that make up the odour of a fruit (Laska et al. 2006; Løtvedt et al., 2012; Nevo et al. 2015).

5.3 Environmental Enrichment

Based on the wide range of behaviours performed during sessions with conspecific urine odour, I conclude that conspecific urine from unknown individuals is a useful form of olfactory environmental enrichment for use with lions and tigers. The tiger marking fluid component, 2-acetyl-1-pyrroline, in contrast, is only a suitable olfactory enrichment with tigers. As urine from healthy animals is usually sterile, there should be no concerns about its use with regard to hygiene. However, it is crucial that conspecific urine to be used must be collected from healthy individuals, to avoid spreading of infections.

Additionally, since the fruity-smelling compound iso-pentyl acetate elicited a high number of interactions towards the wooden logs in the lions, this odorant should also be regarded as a useful olfactory enrichment for use with this species. Further, the use of wooden logs to present olfactory enrichment to large carnivores has been confirmed to be suitable (Nilsson et al 2014).

The effect of freezing and thawing urine on the behavioural responses of animals has been reported with conflicting results in the past, with studies either finding only a weak decreasing effect (Bagley et al 2006; Lenochova et al 2009; Smadja et al 2004; Kwak et al 2009), or finding a strong effect on the behavioural response observed in animals (Hoffman et al 2009).

6 Conclusion

The present study shows that the odour of the tiger marking fluid component 2-acetyl-1-pyrroline elicited the same degree of interest in tigers as the odour of conspecific urine. Additionally, the results show that a single compound can be as effective in eliciting behavioural responses as a complex chemical mixture containing several odorants. In contrast, lions interacted significantly more often with conspecific urine than with 2-acetyl-1-pyrroline. These results support the notion that 2-acetyl-1-pyrroline is a species-specific olfactory communication signal for tigers. Furthermore, conspecific odours presented on wooden logs have been assessed to be suitable to be used as environmental enrichment for captive tigers and lions.

7 Ethical and societal considerations

A better understanding of intra- and interspecific chemical communication can be applied to increase welfare in captive populations of felids. Diverse new forms of enrichment are needed to prevent habituation towards existing methods of enrichment. Further, increased knowledge about the intraspecific olfactory communication in endangered felid species can help with the conservation of wild populations. As territorial behaviour and communication between different prides of felids is mediated by olfactory cues, the results of the present study may contribute to a more effective management of free-ranging felid populations. Further this knowledge can help in developing potential chemical attractants and repellents for use with wildlife.

Zoo animals are kept in captivity for conservational purposes. It is a challenge to study wild individuals as they tend to avoid humans and inhabit large swathes of land making it hard to maintain a controlled environment. Through study of captive individuals at wildlife parks it is possible to transfer what is learned to the conservational effort of wild individuals.

8. Acknowledgments

I would like to thank my supervisor Matthias Laska on all the support throughout the thesis, and the staff at Kolmården wildlife park for all the help.

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Appendix

Appendix 1. Number of behaviours per animal for all stimuli combined.

Species	Sniffing	Pawing	Biting	Licking	Orientating	Guarding	Self-impregnation	Scent-marking	Flehmen	Vocalising
Lions (1)	19.6	33.9	28.1	4.4	2.4	7.4	0.9	0.2	0.7	0.1
Amur tigers (1)	11.8	0.4	2	0.4	5.2	0.2	0	0.2	1.8	0.2
Amur tigers (2)	8.8	0	0.3	0.2	0	0	0.5	0	0	0
Amur tigers (3)	32.5	7.2	9.5	6	2	0	0.2	0.2	0.5	0
Wolves (6)	63.2	1.3	2.8	4.2	1.5	0	0	0	0	0
Wolves (5)	62	12.3	23.9	3.4	3	0	0	0.7	0	0
Meerkats (6)	131.3	55.6	0.5	0	1.1	0.3	0.1	0.3	0	0.1
Dhole (4)	20.8	0.5	9.9	1.6	0.2	0	0.3	0	0	0
African wild dogs (4)	34.7	0.2	6.7	8.9	0	0	1.2	0	0	0
Bushdogs (2)	72.9	0.2	15.3	0.4	0.08	0	1	0.5	0	0.08
Bushdogs (3)	130.5	4.3	10.5	1	7.1	0	0	1.9	1	0

(1 current study, *2 Nilsson 2015, 3 Sjöberg 2013, 4 Nilsson 2014, 5 Norberg 2016, 6 Petterson 2017*

Appendix 2. *The number of interactions per animal directed towards the odorized logs in the present and in previous studies using the same method* ± SE*.*

Species	Odour component	Blood/Urine odour	Fruity odour	Solvent	Total
Lion (1)	6.1 ± 2.2	57.9 ± 22.8	31.2 ± 22.8	2.5 ± 2.5	97.6 ± 40
Amur tiger (1)	8.6 ± 2.2	6.8 ± 2.5	5.6 ± 2.6	1.2 ± 1	22.2 ± 5.7
Amur tigers (2)	2.5 ± 0.5	3.3 ± 1.2	2.3 ± 1.4	2 ± 0.7	10.1 ± 1.4
Amur tigers (3)	27.2 ± 11.8	27.7 ± 8.4	5.7 ± 2.2	4.8 ± 2.9	65.3 ± 31.4
Bush dogs (2)	18.7 ± 5.4	38.7 ± 15.5	15.9 ± 8.1	28.6 ± 14.8	102 ± 35.3
Bush dogs (3)	52.1 ± 39.8	54 ± 51.1	29.7 ± 28.6	22.4 ± 22.7	158.2 ± 50.24
African wild dogs (4)	23.6 ± 14.8	11.8 ± 4.2	10.6 ± 6.5	7.9 ± 5.7	53.9 ± 24
Dholes (4)	14.2 ± 4.3	13.4 ± 6.9	6.3 ± 2.4	4.3 ± 2.1	38.2 ± 17.2
Wolves (5)	45 ± 9.8	48.1 ± 24.1	17.6 ± 10.8	14.4 ± 11.3	125.1 ± 26.6
Wolves (6)	13.8 ± 5.8	34 ± 8.9	14 ± 5.6	11.7 ± 5.1	73.5 ± 25.7
Meerkats (6)	55 ± 35.5	57.1 ± 33.3	34.5 ± 18.6	43.5 ± 22.8	190 ± 40.8

(1 current study, *2 Nilsson 2015, 3 Sjöberg 2013, 4 Nilsson 2014, 5 Norberg 2016, 6 Petterson 2017)*