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RESEARCH ARTICLE

LIP LICKING BEHAVIOR IN CAPTIVE MALAYAN TAPIRS (*Tapirus indicus*): MANIFESTATION OF A STEREOTYPIC OR STRESS RELATED RESPONSE?

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ABSTRACT

Malayan tapirs are highly endangered and wild populations are fast declining. Thus, captive breeding programs in zoos and governmental breeding centers are the most promising conservation strategy for this species. Despite being common, lip licking, a type of oral behavior, has received little attention in the past, and impacts on the welfare of captive Malayan tapirs have not been quantified. Here, we videoed the behavior of seven captive tapirs for eight hours per diem (0900 - 1700) using instantaneous sampling for six months to investigate which stressors in captivity (enclosure type, enclosure size, humidity, visitors) cause increased lip licking behavior. We show that lip licking is induced by unsuitable humidity whereby dry humidity below 65% caused a significant increase in this behavior. We found lip licking behavior in tapirs is not a stereotypic behavior, but it may indicate a stress response towards heat. Hence, we suggest that breeding centers re-evaluate their exhibit design and behavioral enrichments, implementing simple design changes that would help to reduce lip licking and consequently increase the welfare of captive Malayan tapirs.

INTRODUCTION

Stereotypic behaviors and stress responses in wild animals kept in captivity are often used as welfare indicators (Mason, 1991). Stereotypic behaviors are defined as repetitive, invariant and functionless motor responses that occur in domesticated/ captive wild animals as well as in humans (Mason,1991; Houpt *et al.,*1993; Mason *et al.,* 2007), where stress is generally described as an animal's behavioral or physiological response to perceived threats or aversive stimuli (Morgan and Tromborg, 2007). Stereotypic behaviors in captive animals are often considered to be a form of coping behavior in a stressful environment which is triggered by environmental stimuli (Houpt *et al.,* 1993) such as exposure to a sudden change in temperature, physical restraint, threats from conspecifics or the approach of a human (Houpt *et al.,* 1993; Morgan and Tromborg, 2007). Oral stereotypic behaviors such as lip licking, tongue flicking, tongue rolling or tongue curling are common in many Equidae, Canidae, Felidae, Ursidae, Bovidae (Lyons *et al*., 1997; Bashaw *et al*., 2001; Housing, 2002; Vickery and Georgia, 2004; Palestrini *et al*., 2010; Fureix *et al*., 2011; Miranda *et al*., 2012; Protopopova, 2016), where particularly lip licking is associated with distress (Palestrini, 2011). In captivity, it is associated with a variety of stressors, such as enclosure condition, size, complexity (Macedonia, 1987; Lyons *et al*., 1997; Tan *et al*., 2013) and the presence of visitors (Glatson *et al*., 1984; O'Donovan *et al*., 1993; Claxton, 2011).

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For example, studies identified that lip licking behavior in domestic dogs was affected by stress due to fear and poor shelter (Beerda, 1998; Palestrini, 2010; Protopopova, 2016). The percentage of concrete wall in the enclosure surroundings increased mouthing stereotypic behaviors in female Black rhinoceros (*Diceros bicornis*) (Carlstead,1999), and female giant pandas, (*Ailuropoda melanoleuca*) housed in a semi-natural environment spent less time engaged in various stereotypic behavior (not limiting to oral stereotypic) than did females housed in traditional enclosures (Liu *et al*., 2003). However, although licking has been described in tapirs as self-grooming behaviors (Gilmore, 2007), lip licking behavior has been largely overlooked and its potential as a stress -response or stereotypic behavior has not been investigated in this family. The Malayan tapir (*Tapirus indicus*) is listed as 'Endangered' by the International Union for Conservation of Nature (IUCN) Red List with few populations left in the wild. Malayan tapirs are thus currently bred in zoos and breeding centers under special governmental breeding programs to ensure ex-situ conservation (Rose and Roffe, 2013; Traeholt *et al*., 2016). Captive Malayan tapirs are subjected to a variety of potential stressors (Morgan and Tromborg, 2007; Arumugam *et al*., 2018), but reliable stress-indicators have not been established. Therefore any form of distress or new type of behavior need to be observed carefully (Arumugam and Annavi, 2018). In this study we analyze the occurrence and frequency of lip licking (Fig. 1) in captive Malayan tapir and relate it to enclosure type and size, visitor numbers and weather (humidity and temperature). The results from this study will help contribute to a better understanding of Malayan tapir welfare in captivity and will help to develop improved management strategies for this endangered species.

Fig. 1. Picture showing captive Malayan tapir tongue prior to lip lick behavior

MATERIALS AND METHODS

Study Area and Subjects: This study was carried out at three enclosures in Sungai Dusun Wildlife Reserve Centre (GPS reference: 3° 40' 45.3" N, 101° 23' 49.2504" E), one enclosure each in Zoo Negara (3° 12' 25.5996" N, 101° 45' 24.2244" E) and Zoo Melaka (2° 16' 35.5332" N, 102° 17' 56.04" E) in Peninsular Malaysia. The descriptions of each enclosure are given in Table 1. A total of seven adult tapirs between 7 to 14 years of age were observed (male = 4, female = 3). Types of enclosure were classified as either "artificial" or as "semi-natural" enclosure. The artificial enclosures in this study were situated near human settlement, main roads and allowed for visitors, were consequently more prone to noise pollution, whereas the semi-natural enclosures were situated in forested areas which were far from human settlements with restricted visitor access and consequently minimal noise pollution.

Behavioral Observation: The behavioral observations were conducted over a period of 24 weeks (six months) between March and August 2016. The animals were observed at the same time each day between 0900 and 1700 during zoo opening times to quantify visitor effects. Using instantaneous sampling method, the focal behavior was recorded every 20 minutes at 30 seconds interval (Martin and Bateson, 2007; Arumugam *et al*., 2018). Recording was carried out using a digital video camera (Sony, Model: FDR-AXP35) and camera traps (Scout Camera, Model: DTC-560K) with video mode wherever direct observations were not possible and transferred onto a datasheet. The outdoor temperature and humidity were measured with Hygro-Thermometer Clock (Extech Instruments, Model: 445702) at all enclosures and the number of visitors at the tapirs enclosures was counted manually at ZN and ZM.

Data Analysis: Statistical analyses were run in R Statistical Package Version 3.3.2. by fitting generalized linear mixed-effects models using the glmer function in lme4 package and model averaging based on information criteria, AICc (Burnham and Anderson, 2002) in MuMIn package (Barto´n, 2016). The Y-axis represents the frequency of lip lick behavior summed weekly for each individual. The fixed effects included enclosure type, enclosure size, weather, and the number of visitors. Individual identity was included as a random effect in the model to control for individual-specific variation. The number of visitors and weather (temperature and humidity) variables were standardized to a mean of zero and a standard deviation of two (Gelman, 2008). Because the weather variables were inter-correlated (i.e. if temperature (°C) increased, humidity (%) decreased; $r = -0.80$, $p < 0.05$), we included humidity in the model. We used an information-theoretic (IT) approach to select set of plausible models and to estimate the overall importance of each fixed effect (Burnham *et al*., 2011; Annavi *et al*., 2014). Models were ranked by their AICc value, such that the top model had the lowest AICc value (Burnham *et al*., 2011; Annavi *et al*., 2014), and the top model was considered to be the only plausible model if it alone ranked $\Delta AICc \leq 7$. A model's relative Akaike weight (ω) was calculated as the model's relative likelihood ($\exp[-0.5 * \Delta AICc]$), divided by the sum of the likelihoods for all models considered (whether plausible or not). We used the 'average method' (averaged over all plausible models in which the given parameter was included, weighted by the summed weights (ω) of these models; Burnham and Anderson, 2002) to estimate model-averaged parameters. The 95% confident intervals for model-averaged parameter estimates were calculated using the model.avg function in R. The relative importance of each fixed effect was calculated as the total ω of all plausible models that included the fixed effect of interest. If humidity or visitor numbers proved to be significant, we further analyzed if there was a specific range where lip licking behavior occurs using Mann Whitney U Tests grouping humidity or visitor numbers into group 1: with the threshold above the range and group 0: with the threshold below the range.

RESULTS

The model consists of enclosure size and humidity was ranked at top with delta 0.00 and weight 0.234 (Table 2). However based on the model average table, humidity was the only factor showed significant to the lip lick behavior in Malayan tapir as we referred to its 95% confidence interval which did not overlap zero (Table 3) and has the highest relative variable importance at 0.80 compared to enclosure size, 0.64 (Table 3). Therefore, we found tapir engaged more often in lip licking behavior during low humidity and high temperature (Fig. 2). Since, our result shows a significant effect of humidity and lip licking frequency increased at a certain point, we performed a Mann Whitney U test. We computed the frequency of lip licking behavior versus humidity and temperature ranged between group 1: threshold above 65% (humidity)/ below 31.7°C (temperature) and group 0: threshold of below 65% (humidity)/ below 31.7°C (temperature). For both humidity and temperature group 1 and group 0 are non-identical with p-value of 0.7982 and 0.3198 respectively.

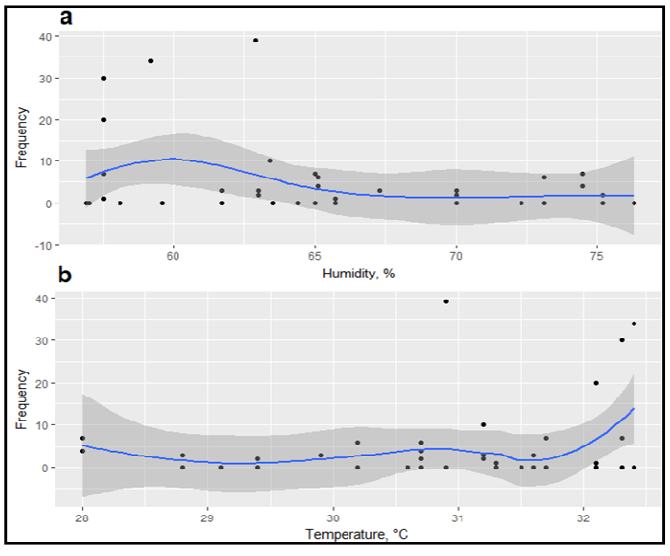

Fig. 2. Relationship of lip licking behavior with (a) humidity and (b) temperature. The behavior increases when the humidity is below 65% and temperature of 31.7°C

Table 1. Description of study sites

Enclosure type	Place	Enclosure size	Fence height (high/low)	Visitors Permitted (yes/no)
Artificial enclosure	Zoo Negara (ZN)	765m^2	high	yes
	Zoo Melaka (ZM)	1189m^2	low	yes
	Sungai Dusun Wildlife Reserve Centre Padlock A (SDA)	728m^2	high	no
	Sungai Dusun Wildlife Reserve Centre Padlock B* (SDB)	937m^2	high	no
	Sungai Dusun Wildlife Reserve Centre Padlock C* (SDC)	733m^2	high	no
Semi-natural enclosure				

*The same male individual was placed in different enclosures at different times: the Wildlife Reserve Centre Padlock B (March-July 2016), Wildlife Reserve Centre Padlock C (August 2016 onwards).

Table 2. Model selection for explanatory parameters on lip lick behavior in Malayan tapir with Delta Akaike's Information Criterion (ΔAICc) < 7

	Intrc	Cncsz	Encty	Hmdt	Vstr	df	Loglink	AICc	Δ	ω
6	1.5970	-0.7285		-0.4735		4	-59.768	128.6	0.00	0.234
7	-3.0950		1.0620	-0.5230		4	-60.015	129.1	0.49	0.183
8	-0.7213	-0.4119	0.5861	-0.4890		5	-59.172	130.0	1.39	0.116
15	-2.7650		0.9321	-0.5995	0.26030	5	-59.245	130.2	1.54	0.108
14	1.5690	-0.7213		-0.5118	0.14370	5	-59.486	130.6	2.02	0.085
2	1.8500	-0.7837				3	-62.116	130.9	2.24	0.076
16	-1.1430	-0.3333	0.6655	-0.5482	0.20740	6	-58.647	131.7	3.08	0.050
3	-3.4160		1.2300			3	-62.824	132.3	3.66	0.037
4	-0.2425	-0.5054	0.5389			4	-61.646	132.4	3.75	0.036
10	1.8420	-0.7805			0.07327	4	-62.040	133.2	4.54	0.024
5	-0.4512			-0.5539		3	-63.627	133.9	5.27	0.017
11	-3.2780		1.1780		0.13690	4	-62.591	134.3	5.65	0.014
12	-0.4277	-0.4727	0.5787		0.11440	5	-61.476	134.6	6.00	0.012

Intrc = intercept, Cncsz = enclosure size, Encty = enclosure type, Hmdt = humidity, Vstr = number of visitors, df = degree of freedom, Δ = delta, ω= weight

Table 3. Model average parameter estimates over all submodels with Delta Akaike's Information Criterion (ΔAICc) < 7, testing the relationship between variables and lip lick behavior

Variables	ß	SE	95% Lower CI	95% Upper CI	RI
Intercept	-0.513	2.521	-5.502	4.552	-
Enclosure size	-0.629	0.340	-1.314	0.049	0.64
Humidity	-0.515	0.229	-0.978	-0.050	0.80
Enclosure type	0.872	0.572	-0.280	2.020	0.56
Visitor	0.190	0.203	-0.213	0.606	0.30

ß = Estimated value, SE = Standard Error, 95% CI = 95% Confidence Interval and RI = Relative Importance. Bold estimates had a confidence interval that did not overlap zero. Fixed effects: Enclosure type (ZN=1; ZM=2; SDA=3; SDB=4; SDC=5); Enclosure size (728m²=1; 733m²=2; 765m²=3; 937m²=4; 1189m²=5

DISCUSSION

Repeated oral stereotypic behaviors may lead to skin damage and eczematous lesions (Gieler *et al*., 2013) and thus reduce animal welfare. In captive Malayan tapirs, lip lick behavior is linked significantly with unsuitable weather. Since, the frequency of lip licking behavior on threshold above and below particular humidity and temperature are not equal, we can conclude that this behavior has direct effect on humidity below 65% and above 31.7^{o}C and was the main reasons for tapirs displaying repetitive lip licking behavior to keep wet. Enclosures in SDA, SDB and SDC, where lip licking was most frequent, contained neither a pool nor mud wallow to help the animals to cool down. Furthermore, in ZN, the highest frequency of lip lick was during period when there was no water available in the pool. In addition, tapirs in SDA, SDB and SDC avoided resting outdoors during hot/dry weather whereas in ZN and two tapirs frequently slept submerged in the water of the pool (Kalai Arasi pers. obs.). Our results therefore indicate that lip lick behavior is a manifestation of heat stress, which can be avoided through the provision of mud wallows, pools and increased amounts of shade surrounding the enclosure to maintain the humidity below 65% that could help the animals to control their body temperature and prevent over-heating (Garcia *et al*., 2012). Because tapirs show this behavior in reaction to particular humidity or temperature, we conclude that this is not a stereotypic behavior but rather a manifestation of stress in response to heat. However, stress related behaviors have a higher likelihood to develop into stereotypic behaviors due to unfavorable environmental stimuli (Houpt *et al*., 1993). Therefore, if no action is taken to provide suitable behavioral enrichment (mainly pool, mud wallow and plenty of tree shades), this endangered animal may also be susceptible to welfare issues such as skin damage and eczematous lesions from extensive lip licking (Gieler *et al*., 2013).

Conclusion

When air humidity was low, and no swimming pool or water hole was provided within the enclosure, tapirs were observed to suffer from heat stress, and thus increased their lip licking behavior. When air humidity was high, and despite being within the suggested outdoor temperature of 35°C, tapirs were still engaged in lip licking. Therefore, plenty of shade trees should be planted, and a pool with clean water as well as a mud wallow should always be provided for tapirs to allow them to regulate their body temperature and prevent over-heating. It is also crucial to take an early step to avoid the advancement of stress related behavior into functionless stereotypic behavior for the betterment of tapirs welfare in captivity.

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Competing interests: KAA, CDB and GA declare that they have no conflict of interest.

Ethics approval consent to participate: All animal handling procedures were approved by the University of Putra Malaysia ethics committee (Reference: UPM/IACUC/AUP-R033/2016).

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