OBSERVATIONAL STUDY OF BREEDING BEHAVIOUR IN MALAYAN TIGERS AT THE NATIONAL WILDLIFE RESCUE CENTRE, SUNGKAI, PERAK.

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CERTIFICATION

This is to certify that we have read this research paper entitled 'Observational Study of Breeding Behaviour In Malayan Tigers At The National Wildlife Rescue Centre, Sungkai, Perak' by Laveda Kelly Logington, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.

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Staff members of NWRC

My family

My friends

Thank You

DEDICATIONS

I dedicate this work and give special thanks to Dr. Choong Siew Shean, for giving me the opportunity to work on this project, for being an inspiration and a role model, for providing guidance to me in time of need and for helping me develop my skills as a veterinary student in the wildlife industry.

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ABSTRACT

The Malayan tiger *(Panthera tigris jacksoni)* is a subspecies of the tiger that originates from Peninsular Malaysia and is now on the verge of extinction. Wildlife rescue centers and zoos play important roles in ex-situ conservation through breeding programs to preserve and increase the population of Malayan tigers. Tigers in captivity are facing challenges in reproduction due to many external factors that affect their reproductive behavior patterns. In addition, the Malayan tiger's behavior is understudied and currently holds high importance due to the dwindling number of these tigers in Malaysia. The purpose of this study is to use non-invasive methods to observe the reproductive behavior of female Malayan tigers to aid in the determination of their estrous cycle. This study was conducted at the National Wildlife Rescue Center (NWRC) in Sungkai, Perak. Two female Malayan tigers were selected, Cindai and Zanah, as they are young and potential candidates for the breeding program. They were observed to assess the relationship between the oestrous cycle and the mating behaviours of the tigresses. This research was conducted for 156 hours in 28 days through visual observation and the outcomes were recorded in focal sampling ethograms and frequency table charts. From the study, Cindai expressed all mating behaviours actively and showed signs of receptivity to mating with the male. Whereas Zanah was not exposed to a male tiger and did not express all mating behaviours. However, the reproductive behaviours expressed by Zanah during the observation period are sufficient to indicate estrus. Therefore, these preliminary results indicated that behaviour expression may be a valuable non-invasive tool to predict estrus in Malayan tigress and animals can be bred naturally through assisted reproductive technology. However, more research needs to be carried out, including longer periods of behaviour study, hormonal profile and reproductive health examination through ultrasonography and others. Combining information from various aspects would give a comprehensive assessment of the

tigress' reproductive health, enabling better planning for the Malayan tiger breeding program at NWRC.

Keywords: Malayan tiger, Malaysia, behaviour study, captive breeding
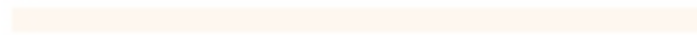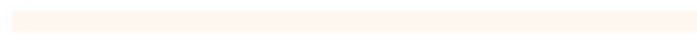
ABSTRAK

Harimau Malaya (*Panthera tigris jacksoni*) merupakan subspesis harimau yang berasal dari Semenanjung Malaysia dan kini berada di ambang kepupusan. Pusat menyelamat hidupan liar dan zoo memainkan peranan penting dalam pemuliharaan luar tapak melalui program pembiakan untuk memelihara dan meningkatkan populasi harimau Malaya. Harimau dalam kurungan menghadapi cabaran dalam pembiakan disebabkan oleh banyak faktor luaran yang mempengaruhi corak tingkah laku pembiakan mereka. Selain itu, tingkah laku harimau Malaya kurang dikaji dan pada masa ini mempunyai kepentingan yang tinggi kerana bilangan harimau ini semakin berkurang di Malaysia. Tujuan kajian ini adalah untuk menggunakan kaedah tidak invasif dalam membuat pemerhatian terhadap tingkah laku pembiakan harimau Malaya betina dan membuat tafsiran kepada penentuan kitaran estrusnya. Kajian ini merupakan kajian pemerhatian untuk memperhatikan tingkah laku mengawan yang diungkapkan oleh dua ekor harimau Malaya betina, untuk mengesan kitaran estrus dan menilai hubungan antara kitaran estrus dan tingkah laku harimau betina. Kajian ini dijalankan di Pusat Menyelamat Hidupan Liar Negara di Sungkai, Perak. Dua ekor harimau Malaya bernama Cindai dan Zanah dipilih kerana masih muda dan berpotensi untuk program pembiakan. Mereka diperhatikan untuk menilai hubungan antara kitaran estrus dan tingkah laku harimau betina. Penyelidikan ini dijalankan selama 156 jam dalam 28 hari melalui pemerhatian visual dan hasilnya direkodkan dalam etogram persampelan fokus dan carta jadual kekerapan. Daripada kajian, Cindai menyatakan semua tingkah laku mengawan secara aktif dan menunjukan tanda-tanda penerimaan untuk mengawan dengan jantan. Manakala Zanah menunjukkan sikap penerimaan yang sangat rendah terhadap harimau jantan dan tidak menyatakan semua tingkah laku mengawan. Oleh itu, keputusan awal ini menunjukkan bahawa Cindai lebih sesuai untuk dikawinkan secara semula jadi, manakala Zanah boleh dibiakkan melalui bantuan

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teknologi pembiakan. Walau bagaimanapun, lebih banyak kajian perlu dijalankan, termasuk tempoh kajian tingkah laku yang lebih lama, profil hormon dan pemeriksaan kesihatan reproduktif melalui ultrasonografi dan lain - lain. Menggabungkan maklumat dari pelbagai aspek akan memberikan penilaian yang lebih baik untuk program pembiakan harimau Malaya di NWRC.

Kata Kunci: Harimau Malaya, Malaysia, kajian pemerhatian, pembiakan tawanan

1.0 Introduction

Tigers are one of the largest members of the Felidae family and a member of the genus Panthera. Felidae is a family of carnivorous mammals which are divided into two subfamilies, the Felinae and the Pantherinae. Felinae comprises small cats that have a bony hyoid, thus they are able to purr but not roar whereas Pantherinae comprise of big cats that possess ossified hyoid bone with elastic tendons that enables their larynx to be mobile and roar. There are over nine subspecies of tigers, this includes Bali tiger *(Panthera tigris balica)*, Caspian tiger *(Panthera tigris virgata)*, Javan tiger *(Panthera tigris sondaica)*, Bengal tiger *(Panthera tigris tigris)*, Indochinese tiger *(Panthera tigris corbetti)*, Malayan tiger *(Panthera tigris jacksoni)*, Siberian tiger *(Panthera tigris altaica)*, South Chine tiger *(Panthera tigris amoyensis)* and the Sumatran tiger *(Panthera tigris sumatrae)* (Dalinsky, 2022).

The Bali, Caspian and Javan tigers have gone extinct while the six other subspecies are remaining. The Malayan Tiger (*Panthera tigris jacksoni*) is native to Peninsular Malaysia. (SeaWorld, 2022). The Malayan tiger population inhabits the southern and central parts of the Malay Peninsula. With their signature red-orange fur and black stripes, tigers have become icons of beauty, power and the importance of conservation. Malayan tigers have been showcased as an animal of national significance in Malaysia's national coat-of-arms. (Kawanishi et al., 2010). Among the six subspecies of tigers, the Sumatran tiger and the Malayan tigers have been classified to be critically endangered on the International Union for Conservation of Nature Red List of Threatened Species. In the 1950s, it was estimated that over 3000 Malayan tigers roamed the forests. Anthropogenic disturbances from human-tiger conflict have been the contributing factor to loss of tiger habitat and tiger prey such as the Sambar deer *(Cervus unicolor).* These disturbances include

deforestation, rapid development, agriculture expansion and widespread hunting mainly using poacher's snares causing a huge drop in the numbers (WWF, 2019).

Currently, an estimation of less than 150 tigers remains in Malaysia (WWF, 2022). Due to this drastic decline in numbers, multiple studies have been conducted to obtain more information and gain insight on the Malayan tigers in order to take as many measures as possible to conserve the species. In the field of conservation, Malaysia has begun to systematically implement the national conservative initiative, the Malaysia National Tiger Conservation Action Plan (NTCAP) in 2009 with the objective of doubling the Malayan tiger population (Abdul & Samsudin, 20222).

In 2018, the Malaysian Government launched the "Save the Malayan Tiger" campaign that is inclusive of five programmes: conservation, enforcement, research, funding, corporate social responsibility, public awareness and promotion campaigns (Ten et al., 2021). The goal of this campaign is to establish the National Tiger Conservation Centre and venture into Malayan tiger captive breeding under conservation and research programmes. There are three basic methods of studying animal behaviour which include observational, experimental and comparative methods. This study is conducted on the reproductive behaviour of Malayan tigresses in Malaysia specifically to observe their oestrous cycle and breeding behaviour. The location of this study is done at the National Wildlife Rescue Centre, Sungkai, Perak.

1.1 Problem statement

According to Tyagi *et al.* (2019), it is proven that tigers, when stressed, release glucocorticoids which allows them to enhance their fitness by energy mobilization and restore homeostasis. However, prolonged release of glucocorticoids jeopardizes their fitness by impacting the quality of physiological processes in relation to reproduction, growth, immunosuppression and muscular atrophy *(Tyagi et al., 2019)*. Therefore, observational studies are done in order to study their behaviour without causing further stress in order to understand them in depth which can help enhance successful mating performance that will increase chances of repopulating the species. The challenges faced by Malayan tigers currently lies within their natural habitat which is now fragmented and made smaller by humans. This has also caused a decline in their main source of food, which is the Sambar deer. These declining numbers have resulted in the tigers being driven out of their natural habitat in search of prey for their survival. Thus, leading to more human – tiger conflict. Tigers are also on high demand for their body parts by poachers and for traditional Chinese medicinal purposes (Hays, 2014).

The National Wildlife Rescue Centre of Sungkai is now taking up measures to rescue, rehabilitate and carry out breeding programmes to breed more Malayan tigers to overcome the crisis of extinction. However, the tigers in captivity also face challenges in breeding mainly due to the limited size of reserve area, lack of access to its natural habitat, limited number of male tigers to be bred, the absence of exposure to other tigers and lack of experienced staff members. In the absence of the tiger's parent or limited exposure to other tigers, these big cats kept in captivity are not able to acquire learned behaviours. Thus, this is the recent challenges faced in rescue centres in Malaysia. Currently, several studies using non-invasive methods have already been conducted in Malaysia in several animals but as for the Malayan tigers that are showing a rapid decline in

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numbers, more research is required to help aid the species to successful breeding in order to overcome extinction and ensure continued existence of these tigers.

1.2 Research questions

1. What are the breeding behaviours displayed by the Malayan tigresses?

2. Do behaviours relate to different phases of the oestrous cycle in Malayan tigresses?

3. Do Malayan tigress exhibit behaviours that can be used to predict estrus?

1.3 Research hypothesis

1. There are various breeding behaviours exhibited by Malayan tigress.

2. The breeding behaviour of Malayan tigresses differs at each oestrous phase.

3. Breeding behaviour can be used to predict oestrus in Malayan tigress.

1.4 Research objectives

1. To observe breeding behaviours displayed by Malayan tigresses.

2.To assess the relationship between the oestrous cycle and behaviour of Malayan tigresses.

3. To predict oestrus based on behaviour of Malayan tigresses.

2.0 Literature review

2.1 Animal behaviour

An animal behaviour according to Upen (2019), is an evoked response of an animal towards a stimulus Animal behaviours can be divided into two categories which are learned or innate. A learned behaviour is acquired from experience or the environment and is expressed by animals based on learning from surrounding or parent, also done by training. Whereas innate behaviour is inherent and comes with natural instincts of an animal when exposed to stimulus. Innate behaviours are genetically hard-wired and are of high importance as they contribute to the survival and proper functioning of an animal. In addition, innate behaviours are not easily altered like learned behaviours (Upen, 2019). There are vast behaviours that vary in every species of animal depending on the exposure to stimuli.

2.2 Ethology

Ethology is the scientific study of animal behaviour in natural conditions (Bioknowledge, 2022). It encompasses the examination of instinctive and adaptive nature of animals including the observation of natural processes such as communication, courtship, feeding, mating and self-defense. In this study, the behaviour of the Malayan tigresses is studied **during breeding** along with the detection of their oestrous cycle.

2.3 Tiger reproduction

Tigers reproduce sexually by copulation after reaching sexual maturity. The lifespan of Malayan tigers ranges from 18 - 25 years (AZ Animals, 2021) in which female tigers reach this maturity at the age of three to four years and males become sexually mature at age four to five years (Seaworld, 2022). Although tigers are solitary animals, they come into interaction with other tigers during mating season and to share their kill. Tigers are known to be polygynous meaning a male will mate with several females (Russian Academy of Sciences, 2023). The breeding season of Malayan tigers falls between November and March but they are known to mate year-round indicating that these big cats are poly-oestrous animals (AZ Animals, 2021). During the time of breeding, tigers display a variety of behaviours in stages that indicates their receptivity and readiness for courtship.

2.4 Oestrous cycle in felines

The oestrous cycles of felines starts with proestrus, oestrus, interoestrus, dioestrus and anoestrus (Brenda Griffin, 2001). Proestrus is the phase when the follicles of the ovary experience growth under the influence of oestrogen (Britannica, 2023). At this phase females are not yet sexually receptive. Oestrus is the phase when females are sexually receptive or "in heat" (Weir, 2023). This is when the females will exhibit sexually receptive behaviours that signals the males and results in copulation. A female tiger is receptive for three to six days and comes into oestrous at intervals in three to nine weeks every twenty-five days (Seaworld, 2022). Females are also induced ovulators whereby the act of mating causes the neurotransmitters that are released into the bloodstream to trigger the release gonadotropin releasing hormones (GnRH) and luteal hormones

(LH) that results in ovulation by the fertilization of eggs in the tigress (Bhattacharjee et al., 2015). Despite this, there have been reported cases of tigers being spontaneous ovulators in which the females ovulate naturally in response to hormonal and environmental factors such as photoperiod. In terms of photoperiod, melatonin plays the main role in which an increased period of exposure to light increases melatonin and therefore promotes cyclicity (ViviD & Bentley, 2018).

As tigers are poly-oestrous and do not consistently ovulate after every oestrus phase, an interoestrus phase follows oestrus (Seaworld, 2022). In this phase, intervals of sexual inactivity will take place. If ovulation occurs during the phase of oestrous, progression into dioestrus phase will take place in which females no longer remain sexually receptive. According to Brenda Griffin, 2001 the corpus luteum of the female will start to form and there will be secretion of progesterone hormone for maintenance of pregnancy. However, in absence of pregnancy the dioestrus phase will terminate with the regression of the corpus luteum and will proceed into the phase of anoestrus. This is the rest phase of cyclicity before the females can go into the next phase of proestrus with absence of sexual receptivity (Brenda Griffin, 2001).

2.5 Breeding behaviours in tigers

Sexually mature tigresses are usually receptive for three to six days for mating (Seaworld, 2022). During the different phases of their oestrous cycle, female tigers display various types of breeding behaviours.

I. **Scent-markings**: A few days prior to the entry to oestrous phase, female tigers will become restless and start scent-marking their enclosure frequently via urination in order for males to

detect them by sniffing the markings followed by grimacing or showing Flehmen response with wrinkled nose and protrusion of tongue (David Smith et al., 1989).

II. **Vocalisation**: During oestrous, tigresses will be sexually receptive and display behaviours such as vocalization by producing long low intensity sounds by expelling air through parted lips and nostrils termed as prustening or chuffing. This acts as a communicative behaviour that signals the male tigers that the female is ready for mating (Carnegiemnh, 2020).

III. **Lordosis**: Female tigers will lastly exhibit the behaviour of lordosis which is a body posture for sexual receptivity to copulation by lowering the forelimbs while hindlimbs are extended with raised hips, ventral arching of the spine and raising or sideward displacement of the tail. Once permitted for mounting, male tigers usually bite the nape of the female's neck to immobilize them and mount from behind. During copulation, both female and male tigers vocalize by moans, groans, grunts, growls or hisses (Guarraci & Frohardt, 2019).

IV. **Aggression**: Females may also display aggression towards males as the act of copulation can be painful from the bites given from male tigers and due to the anatomical structure of the male's penis that has keratinized penile spines or spikes. The dismounting of the male will induce pain and in response, the females may growl, jump, or scratch the males (Edwards, 2022).

V. **Other behaviours**: Other behaviours exhibited include circling, pacing, cheek rubbing, head butting, flank rubbing, rolling and tail lifting. All of which are signs shown by the tigresses to announce it's readiness to be mated with (San Diego Zoo Wildlife Alliance, 2022).

3.0 Materials and methods

The wildlife research was conducted at the National Wildlife Rescue Centre in Sungkai, Perak. The research obtained approval from the Department of Wildlife and National Parks (DWNP), Malaysia. The approval code obtained was ref: JJPHTN.600-6/1/4 JLD2 (85). This research did not require the approval of institutional animal care and use committee (IACUC) as the study is an observational study that does not involve invasive procedures nor sample taking from the subject of study which is the Malayan tigers.

3.1 Study area

The study area of this observational study was located at the National Wildlife Rescue Centre (NWRC) of Sungkai, Perak, which was officially opened in 2018 established within the Sungkai Wildlife Reserve. This wildlife reserve was founded in 1928 with a land area over 2,000 hectares (DWNP, 2021). The rescue centre was established for the purpose of rescuing wildlife from poachers, illegal smugglers, wildlife caught in snares or traps and involved in road accidents. NWRC also offers protection, rehabilitation, and the releasing of some of the animals back into their natural habitat. The tiger unit is divided into five blocks that consists of a total of 20 night stalls with 10 exercise yards. In the year 2020, NWRC housed 16 tigers and currently houses 19 tigers. The tigers are mostly kept in the night stalls and released into the exercise yard at 9:00 am till 3:00 pm. The night stalls are designed with double tiered tables for the tigers to climb and lay on logs for enrichment purposes. The logs are placed as pillars and laid horizontally as well along with one or two enrichment items. Each night stall also consists of a drinking sink and a narrow drain for drainage during cleaning. The daily routines at NWRC involved cleaning of the night stalls every morning before 9:00 am. The exercise yard is a natural enclosure that is fenced with

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electrical lines and tall fences to prevent the tigers from gaining access that can endanger the safety of the workers.

3.2 Study design

This study was designed to record all reproductive behaviours of Malayan tigress between 9:00 am to 5:00 pm during the NWRC operating hours. This study was conducted for a period of 28 days in order to be able to observe one cycle as a female tiger will come into oestrous at intervals in three to nine weeks every twenty-five days. Observation was done in intervals with alternate timing to allow recording of each tigress's behaviour during the morning and late noon. A broad category of behaviours with relation to the tigresses' oestrous cycle was observed, such as circling, pawing, scent marking, vocalization, rubbing, rolling, tail lifting, lordosis and aggression. The outcome of the observation was tabulated in an ethogram and a tally chart table pending further analysis.

3.3 Study population

The study population of this study were two young captive Malayan tigresses that have not been bred,resided within NWRC. Tiger A, Cindai, was one of the first-born Malayan tigers in NWRC and was 8 years of age as of the year 2022. She weighs 90kg and spent her life growing up within the facilities of NWRC. Cindai was paired with a male tiger named Yeop. Tiger B, Zanah, was transferred from the Langkawi Wildlife Park on 27th of July in 2018 and she weighs over 100kg. She was estimated to be over 10 years of age. She was not paired together with a male

in one night stall; however Zanah was placed in a night stall next to a male tiger named Lobo. Table 1 below shows the data of the study group and the daily routines carried out at the night stalls every day in the morning and afternoon.

Table 1: Study population data

Tiger	Age	Weight (kg)	Origin	Unit of night stall	Pairing with male	Daily routines conducted in night stall
A: Cindai	8 years	90kg	NWRC	Unit C	Paired	● Daily cage cleaning ● Daily feeding ● Release into exercise yard
B: Zanah	>10 years	>100kg	Langkawi Wildlife Park	Unit E	Not paired	● Daily cage cleaning ● Daily feeding ● Release into exercise yard

From the table above (Table 1), the daily routines such as night stall cleaning were carried out by at least two staff members by first transferring the tigers to the night stall beside for safety purposes. Feces were disposed of along with leftover feed. The floor, walls and drinking sink of the night stall were washed and scrubbed until clean from urine or dirt and water was refilled into the drinking sink. The tigers are fed every six days in a week at 3:00pm with fresh meat of either

whole chicken or beef. They are also fed with offals for nutritional purposes. Feed given was 10%
of each tiger's bodyweight.

3.4 Sampling Technique

Behaviour sampling technique was used to monitor the oestrous cycle and breeding behaviour
in this study. . In this technique, a focal sampling method was conducted using an ethogram and a
frequency table to record the activities or behavioural state of the animals for a specific period.
From the behaviours displayed by the animal, the information was interpreted to predict the
different phases of the oestrous cycle, particularly oestrus.

3.5 Data collection tools

- Camera
- Stationery and clipboard
- Timing device (watch/timer)
- Ethogram worksheet
- Focal sampling worksheet
- Frequency table chart sheet

3.6 Sampling Procedure

Information regarding the animal such as species, common name, date of study and the time of day when observation commenced was first recorded in all the data collection sheets. The list of behaviour patterns exhibited by the tigresses were then visually observed and recorded on the focal sampling worksheet and ethogram from 9:00 am till 5:00 pm with an interval of three hours from 12:00pm - 3:00pm. In addition to that, the number of times each behaviour was expressed was also recorded on the frequency table using a tally numeration system. Other modes of data collection were obtained through photographs and video recordings of the animal of interest. Based on the behaviours observed, correlation was made with the phases of the oestrous cycle by data analysis on the flow of pattern of behaviors displayed at various time that were converted into charts.

4.0 Results

Based on the ethogram chart, statistical analysis was not considered for this project due to the small number of data collected. Interpretation of raw data from the ethogram will be conducted.

Results for Cindai were obtained from frequency tables using a tally numeration system and ethogram worksheet. The findings were tabulated in the form of bar charts and line graphs as attached below.
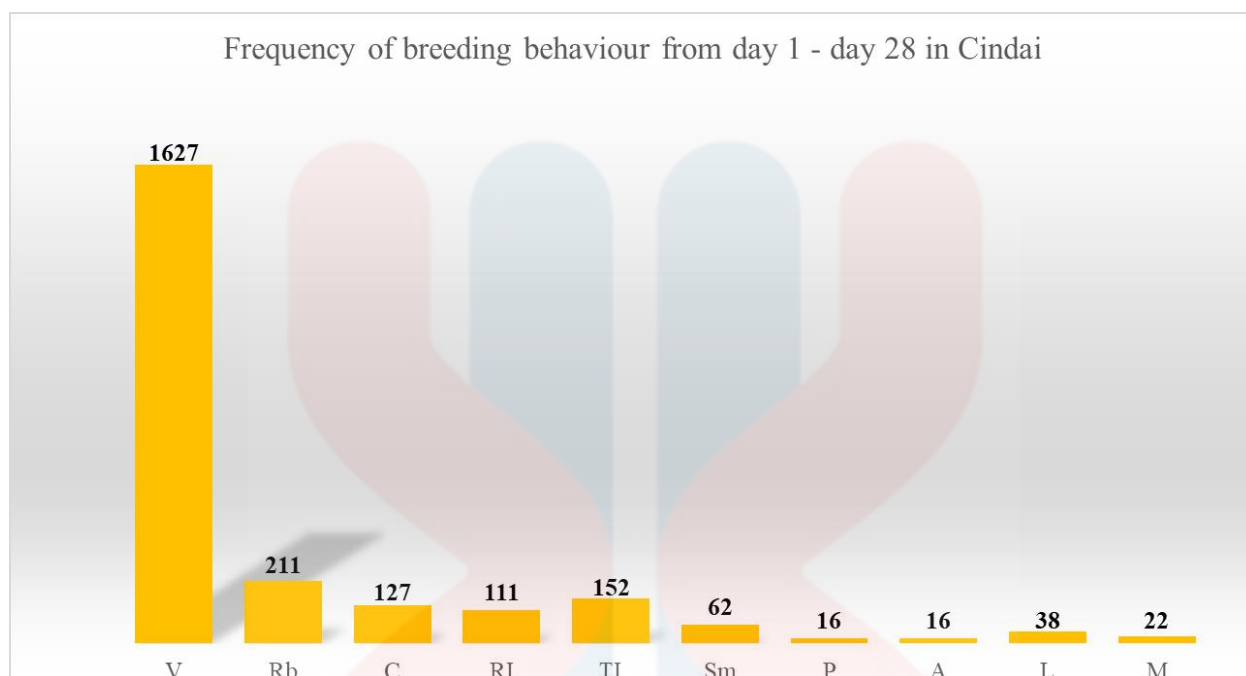
Figure 1: Frequency of breeding behaviour in Cindai in 28 days

(V-vocalization, Rb - rubbing, C - circling, Rl - rolling, Tl - tail lifting, Sm - scent marking, P - pawing, A - aggression, L - lordosis, M - mounted)

The bar chart above was tabulated based on the behaviours that were observed along with the number of mountings against the frequency of the behaviours expressed throughout 28 days. Cindai expressed vocalization behaviour the most up to 1627 times in forms of prustening at the male, growling and roaring (Figure 1). The second most expressed behaviour was rubbing onto walls of the enclosure, mostly cheek and flank rubbing onto the male when paired together for 211 times. Rolling was mostly seen after being mounted by the male in which aggression was first displayed in forms of roaring and pawing at the male immediately after dismounting of male from Cindai. Tail lifting was often seen expressed concurrently with scent marking whereby Cindai would lift her tail toward a log pillar or wall located in the night stall or a tree located in the exercise yard before letting out a spray onto it. Circling was seen almost every day which was not significant to Cindai's oestrous cycle. Pawing and aggression were also not accurate as they were influenced

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by other factors. Lordosis was displayed by Cindai 38 times during her phase of oestrous prior to mounting up to 22 times as a sign of receptivity to the male. Mounting then followed after lordosis as to when the male positioned himself onto Cindai to copulate.

Line graphs were formed to show the frequency of each behaviour that was expressed by Cindai in 28 days in a total of 78 hours (Figure 2-8).

Vocalisation behaviour from Day 1 - day 28 in Cindai
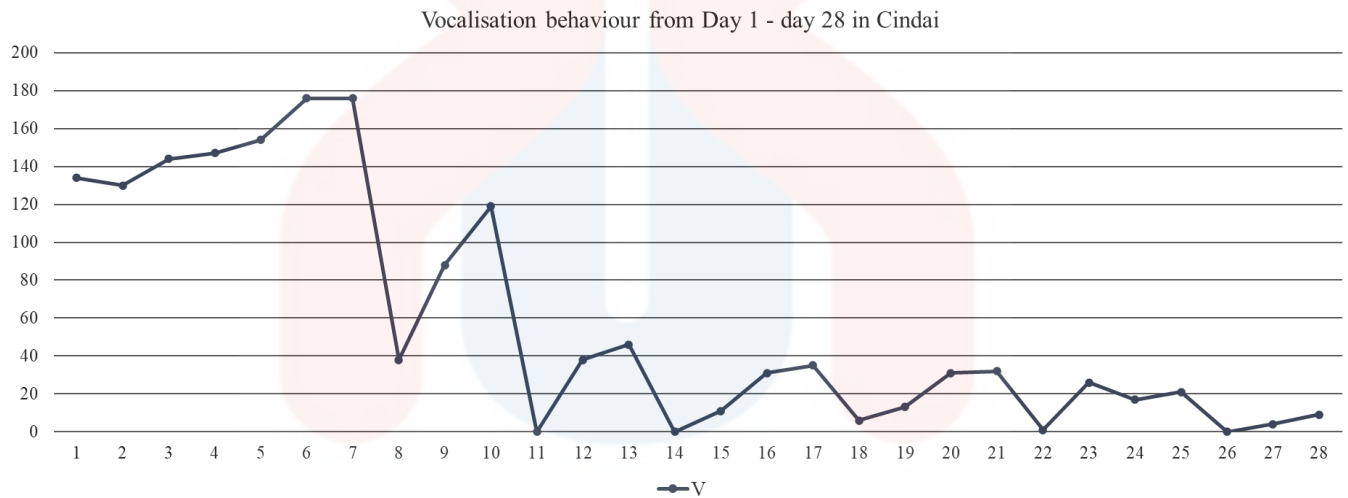
Figure 2: Line graph of vocalization behaviour in Cindai (V - vocalization)

Rubbing behaviour from Day 1 - day 28 in Cindai
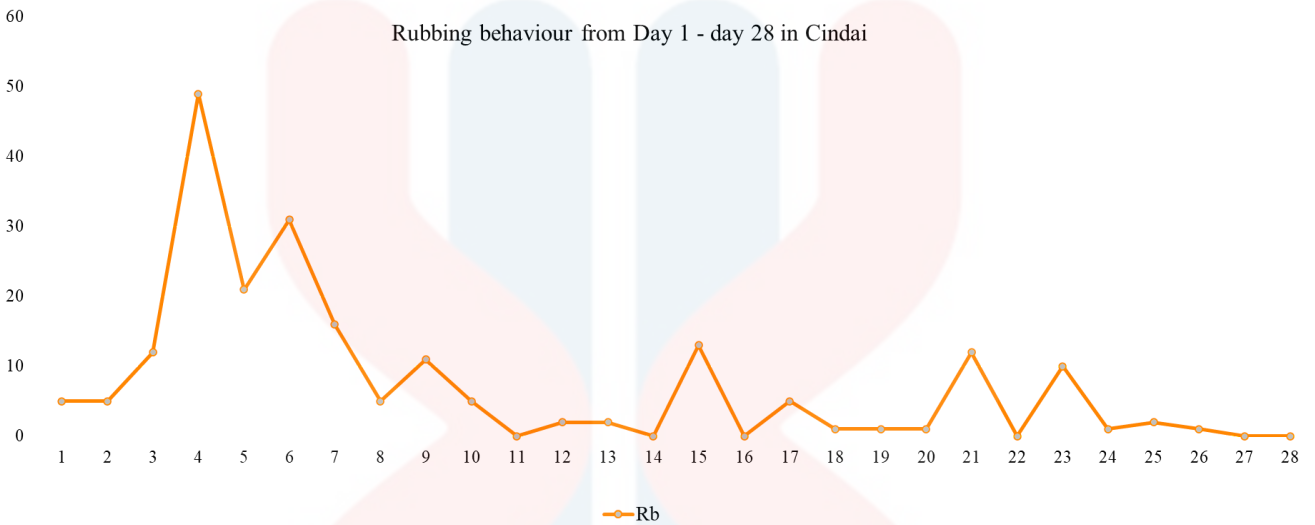
Figure 3: Line graph of rubbing behaviour in Cindai (Rb - rubbing)

Rolling behaviour from Day 1 - day 28 in Cindai
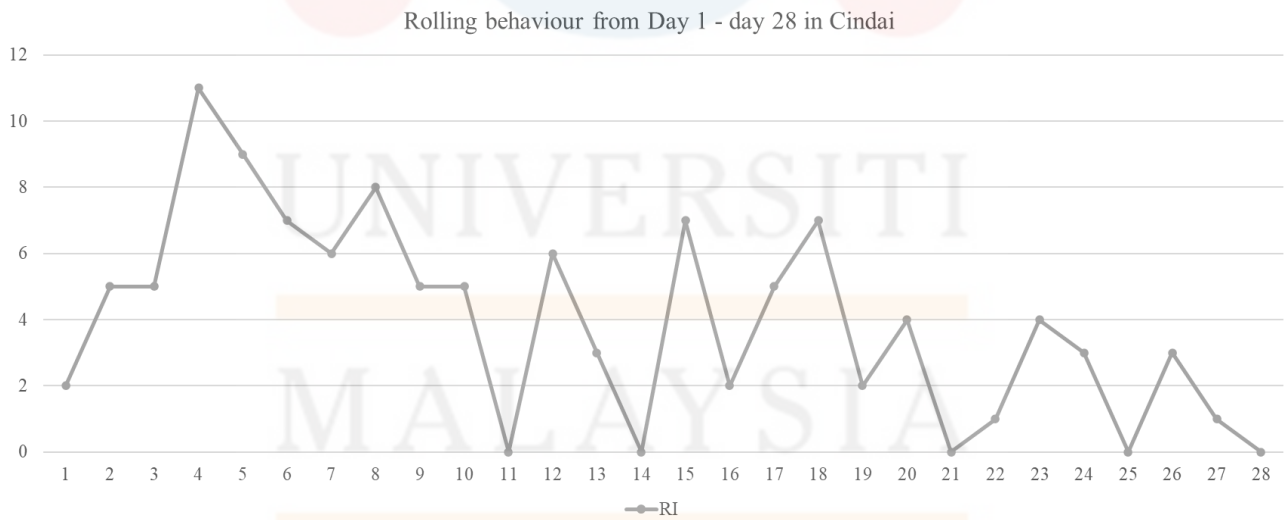
Figure 4: Line graph of rolling behaviour in Cindai (Rl - rolling)

Tail lifting behaviour from Day 1 - day 28 in Cindai
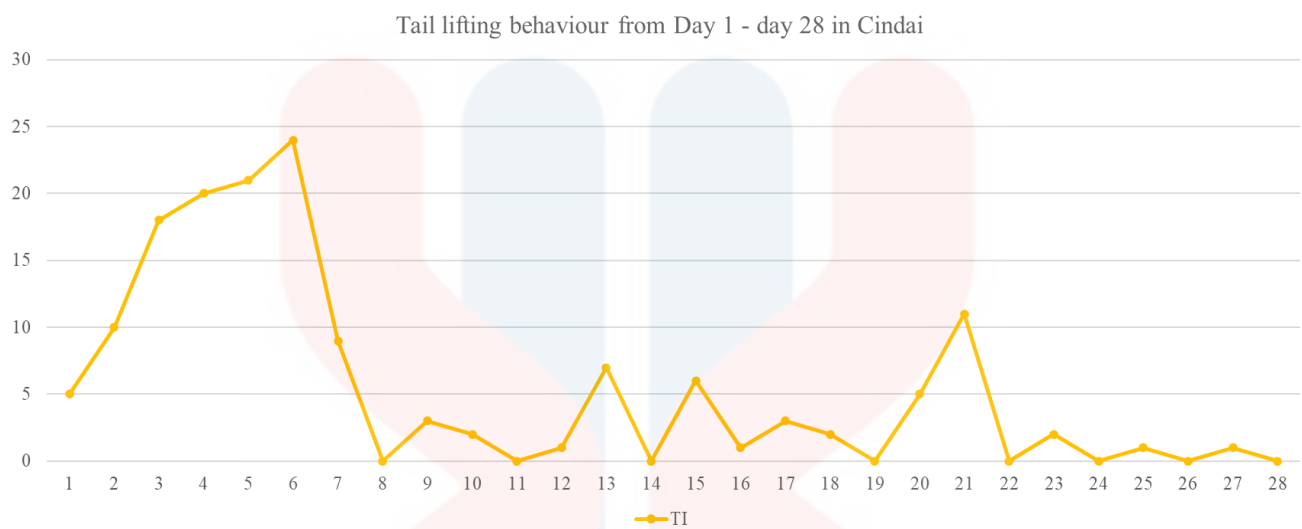
Figure 5: Line graph of tail lifting behaviour in Cindai (Tl - tail lifting)

Scent marking behaviour from Day 1 - day 28 in Cindai
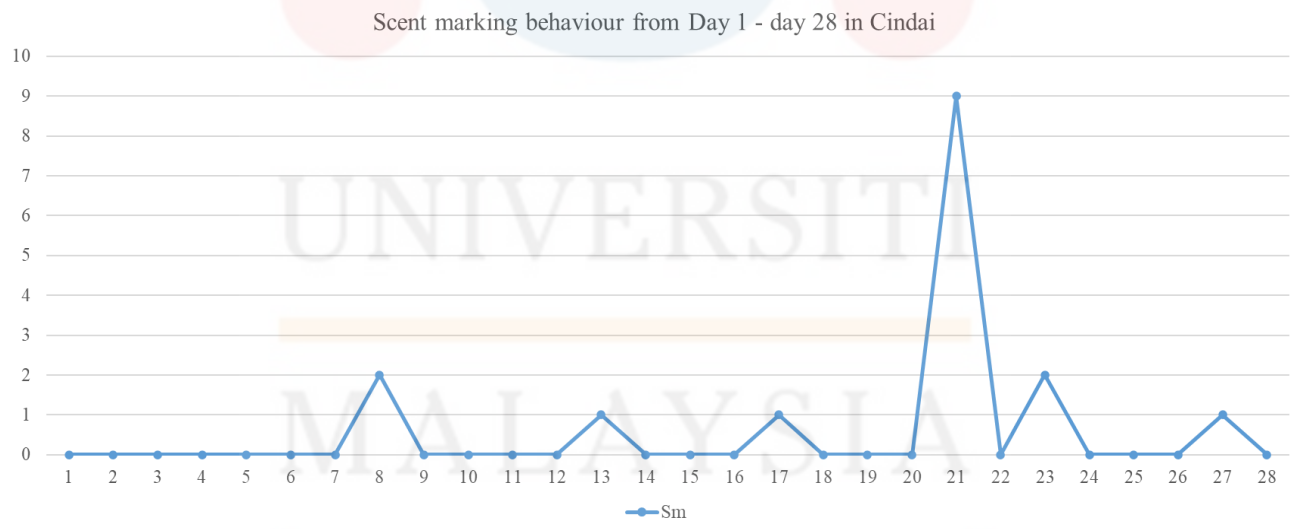
Figure 6: Line graph of scent marking behaviour in Cindai (Sm - scent marking)

Lordosis behaviour from Day 1 - day 28 in Cindai
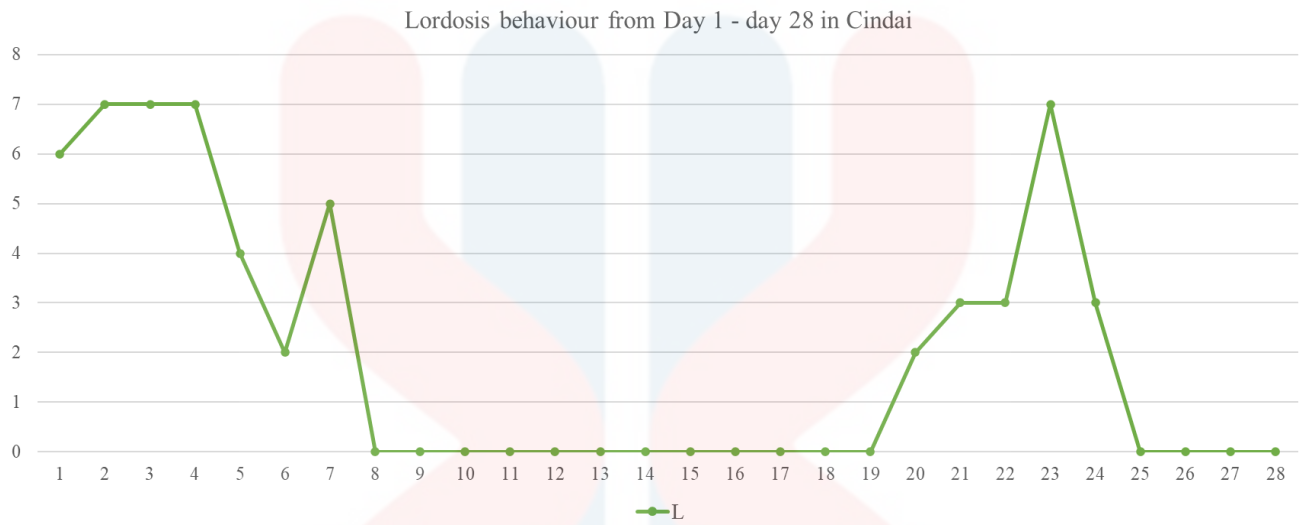
Figure 7: Line graph of lordosis behaviour in Cindai (L - lordosis)

Mounted from Day 1 - day 28 in Cindai

Figure 8: Line graph of mountings in Cindai (M - mountings)

From the line graphs above, Cindai showed an increase in vocalization, rubbing, rolling and tail lifting behaviours (Figure 2 - 5) on day 3 - day 6 except for scent marking, lordosis and being mounted (Figure 6 - 8). This shows Cindai was approaching the end of her oestrous. Scent markings are more often seen when females are in heat or marking their territory which answers to this behaviour being expressed in lower frequency. On day 7 - day 8, a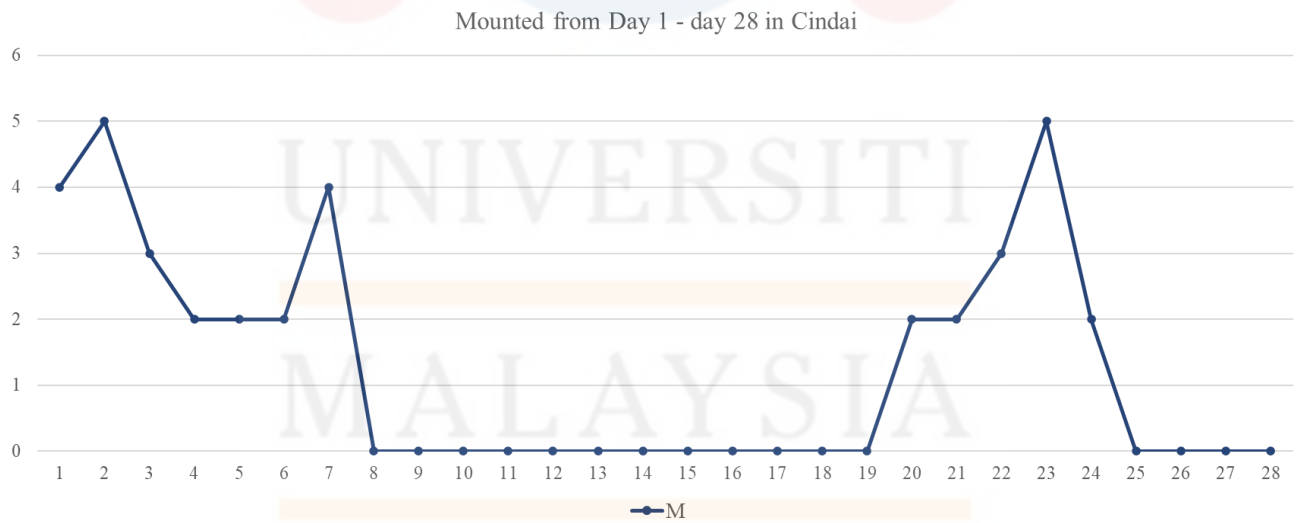ll of the behaviours showed a decline in number which indicated the end of the oestrous phase (Figure 2 - 8) and Cindai undergoing ovulation. On day 8 - day 14, Cindai was in the phase of anestrus as there were zero signs of lordosis and no mounting took place (Figure 7 and figure 8). Cindai started showing a slight increase in expressing breeding behaviours again from day 15 up till day 20 as she was entering a new cycle of proestrus. On the 19th day Cindai continuously displayed lordosis and was mounted multiple times up till day 24 which indicated her second phase of oestrous. Lastly, on day 25 - day 28, there were absence or lordosis and mounting that took place indicating the end of oestrous phase after 5 days of being in the phase of oestrous. Cindai's absence of receptiveness to mate indicated her entry into the anestrus phase. The remaining behaviours displayed showed a decline as well.

Results for Zanah were also obtained from frequency tables using a tally numeration system and ethogram worksheet. The findings were tabulated in the form of bar charts and line graphs as attached below.
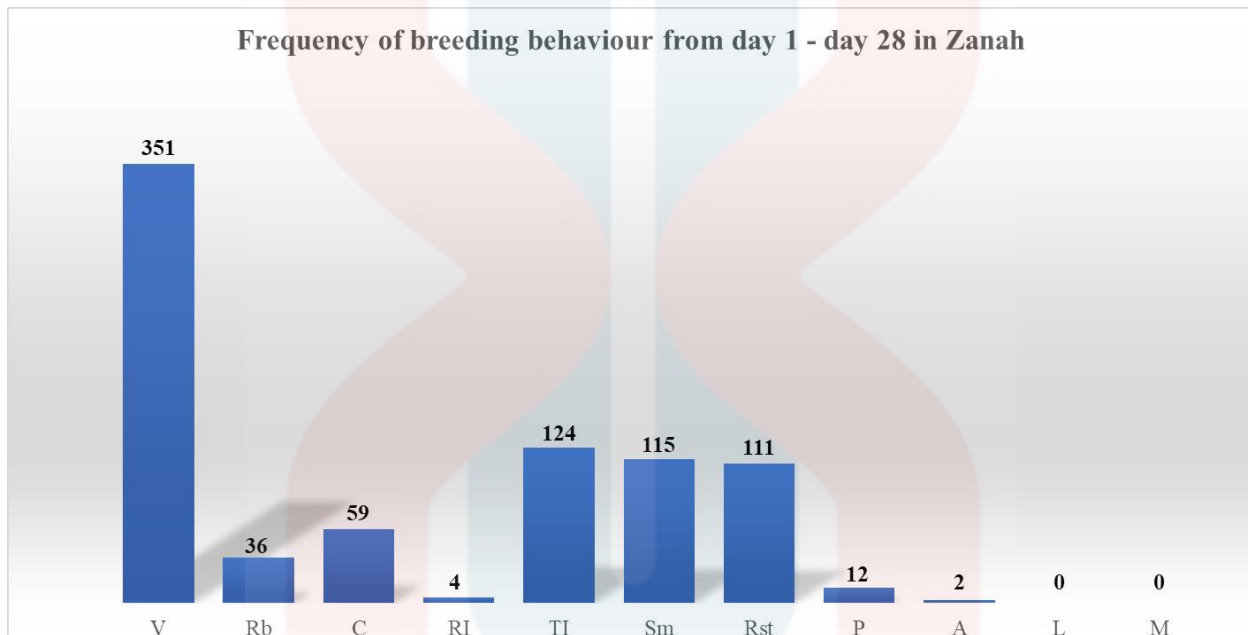
Figure 9: Frequency of breeding behaviour in Cindai

(V-vocalization, Rb - rubbing, C - circling, Rl - rolling, Tl - tail lifting, Sm - scent marking, P - pawing, A - aggression, L - lordosis, M - mounted)

The bar chart above was tabulated based on the behaviours that were observed against the frequency of the behaviors expressed throughout 28 days. Zanah expressed vocalization the most in forms of prustening at the staff members and low moans when in close proximity to humans in a total amount of 351 times. The second most expressed behaviour was tail lifting at 124 times and scent marking at 115 times which was mainly influenced by Zanah's oestrous cycle during the phase of oestrous and proestrus. Resting was observed almost every day as Zanah had lack of enrichment within the night stall and was not paired with the male. Circling behaviour was

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displayed the most during the phase of oestrous and proestrus as females become restless and anxious during this period of time. Aggression was the least expressed behaviour as Zanah was not in close proximity to the male tiger and was considered to have a more docile temperament compared to Cindai as she was easily approached by the staff members and will not react fiercely or aggressively unless provoked.

Figure 10: Line graph of vocalization behaviour in Zanah (V - vocalization)

Figure 11: Line graph of rubbing behaviour in Zanah (Rb - rubbing)
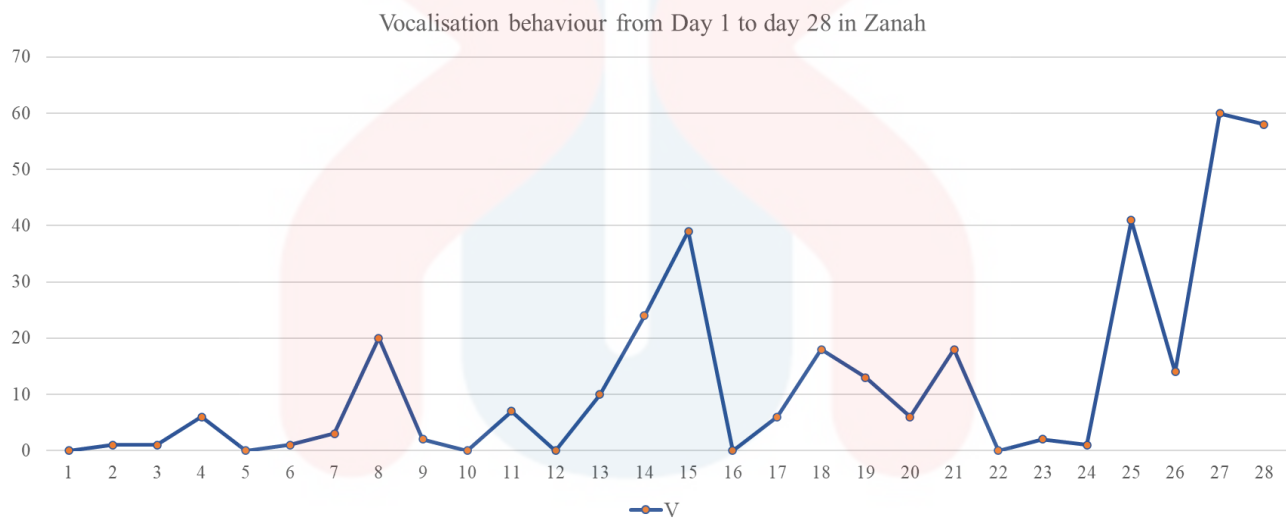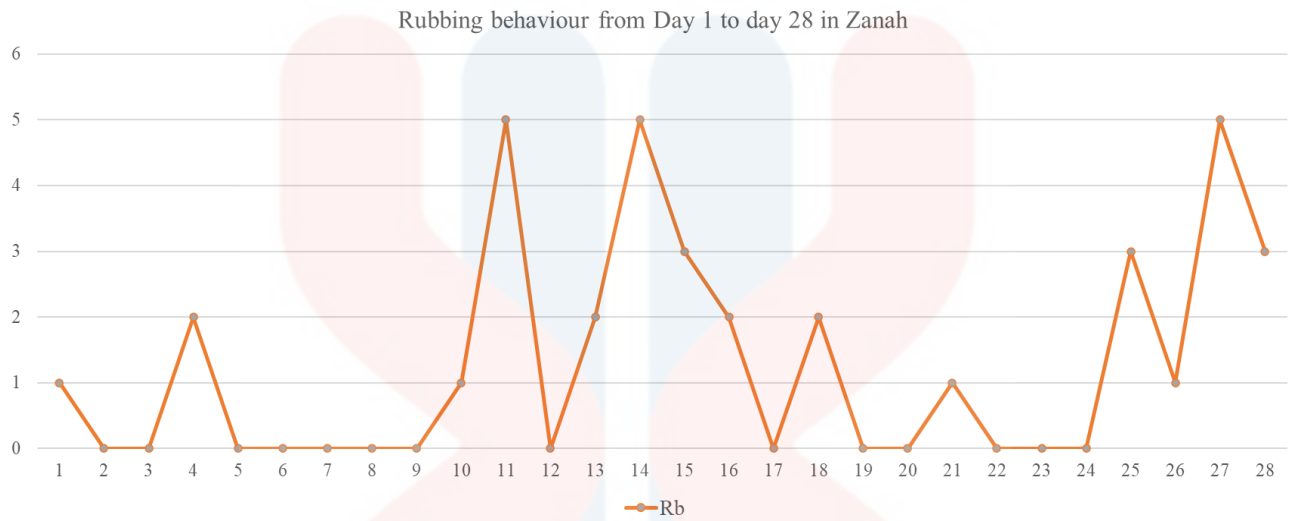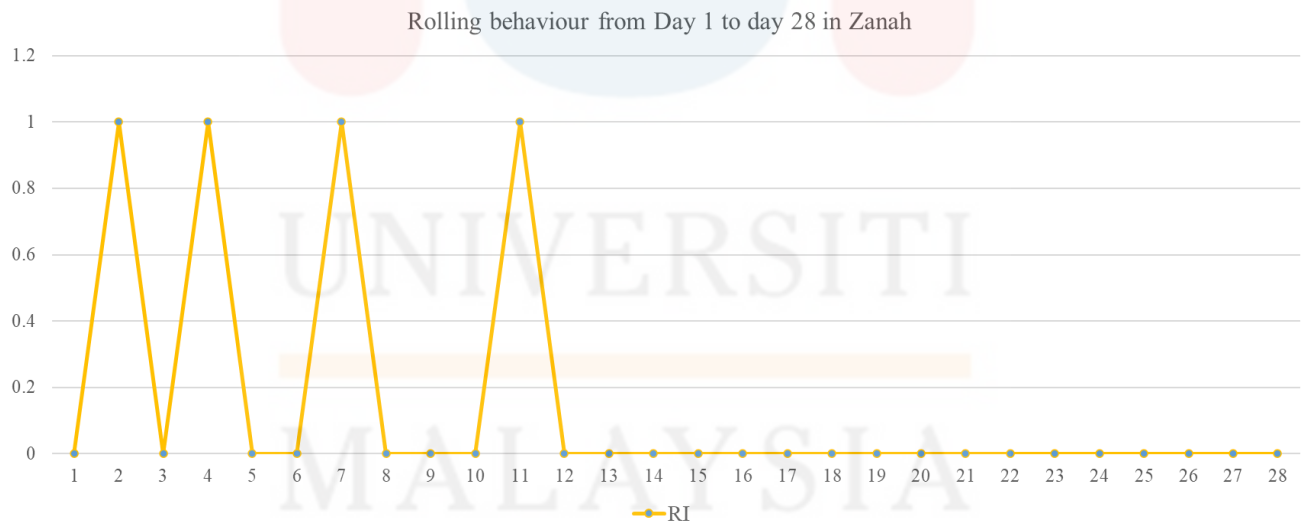

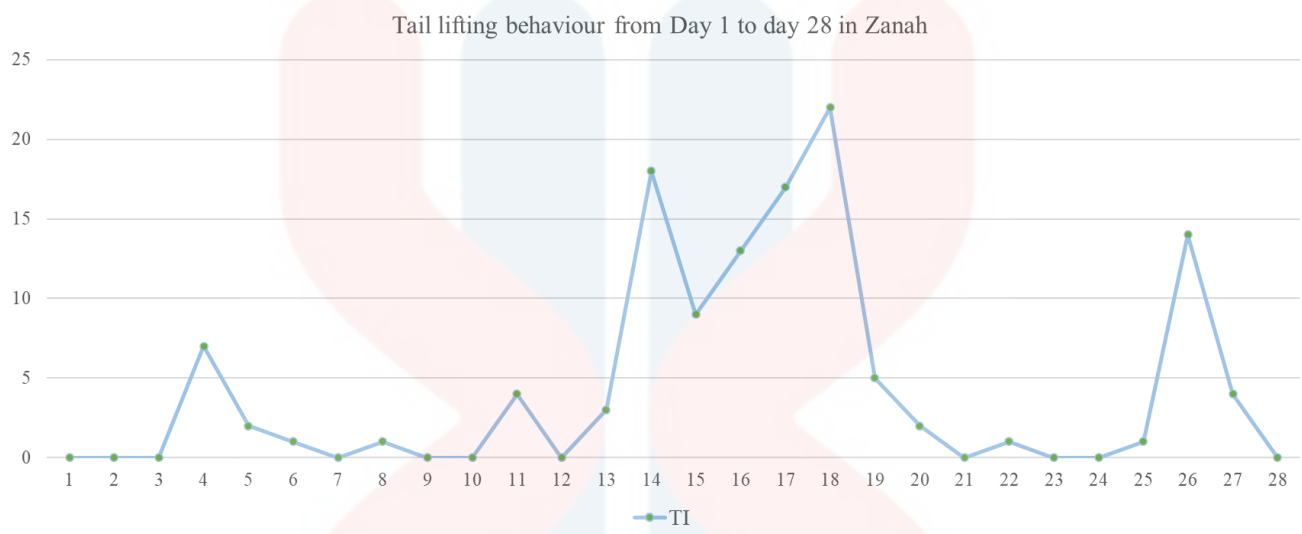**Figure 12:** Line graph of rolling behaviour in Zanah (Rl - rolling)

Figure 13: Line graph of tail lifting behaviour in Zanah (Tl - tail lifting)
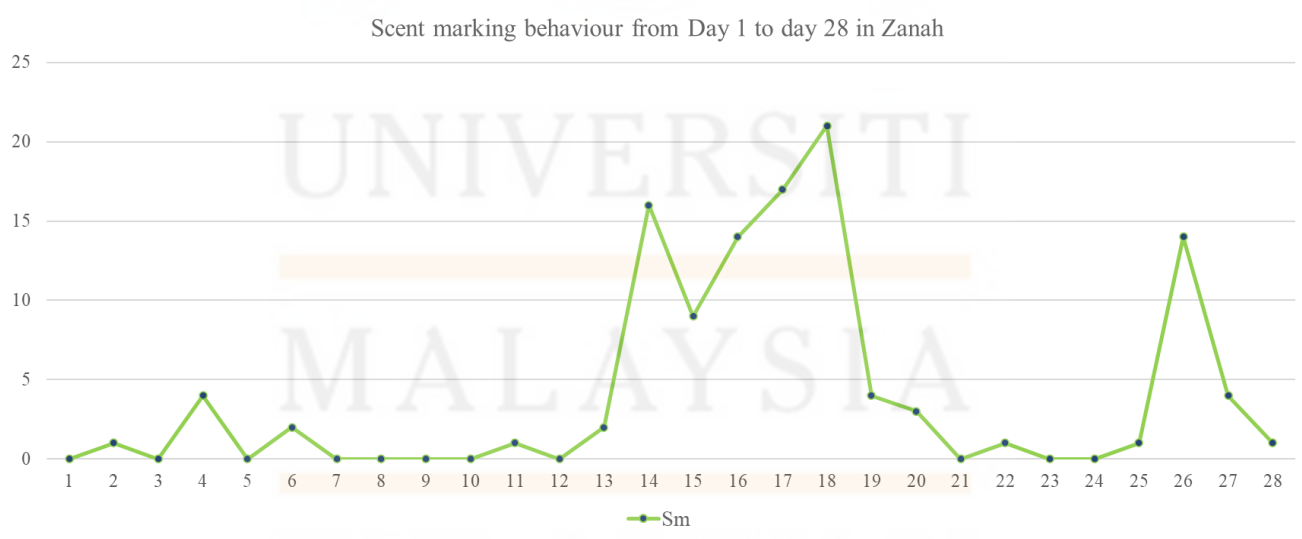
Figure 14: Line graph of scent marking behaviour in Zanah (Sm - scent marking)

The line graph above shows the frequency of each behaviour that was expressed by Zanah in the same amount of time that was spent on observing Cindai (Figure 10 - 14). On day 1 - day 5 there was low activity yet with some breeding behaviours still being expressed indicating Zanah was no longer in heat. On day 6 - day 9, there were minimal to no breeding behaviours expressed as this was strongly indicating Zanah's anestrus phase where she was sexually unreceptive. However, on day 10 - day 12, there were signs of increase in all the mating behaviours displayed which shows the beginning of the proestrus phase. Zanah expressed a spike in mating behaviours on day 13 - day 20 as she entered her phase of oestrous except for rolling. A decline in expressed behaviors was seen from day 18 - day 19 which was indicative of the end of Zanah's oestrus. She was then under the anestrus phase with minimal activity for 3 - 4 days till day 24. On day 25 till day 28, there was a spike in frequency of mating behaviours being expressed once again which shows Zanah's second cycle of estrous till end of the study.

5.0 Discussion

Breeding behaviours in tigresses vary in accordance with the oestrous cycle that comes in different phases. The common behaviours include vocalization, rubbing, rolling, scent marking, tail lifting, lordosis, and the act of mounting. In the oestrous cycle, felines first experience proestrous which takes 1 - 3 days. This is the time where felines will start expressing some breeding behaviours as an early indication of being receptive for mating or finding a mate. The second phase will be estrous that averagely takes 4 - 7 days to occur but can last up to 21 days in which females will express more breeding behaviours such as lordosis indicating they are sexually receptive to be mated and mounted. Once this phase comes to an end, they will undergo ovulation that will take

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up to 24 - 48 hours as the act of mating will stimulate the release of a mature egg from the ovary to undergo fertilization. If ovulation is successful, there will be progression into the dioestrus phase in which females will no longer remain sexually receptive as physiologically their body will undergo pregnancy maintenance to undergo gestation. However, if this fails to occur, the dioestrus phase will terminate with the regression of the corpus luteum and will proceed into the phase of anoestrus. This is the rest phase of cyclicity before the females can go into the next phase of proestrus.

In the observational study conducted at NWRC, all the behaviours were successfully expressed by Cindai whereas Zanah showed partial expression of breeding behaviours. This is because Zanah was not paired with a male throughout the 28 days of observation as she was reported to have a traumatic experience from previous attempts of being paired with another male tiger that showed aggression in which Zanah was nearly killed. The behaviours expressed by Cindai were seen in cycles of oestrous and converted into a line graph attached on Figure 16 below. The line graph indicates the number of days in which this study was conducted against the number of times or frequency of behaviours expressed.
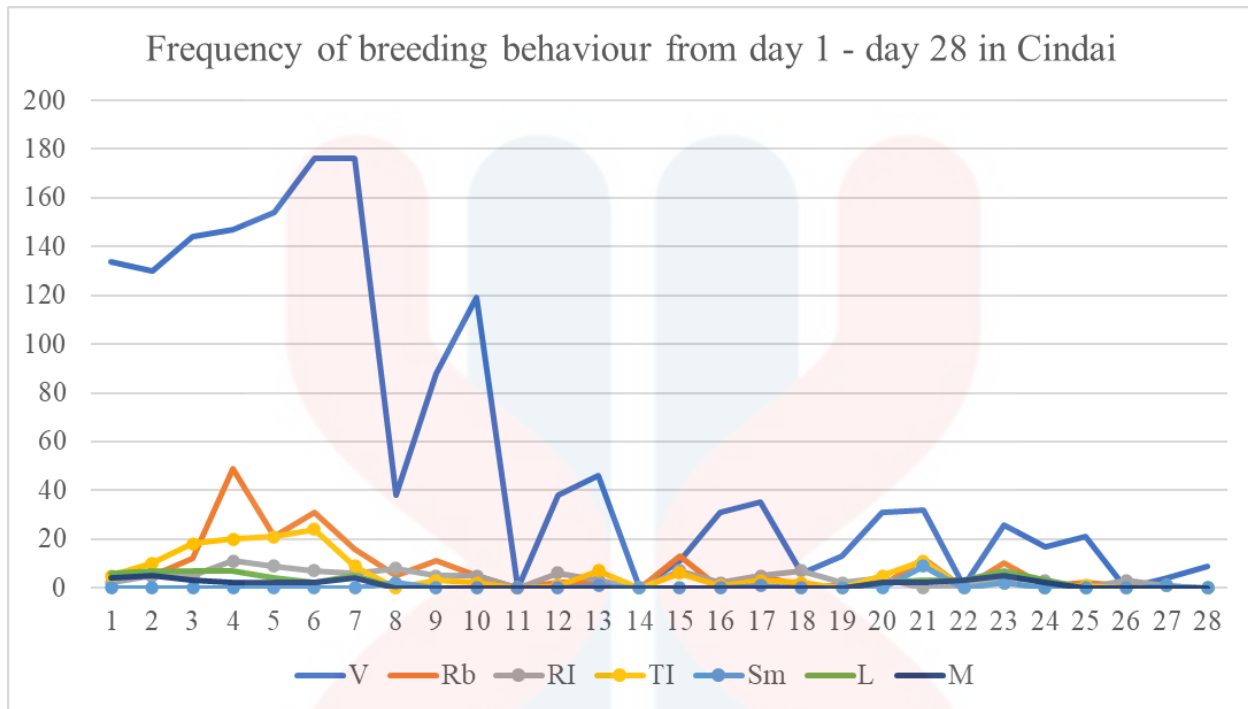
Figure 15: Line graph of breeding behaviour frequency in Cindai for 28 days

(V-vocalization, Rb - rubbing, Rl - rolling, Tl - tail lifting, Sm - scent marking, L - lordosis, M - mounted)

From the line graph above (Figure 15), Cindai successfully mated with the male tiger that was paired and showed sexual receptivity to being mounted. Cindai expressed all of the breeding behaviours at the highest frequency on an average of day 3 - day 6 which showed that she was in the phase of estrous which is the second stage in the estrous cycle after proestrus for a total of 4 days which falls within the average number of days for felines. In the early phase of oestrous, Cindai was restlessly circling around the night stall repetitively. She was seen showing aggression by growling, fiercely snarling, and pouncing towards the staff members on the gates everytime she was approached for cage cleaning. The following behaviour expressed was also tail lifting against the surface of walls or logs and scent marking by spraying or urinating. Tigresses spray in order to release their pheromones in order to signal males of their receptivity. At this time, both the male

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and female tigers were seen spraying or urinating and sniffing each other's secretions which was followed by a flehmen response.

Cindai was seen showing flehmen response multiple times during her oestrous phase by first sniffing the male's urine or secretions sprayed within the night stall and curls back her upper lip with her front teeth and gums exposed tongue drooping down with her head facing upwards. This is an indicator of Cindai showing interest in her mate. According to (MOWATT, 2016), tigers display flehmen response to allow the scent to reach the roof of its mouth where the Jacobson organ is located. This organ is a part of the olfactory system and plays a major role in chemoreception for mating purposes, territory markings and communications.

Upon close contact, Cindai prustened many times repetitively at the male right before rubbing her head, cheeks, and flank against her partner. This occurrence was seen mostly during the phase of proestrus. Once Cindai was sexually receptive to being mounted, the above behaviours were displayed at the highest frequency and Cindai started displaying lordosis to present herself before her mate. Cindai expressed lordosis by laying sternally in front of the male with her posterior pelvic elevated dorsally, her forelimbs lowered while hindlimbs were extended with and her tail displacement sidewards. Once she was mounted, a low moan was emitted by Cindai as copulation took place. After 1-3 seconds when the male dismounted, Cindai let out a loud roar and showed immediate aggression by swiping her paws towards the male. This was caused by a painful sensation that was felt from copulation due to the anatomical structures of the male's penis that is spiky.

On Day 7 - day 8 Cindai showed a slight drop in expressing breeding behaviours after being mounted which shows that she was undergoing ovulation. Day 8 - day 14 revealed lowly expressed breeding behaviours for a total of 7 days which indicated progression into anoestrus phase. On day 15, there was a slight spike in breeding behaviours shown by Cindai which indicated her progression into the next cycle of proestrus as she expressed vocalization by multiple prustenings, rubbing, rolling and tail lifting. Cindai then showed signs of receptivity to be mated by frequent scent markings, lordosis and was mounted on day 20 up till day 24. Each mating lasted 1 - 3 seconds. Thus, Cindai's second cycle of estrous which took place for a period of 5 days was detected. From day 25 till day 28 Cindai showed minimal signs of mating behaviours which concluded she was in the anestrus phase of the oestrous cycle. In a nutshell, Cindai experienced two cycles of oestrous throughout 28 days with high frequency of mating behaviours expressed.

In the case of Zanah, she was never paired with a male tiger thus the results obtained differed from Cindai. All the behaviours expressed were converted into a line graph attached below. From the line graph, Zanah did not successfully express all the breeding behaviours and was showing very weak signs of receptivity towards the male.
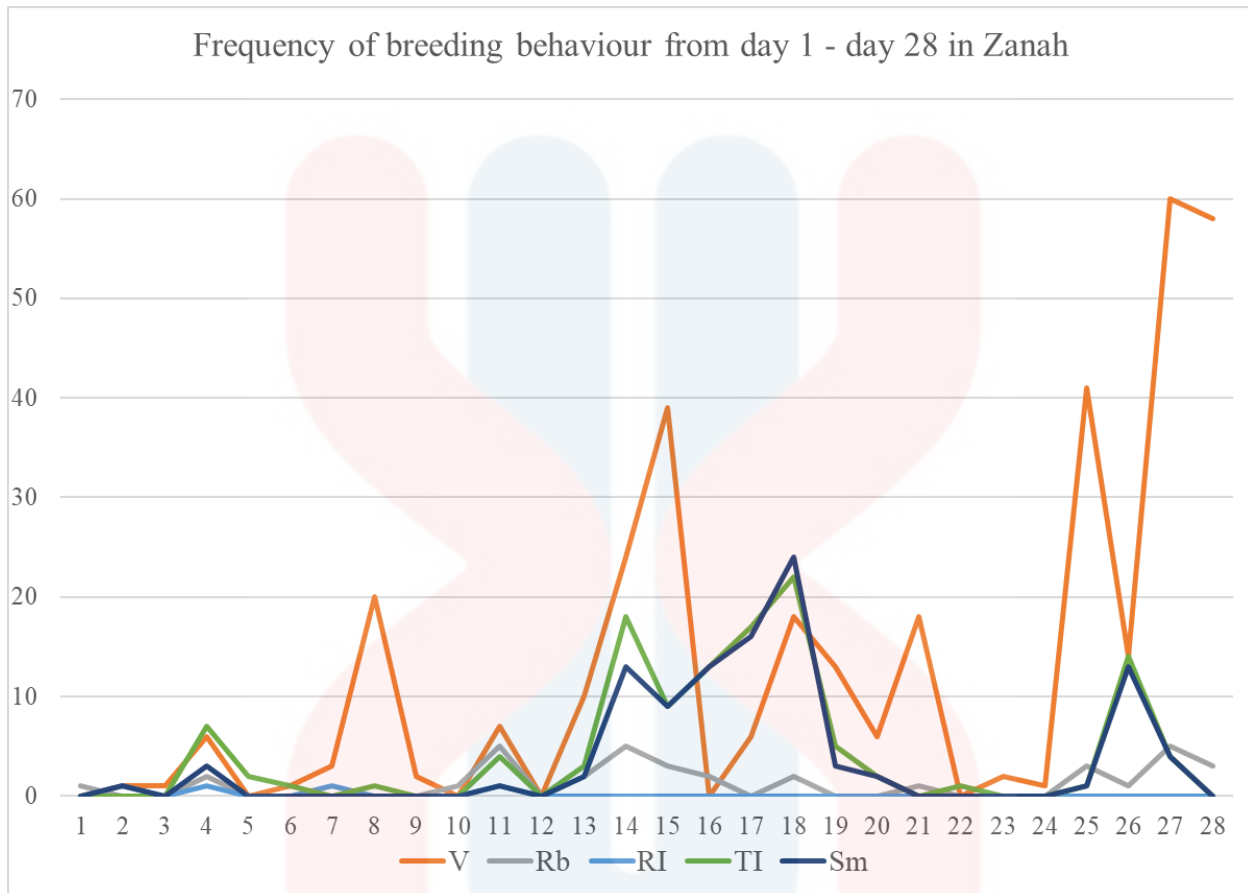
Figure 16: Line graph of breeding behaviour frequency in Zanah for 28 days

(V-vocalization, Rb - rubbing, Rl - rolling, Tl - tail lifting, Sm - scent marking)

From the line graph above, Zanah expressed some of the breeding behaviours which were not entirely influenced by the presence of the male tiger placed next to her night stall. In addition to that, the absence of male placed in the same night stall was a factor that resulted in absence of lordosis behaviour and Zanah not being mounted. Thus, lordosis and mounting were not included in the line graph above. Upon observation, during day 1 - day 5, Zanah showed a decline in expressing mating behaviours which indicates the end phase of her oestrous. On day 6 - day 9, Zanah showed very minute signs of mating behaviours that indicated her progression into the anestrus phase. Zanah entered the proestrus phase on day 10 - day 12 as there was a mild increase

in vocalization, rubbing, tail lifting and scent marking. She then entered the oestrous phase on day 13 - day 17 which lasted for 5 days in which she had highly expressed prustening as a form of vocalization, tail lifting and scent marking by spraying onto surrounding walls and logs within the night stall. On day 18 - day 19 there was a drop in the behaviours associated with Zanah undergoing ovulation for a day. Zanah then progressed into the anestrus phase on day 21 - day 24 as she showed minimal signs of activity and was mostly resting. Four days later she entered her third cycle, which is the proestrus phase on day 25 - day 28 as she began displaying vocalization, rubbing, tail lifting and scent marking behaviours. In conclusion, Zanah experienced two and a half cycles of oestrous up till day 28.

In this study, the remaining behaviours such as circling, pawing, aggression and resting were not added as it was affected by external factors. External factors that interfered with the behaviours were exposure of the tigresses to staff members that approached the night stalls for cleaning, transferring of tigers and feeding. This resulted in the tigress responding with a display of aggression and excessive circling. Circling was also influenced by hunger in which both the females were seen circling at a high frequency prior to feeding time. Pawing behaviour was also contributed by the lack of enrichment implemented in the night stalls and exercise yard that resulted in the tigress clawing logs from boredom and to sharpen their claws. On days where Cindai was not paired with the male tiger by the keepers, she would express higher rest periods as there was no other form of enrichment or company. Zanah showed the highest number of times in resting as she was never once paired with a male. Thus, these four behaviours could not be analyzed with sufficient accuracy.

According to Rioldi, 2013, captive breeding of animals can cause stress that leads to the expressions of abnormal behaviour and loss of fitness. Animals in captivity that are provided with

enclosures resembling their natural environment, allow them to perform natural behaviours and show a decrease in stereotypes and stress. Upon providing the appropriate management and enclosures, animals can also show a decrease in abnormal behaviour and an increase in fitness and health which influences their reproductive success. In the case of Zanah, she was physically inactive and mostly sleeping compared to Cindai. It was observed that her body condition score was 4.5 out of 5 which falls in the overweight category. This was determined from visual observation and photographs taken in which showed Zanah having fat deposits on her hips, stomach and significantly reduced definition on her pelvic, abdomen, shoulders and neck region. Zanah was sometimes seen expressing stress associated behaviours such as excessively pacing to and fro for over one hour multiple times within a day. This can be attributed to the lack of enrichment and infrequent introduction of enrichment items into the night stall and exercise yard. Thus, Zanah displayed low frequency of breeding behaviours and abnormal repetitive behaviors.

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion, the detection of the Malayn tigress oestrous requires a baseline understanding of breeding behaviours expressed. Although no studies have been conducted on the breeding behaviours of the Malayan tigers of Malaysia, there have been studies carried out in tigers of different breeds in other countries such as Europe, Indonesia, India, and Italy. The Malayan breeding behaviours n tigresses were successfully displayed such as circling, vocalization, rubbing, rolling, tail lifting, scent marking, pawing, lordosis, and aggression in Cindai (Tiger A). However, Zanah (Tiger B) showed lack of interest toward the male tiger and was less active as compared to Cindai. The behaviours expressed were relatable to the different phases of the oestrous cycle in the Malayan tigresses and they successfully displayed behaviours that were used in the prediction of oestrous. However, due to external factors, behaviours such as circling, pawing, aggression and resting were affected and could not be accurately analysed.

6.2 Recommendations

To achieve an in-depth understanding of the Malayan tiger behaviour, a 24-hour observation study should be done in order to obtain a complete insight into the activities carried out by the tigers. Physiological and behavioural traits need to be correlated for a well-rounded comprehension of tiger behaviour and oestrous cycles. Enrichment factors should be given priority to ensure the overall well-being and performance of these tigers. Assisted reproductive technology can be done to overcome problems involved in unsuccessful natural mating.

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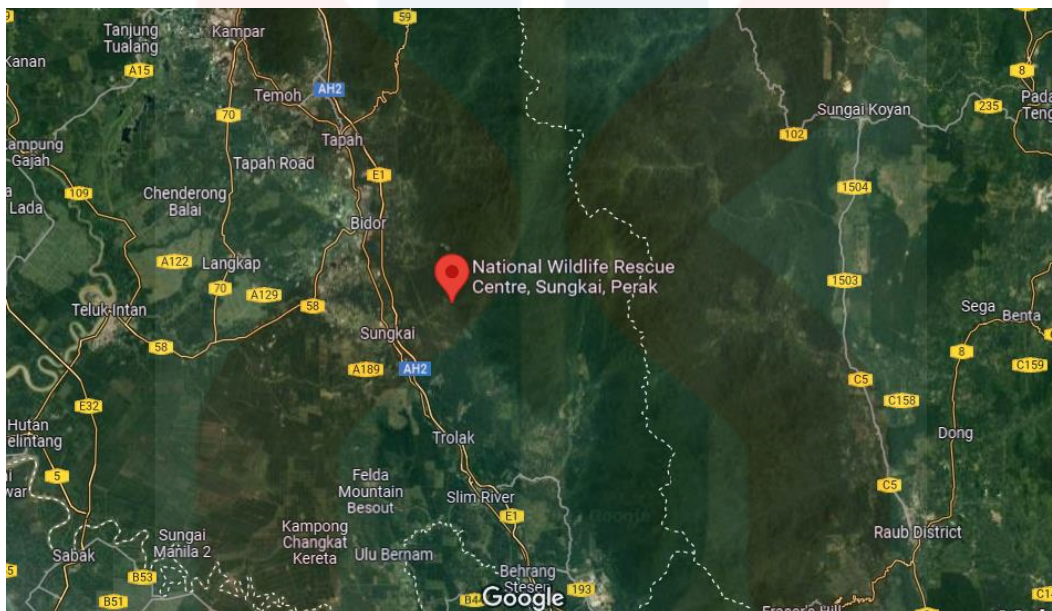
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APPENDIX A

Appendix A.1: A satellite view of the National Wildlife Rescue Centre, Sungkai

Appendix A.2: Administration office of the National Wildlife Rescue Centre

Appendix A.3: Malayn tiger Unit of NWRC

Appendix A.4: Malayn tiger night stall

Appendix A.5: Interior design of night stall with natural logs and elevated platforms

Appendix A.6: Walkway from which study was conducted
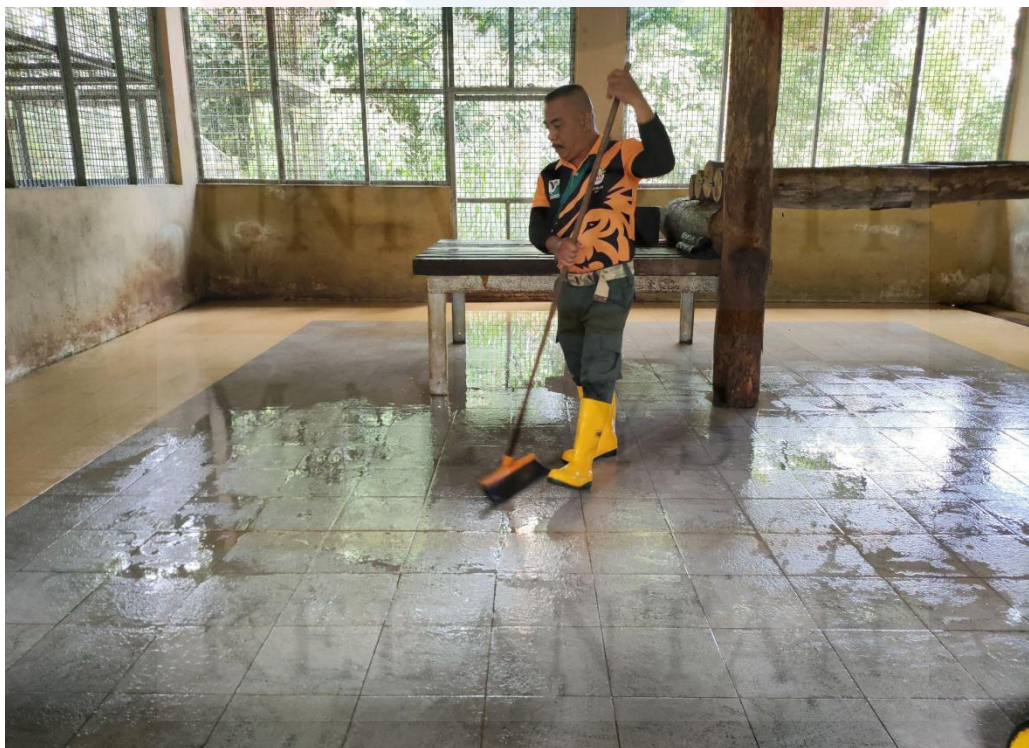
Appendix A.7: Daily cleaning routine of night stall

Appendix A.8: Location of exercise yard next to night stall

Appendix A.9: Malayan tigress A resting with paired male

Appendix A.10: Cindai and male partner after mating

Appendix A.11: Display of aggression in Cindai when approached

Appendix A.12: Rolling behaviour seen in Cindai towards male

Appendix A.13: Cindai seen constantly approaching male tiger and rubbing onto him
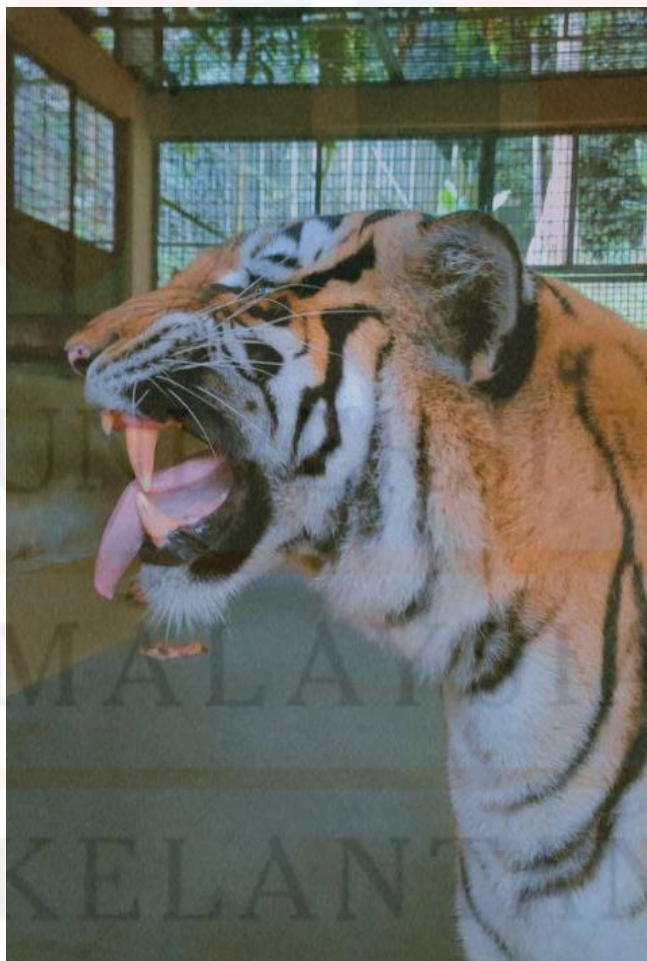
Appendix A.14: Cindai displaying flehmen response after sniffing the urine of the male

Appendix A.15: Cindai expressing tail lifting behaviour prior to spraying

Appendix A.16: Cindai presenting herself before the male by display of lordosis

Appendix A.17: Cindai mounted by her male

Appendix A.18: Cindai and male tiger mating on elevated platform

Appendix A.19: Cindai attacks male as soon as he dismounts after copulation

Appendix A.20: Cindai seen resting in exercise yard

Appendix A.21: Malayan tigress B seen sleeping after eating
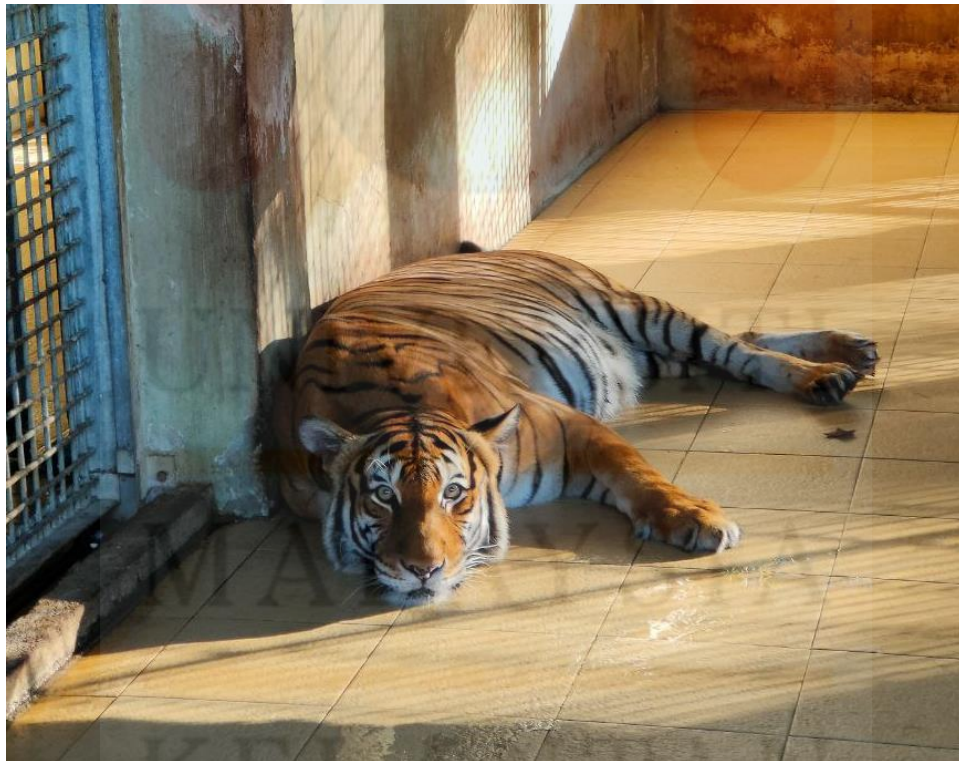
Appendix A.22: Zanah seen sleeping in the morning

Appendix A.23: Zanah seen sleeping during early afternoon

Appendix A.24: Zanah seen pawing on log in exercise yard

Appendix A.25: Zanah seen resting in exercise yard

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12.2 Focal sampling worksheet of breeding behaviours in female Malayan tigers (*Panthera tigris jacksoni*)

Observer :   Animal ID :   Sex :   Date :   Start time: End time :

Time	Circling	Pawing	Scent marking	Vocalisation	Rubbing	Rolling	Tail lifting	Lordosis	Aggression	Resting	Other

Appendix A.26: Focal sampling worksheet used for data collection

Observer: Animal ID: Sex: Date: Start to end time :

Ethogram and frequency chart of breeding behaviour in the Malayan tigers (*Panthera tigris jacksoni*)

Breeding behaviour	Vocalising	Rubbing	Circling	Rolling	Tail lifting	Scent marking	Resting	Pawing	Aggression	Lordosis
Code	V	Rb	C	Rl	Tl	Sm	Rst	P	A	L

Non-breeding behaviour	Grooming	Lying down (sternal)	Lying down (lateral)	Yawning	Flehmen	Eating	Drinking	Defecating
Code	G	Lds	Ldl	Y	F	E	Dr	Df

Activity:		Tally marks recorded of breeding behaviours	Frequency
	V		
	Rb		
	C		
	Rl		
	Tl		
	Sm		
	Rst		
	P		
	A		
	L		
	Note:		

Appendix A.27: Frequency table chart using tally numeration system for data collection

12.1 Ethogram of breeding behaviours in female Malayan tigers

(*Panthera tigris jacksoni*)

Behavioural acts	Description	Duration
Circling	Walking repetitively in circles Note:	
Pawing	Using paws or claws to scratch Note:	
Scent marking	Urinating or spraying Note:	
Vocalising	Producing vocal sounds Note:	
Rubbing	Rubbing cheeks, head/flank on surfaces around enclosure Note:	
Rolling	Lying on the back and turning over Note:	
Tail lifting	Upward or erect position of tail away from perineal region Note:	
Lordosis	Lowering forelimbs, extended hindlimbs, raised hips, ventral arching of spine, raising/sideward displacement of the tail Note:	
Aggression	Scratching growling, jumping Note:	
Resting	Lying down with minimal to no activity Note:	
Other		

Appendix A.28: Ethogram worksheet for data collection

Appendix A.29: Time recording of behaviours expressed recorded into worksheet upon observation
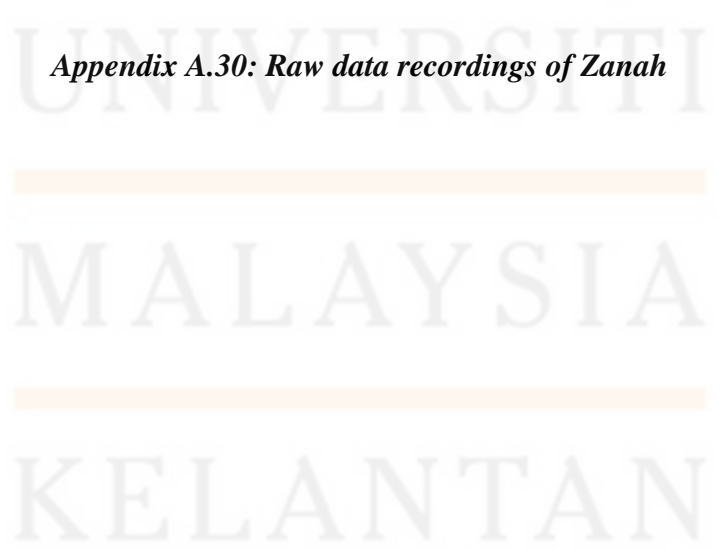
Tally frequency worksheet of breeding behaviour in Zanah from day 1 - day 28

Date	DAY	V	Rb	C	RI	TI	Sm	P	A	L	M
9/9/2022	1	0	1	1	0	0	0	0	0	0	0
12/9/2022	2	1	0	2	1	0	1	1	0	0	0
13/9/2022	3	1	0	0	0	0	0	0	0	0	0
14/9/2022	4	6	2	2	1	7	4	0	0	0	0
15/9/2022	5	0	0	1	0	2	0	1	0	0	0
16/9/2022	6	1	0	2	0	1	2	1	0	0	0
17/9/2022	7	3	0	2	1	0	0	0	0	0	0
18/9/2022	8	20	0	2	0	1	0	1	0	0	0
19/9/2022	9	2	0	1	0	0	0	0	0	0	0
20/9/2022	10	0	1	0	0	0	0	0	0	0	0
21/9/2022	11	7	5	2	1	4	1	2	0	0	0
22/9/2022	12	0	0	0	0	0	0	0	0	0	0
23/9/2022	13	10	2	4	0	3	2	0	0	0	0
24/9/2022	14	24	5	4	0	18	16	0	0	0	0
25/9/2022	15	39	3	2	0	9	9	2	0	0	0
26/9/2022	16	0	2	4	0	13	14	0	0	0	0
27/9/2022	17	6	0	4	0	17	17	0	1	0	0
28/9/2022	18	18	2	5	0	22	21	2	1	0	0
29/9/2022	19	13	0	3	0	5	4	0	0	0	0
30/9/2022	20	6	0	4	0	2	3	0	0	0	0
1/10/2022	21	18	1	2	0	0	0	0	0	0	0
2/10/2022	22	0	0	0	0	1	1	0	0	0	0
3/10/2022	23	2	0	1	0	0	0	0	0	0	0
4/10/2022	24	1	0	0	0	0	0	1	0	0	0
5/10/2022	25	41	3	1	0	1	1	0	0	0	0
6/10/2022	26	14	1	6	0	14	14	0	0	0	0
7/10/2022	27	60	5	1	0	4	4	1	0	0	0
8/10/2022	28	58	3	3	0	0	1	0	0	0	0

V - vocalization, Rb - rubbing, C - circling, RI - rolling, TI - tail lifting, Sm - scent marking, P - pawing, A - aggression, L - lordosis, M - mountings
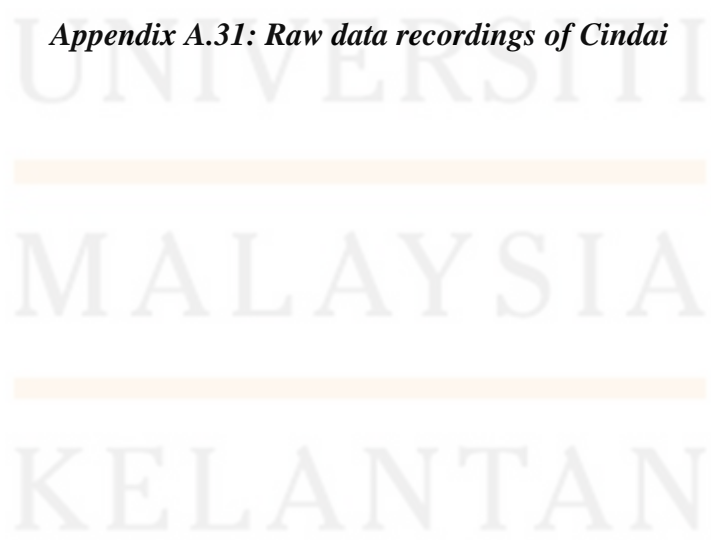
Appendix A.30: Raw data recordings of Zanah

Tally frequency worksheet of breeding behaviour in Cindai from day 1 - day 28

Date	DAY	V	Rb	C	RI	TI	Sm	P	A	L	M
9/9/2022	1	134	5	2	2	5	0	1	1	6	4
12/9/2022	2	130	5	3	5	10	1	0	0	7	5
13/9/2022	3	144	12	5	5	18	2	1	1	7	3
14/9/2022	4	147	49	4	11	20	7	0	0	7	2
15/9/2022	5	154	21	5	9	21	13	3	0	4	2
16/9/2022	6	176	31	4	7	24	3	0	2	2	2
17/9/2022	7	176	16	5	6	9	3	3	1	5	4
18/9/2022	8	38	5	5	8	0	0	1	0	0	0
19/9/2022	9	88	11	6	5	3	2	2	1	0	0
20/9/2022	10	119	5	2	5	2	2	0	0	0	0
21/9/2022	11	0	0	0	0	0	0	0	0	0	0
22/9/2022	12	38	2	4	6	1	0	0	1	0	0
23/9/2022	13	46	2	9	3	7	2	0	1	0	0
24/9/2022	14	0	0	1	0	0	1	0	0	0	0
25/9/2022	15	11	13	6	7	6	0	0	0	0	0
26/9/2022	16	31	0	4	2	1	1	0	1	0	0
27/9/2022	17	35	5	6	5	3	4	0	0	0	0
28/9/2022	18	6	1	3	7	2	2	2	0	0	0
29/9/2022	19	13	1	3	2	0	0	0	0	0	0
30/9/2022	20	31	1	6	4	5	2	0	0	0	0
1/10/2022	21	32	12	7	0	11	12	1	1	0	0
2/10/2022	22	1	0	3	1	0	0	0	1	0	0
3/10/2022	23	26	10	8	4	2	3	0	2	0	0
4/10/2022	24	17	1	11	3	0	0	2	2	0	0
5/10/2022	25	21	2	2	0	1	0	0	0	0	0
6/10/2022	26	0	1	4	3	0	1	0	1	0	0
7/10/2022	27	4	0	6	1	1	1	0	0	0	0
8/10/2022	28	9	0	3	0	0	0	0	0	0	0

V - vocalization, Rb - rubbing, C - circling, RI - rolling, TI - tail lifting, Sm - scent marking, P - pawing, A - aggression, L - lordosis, M - mountings

Appendix A.31: Raw data recordings of Cindai

Appendix A.32: Approval form from DWNP