

# Mineral contents of salt-lick water and mammal visitation to salt-lick in tropical rainforests of Peninsula Malaysia

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## Research Article

**Keywords:** salt-lick, mineral contents, camera trapping, herbivore, Peninsular Malaysia

**Posted Date:** August 18th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1960026/v1>

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**Additional Declarations:** No competing interests reported.

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**Version of Record:** A version of this preprint was published at European Journal of Wildlife Research on April 12th, 2023. See the published version at <https://doi.org/10.1007/s10344-023-01674-2>.

# Abstract

In order to examine the possible mineral supplementing function of salt licks for mammals in tropical rainforests of Peninsular Malaysia, we analysed mineral concentration of water from the salt-licks mainly visited by herbivorous mammals to drink water, and relationship between the mineral concentration and salt-lick visit by mammals. Among analysed minerals, only the concentrations of sodium and calcium were significantly higher in the water from all studied salt-licks than those of the nearby streams in both dry and rainy seasons, indicating that the mammals could supplement these minerals by drinking the salt-lick water. The herbivores most frequently visited the salt-lick with the sodium concentration significantly higher than other salt-licks. In contrast, the omnivores and the carnivores didn't show such tendency. Among four herbivore species that visited the salt-licks most frequently, red muntjac and sambar, the first and second most-frequent visitors (41.7% and 20.8% of the visitation record by mammals), most frequently visited the salt-lick with highest sodium concentration, but Malayan tapir and white-thighed langur frequently visited the salt-lick with lower sodium concentration. The results are consistent with the hypotheses that sodium supplementation is an important purpose of salt-lick visit by herbivores, but also indicated that the factors other than sodium concentration also affect the selection of the salt-licks to visit by herbivores.

## Introduction

In forests from temperate to tropical areas, there are specific sites where many terrestrial and some arboreal mammals have been recorded to eat soil and/or drink seeping water at these sites (Corlett 2009). These sites are called "salt-licks" (Molina et al. 2014), "mineral licks" (Moe 1993), "natural licks" (Matsubayashi et al. 2007a), or "mineral spring" (Bechtold 1996), etc. The salt-licks are considered to be important places for conservation of forest animals since various animals including endangered species are observed there. However, the function of salt-lick visits for animals were still not well studied in many regions, especially in Southeastern Asia.

Several hypotheses on the functions of salt-lick visits have been proposed, such as mineral supplementation, detoxification of plant secondary compounds, or alleviation of gastrointestinal problems (Ayotte et al. 2006, Bechtold 1996, Wakibara et al. 2001). The mineral supplementing function, in particular sodium supplementation, was suggested by a number of studies (Ayotte et al. 2006, Clayton and MacDonald 1999, Matsubayashi et al. 2011, Owen et al. 2014). This hypothesis supposes that herbivores need to supplement sodium at the salt-licks because plants don't contain enough sodium for animal physiological functioning (Corlett 2009).

In Southeastern Asia, Matsubayashi et al. (2007a) reported that various mammals in tropical rain forests of Borneo, including endangered species such as orangutan, drank the salt-lick water with much minerals such as sodium and calcium, suggesting the mineral supplementing function of the salt-licks and importance of the salt-licks in wildlife conservation. In tropical rainforests of Peninsular Malaysia, the Department of Wildlife and National Parks (DWNP) has developed artificial salt-licks which provide various minerals such as sodium, phosphorous, and calcium in the protected areas under its jurisdiction from 2011–2012 (Magintan et al. 2015), supposing the mineral supplement function of the salt-licks. At these artificial salt-licks, some ungulate species such as wild boars (*Sus scrofa*), red muntjacs (*Muntiacus muntjak*), and Malayan tapirs (*Tapirus indicus*) were reported to drink water frequently (Simpson et al. 2020). On the function of natural salt-licks of this area, however, we still have limited studies.

At wet-type natural salt-licks in the tropical rainforests of Belum-Temengor Forest Complex (BTFC), Peninsular Malaysia, the present authors analysed the behaviour of medium-large mammals recorded there, and showed that 95.3% of all records of animals at the salt-licks were herbivores, while the records of omnivores and carnivores were only 3.5% and 1.2%, respectively (Tawa et al. 2022). In addition, they reported that water-drinking behaviors were recorded significantly more frequently in herbivores (73% of video captures) than in omnivores (28%) and carnivores (0%) at the salt-licks (Tawa et al. 2022). Their results suggested that animals, especially herbivores, visited the salt-licks to supplement minerals which are deficient in plant-based diets and/or alleviate gastrointestinal acidosis related to plant-based diets by drinking the salt-lick water. It is still not clear, however, whether the water of these salt-licks contains higher concentration of minerals and/or bicarbonate which have been reported to alleviate gastrointestinal acidosis by neutralizing increased rumen acidity (Ayotte et al. 2008; Davies and Baillie 1988) than the water of other places or not.

In this study, to clarify if the salt-licks in BTFC actually have the mineral supplementing function for herbivorous mammals of this area and which mineral is important for their mineral supplementation, we compared the mineral concentrations of the water from the salt-licks and the nearby streams. We also analysed the relationship between mineral concentration and frequency of salt-lick visit by animals, supposing that herbivores would visit the salt-licks with higher mineral concentration more frequently if the mineral supplementation is a major reason for their salt-lick visits. In this study, we did not discuss the possible function of the salt-lick water to alleviate gastrointestinal acidosis of herbivores because we did not analyse bicarbonate concentration in the water.

## Materials And Methods

### Study area

This study was conducted in the Belum-Temengor Forest Complex (BTFC) located in the State of Perak in northern Peninsular Malaysia (5°30' N, 101°20' E) (Fig. 1), which is mainly covered by upland and lowland dipterocarp forests (Misni et al. 2017). The BTFC consists of two sections, the Royal Belum State Park (RBSP) in the north and Temengor Forest Reserve (TFR) in the south, separated by the East-West Highway (Gerik-Jeli Highway). The RBSP covers an area of 1,175 km<sup>2</sup> (260–1,533 m a.s.l.) and consists of primary forests. By contrast, TFR (1,489 km<sup>2</sup>, 260–2,160 m a.s.l.) is classified as a production forest where selective logging has been ongoing since the 1970s (Ching and Leong 2011). According to the data of rainfall at Kg. Lalang about 30 km west of the study site) provided by Water Resources Management and Hydrology Division, the rainy season around BTFC was from April to December with the break from June to August during the study period (the graph of monthly rainfall is shown in Tawa et al. (2022) as Supplementary Information).

### Locations of studied salt-licks

There are at least 12 salt-licks known by local people in the BTFC and some of them are shown in the sightseeing maps of this area. Automated infrared sensor cameras were established around four salt-licks easily accessible for humans. All the four salt-licks are of the “wet” type; i.e., salt-licks where water that may contain minerals is seeping or flowing from the ground. These salt-licks are relatively open areas surrounded by forest, lacking large trees and where the underbrush is not very dense. One of the four studied salt-licks, called the Tersau salt-lick (5°19' N, 101°22' E), is located 16 m from the shoreline of Temengor Lake in TFR. This area is mainly covered by upland dipterocarp forest (Misni et al. 2017). The other three salt-licks are located along the Tiang River in RBSP. These salt-licks are called (A) Sira Kuak (5°42' N, 101°27' E), (B) Sira Batu (5°43' N, 101°27' E), and (C) Sira Tanah or Sira

Dinding (5°43' N, 101°27' E) by the local people; however, in this study, we hereafter refer to them as “Tiang A,” “Tiang B,” and “Tiang C,” respectively. This area is covered by mixture of upland and lowland dipterocarp forest (Misni et al. 2017). The detail information on the studied salt-licks is described in Tawa et al. (2022) as Supplementary Information. The distance between Tiang A and Tiang B, Tiang A and Tiang C, and Tiang B and Tiang C is about 2.4 km, 2.2 km and 0.44 km, respectively. Thus, the medium-large mammals in this area might easily access all of these salt-licks. In contrast, it is more than 40 km between the salt-licks in Tersau area and those in Tiang area which are also separated by a highway (Fig. 1). Thus, Tersau salt-lick was probably visited by mammal populations different from those that visited the studied salt-licks in Tiang area. Therefore, to analyse the relationship between mineral concentrations and frequency of salt-lick visit by medium-large mammals, we compared three salt-licks in Tiang area, RBSP excluding the salt-lick in Tersau area, TFR.

#### Mineral analyses in the water of salt-licks

Mineral contents were compared between the water of the salt-licks and the nearby streams. The method of sample collection followed Matsubayashi et al (2007a). The water samples were collected at four salt-licks in 4–5 February (dry season) and 18–19 November (rainy season) 2015. At Tersau salt-lick, the water samples were collected right near the water-spring vents on the bottom of puddle from where the bubbles were rising up. At three salt-licks in Tiang area, the water-spring vent was not detected, and thus the water samples were collected randomly from the puddle. The water of streams near each salt-lick were also collected. The streams were at a distance of 10–20 m away from each salt-lick, but they were not affected by the water from salt-licks. Nine or 10 samples were collected using a syringe (Nipro syringe 20 ml 08753, Nipro corporation, Osaka, Japan) at each salt-lick and stream. All the samples were filtered through a syringe filter of 0.20 µm pore size (Sartorius, Minisart 17597, Sartorius AG, Goettingen, Germany) to remove debris and particulates. The concentration of sodium, potassium, magnesium, and calcium in the water samples were measured using Atomic Absorption Spectrometer (AAnalyst 800, Perkin Elmer, Shelton, USA).

#### Camera trapping

We placed motion-sensitive infrared-triggered digital cameras at the four salt-licks between 25 February 2014 to 24 February 2016 (730 days). At each of Tersau, Tiang A, and B salt-lick, three cameras were deployed. At Tiang C salt-lick, two cameras were deployed. The models of the cameras used in this study were Trophy Cam HD 2013 (Bushnell Outdoor Products, Kansas, USA), Stealth Cam Sniper Shadow STC-SNX1 (Stealth Cam LLC, Texas, USA) and TREL 20J (GISupply Co. Ltd., Hokkaido, Japan). The cameras were deployed originally to record the behaviors of Malayan tapirs in the other study by the present authors (Tawa et al. 2021). We used these cameras in video mode and collected 1-min-long videos per trigger event. The triggering interval was a minimum of 20 seconds. All camera units were mounted on trees at approximately 1 m above the ground to record medium- to large-sized mammals. Two or three cameras were placed at each salt-lick to cover a large portion of the open area; however, some parts of each salt-lick were often not adequately covered due to issues with the faulty cameras, SD memory cards, or batteries.

In this study, videos recorded continuously at intervals of 30 minutes or less were considered to be not independent and counted as “one capture” to avoid repetitive count of many images by continuous shooting during a single visiting event by same animals. Even if multiple cameras at the same salt-lick recorded an animal and/or group of animals walking into the salt-lick, the video records of this event were counted as “one capture”, regardless of the

group size. The capture frequencies (the number of captures per day with active camera) of each studied salt-lick and each species were calculated.

To analyse the relationship between mineral concentrations and frequency of salt-lick visit by medium-large mammals, we compared the mineral concentration in the salt-lick water and the capture frequencies of animals among the three salt-licks closely located each other in Tiang area excluding the salt-lick in Tersau area separated by a high way from other studied salt-licks.

### Statistical analyses

Wilcoxon rank sum test was used to compare the mineral concentration between the water from salt-licks and nearby streams. Steel-Dwass test was used to compare the mineral concentration among the three salt-licks at Tiang area. Fisher's exact test was used to compare the record frequency of mammals among three salt-licks at Tiang area. These tests were computed in R version 4.2.0. For all tests, a p-value of  $< 0.05$  was taken to indicate statistical significance. Data are presented as the mean  $\pm$  standard deviation (*SD*).

## Results

### Mineral concentration of the salt-lick water and stream water

Table 1 compares the mineral concentrations of each salt-lick water and those of stream water in each season. The sodium concentration was significantly higher at all four salt-licks studied than the average of stream water both in dry and rainy season (Wilcoxon rank sum test, p-values are shown in Table 1). The calcium concentration was also significantly higher at the most salt-licks than the stream water in both seasons, except at Tiang A in rainy season. On the other hand, the potassium and magnesium concentrations were not always significantly higher at the salt-licks than the stream water. Thus, we focused on the concentrations of sodium and calcium in the rest of our analyses.

Table 1

Mineral concentration of each salt-lick and the average of all streams. The asterisks (\*) indicate that the mineral concentration of the salt-lick was higher than that of all stream sites at  $P < 0.05$ ; double asterisks (\*\*) at  $P < 0.001$  (Wilcoxon rank sum test)

		Area	Salt lick (ppm)	(Range, N)	Ave. of streams (ppm)	(Range, N)	Test results
Na	Dry season	Tersau	38.30 ± 7.97**	(24.36–45.55, n = 10)	5.96 ± 2.11	(2.81–10.57, n = 39)	W = 390, P < 0.001
		Tiang A	11.00 ± 1.22**	(10.17–14.05, n = 10)			W = 384, P < 0.001
		Tiang B	41.02 ± 31.90**	(11.22–79.01, n = 9)			W = 351, P < 0.001
		Tiang C	79.19 ± 3.87**	(73.28–84.85, n = 10)			W = 390, P < 0.001
	Rainy season	Tersau	36.53 ± 7.75**	(26.38–44.47, n = 10)	5.85 ± 5.72	(2.25–24.95, n = 40)	W = 400, P < 0.001
		Tiang A	10.66 ± 9.18*	(4.69–27.75, n = 10)			W = 320, P = 0.003729
		Tiang B	13.19 ± 12.82*	(5.74–46.38, n = 9)			W = 300, P = 0.002015
		Tiang C	45.00 ± 12.01**	(31.49–71.28, n = 10)			W = 400, P < 0.001
K	Dry season	Tersau	2.13 ± 0.07	(2.01–2.20, n = 10)	2.34 ± 0.25	(1.90–2.67, n = 39)	W = 102.5, P = 0.02243
		Tiang A	3.13 ± 0.09**	(2.99–3.28, n = 10)			W = 390, P < 0.001
		Tiang B	8.86 ± 19.14	(0.93–59.69, n = 9)			W = 125.5, P = 0.1909
		Tiang C	6.20 ± 1.54**	(3.95–8.42, n = 10)			W = 390, P < 0.001
	Rainy season	Tersau	1.80 ± 0.08	(1.68–1.90, n = 9)	2.03 ± 0.35	(1.45–2.64, n = 40)	W = 90, P = 0.02075
		Tiang A	2.66 ± 1.05	(1.54–4.18, n = 10)			W = 260, P = 0.1487
		Tiang B	9.19 ± 24.00	(0.51–77.45, n = 10)			W = 140, P = 0.1487
		Tiang C	41.16 ± 38.17**	(4.06–77.48, n = 10)			W = 400, P < 0.001
Mg	Dry season	Tersau	0.21 ± 0.04	(0.16–0.29, n = 10)	0.94 ± 0.64	(0.17–2.04, n = 39)	W = 44.5, P < 0.001

		Area	Salt lick (ppm)	(Range, N)	Ave. of streams (ppm)	(Range, N)	Test results
		Tiang A	2.52 ± 0.52**	(1.99–3.10, n = 10)			W = 384, P < 0.001
		Tiang B	1.80 ± 0.94*	(0.25–3.02, n = 9)			W = 274, P = 0.009617
		Tiang C	1.25 ± 0.27	(0.86–1.63, n = 10)			W = 246, P = 0.21
	Rainy season	Tersau	0.26 ± 0.11	(0.16–0.44, n = 10)	1.50 ± 0.77	(0.26–2.59, n = 40)	W = 41, P < 0.001
		Tiang A	1.18 ± 0.60	(0.55–2.13, n = 10)			W = 146.5, P = 0.1985
		Tiang B	2.40 ± 1.09*	(0.79–3.62, n = 10)			W = 305, P = 0.01123
		Tiang C	2.03 ± 2.55	(0.45–8.70, n = 10)			W = 206, P = 0.8938
Ca	Dry season	Tersau	3.69 ± 0.46**	(2.69–4.29, n = 10)	1.20 ± 0.74	(0.22–2.05, n = 39)	W = 390, P < 0.001
		Tiang A	2.78 ± 0.14**	(2.60–2.95, n = 10)			W = 390, P < 0.001
		Tiang B	2.61 ± 0.95**	(1.19–3.81, n = 9)			W = 309, P < 0.001
		Tiang C	3.75 ± 0.53**	(3.16–4.51, n = 10)			W = 390, P < 0.001
	Rainy season	Tersau	3.33 ± 0.51**	(2.65–3.86, n = 10)	1.72 ± 0.90	(0.23–3.01, n = 40)	W = 390, P < 0.001
		Tiang A	1.54 ± 1.02	(0.51–3.15, n = 10)			W = 200.5, P = 1
		Tiang B	3.36 ± 1.27*	(1.44–5.72, n = 10)			W = 333.5, P = 0.001212
		Tiang C	4.78 ± 3.10**	(2.70–12.79, n = 10)			W = 388.5, P < 0.001

The relationship between mineral concentration and capture frequency of mammals

Table 2 shows the number of active cameras, captures with mammal records, days with active camera (total days when at least one camera was active at each studied salt-lick), and the capture frequency at each salt-lick. The mammal species most frequently recorded at the studied salt-licks was red muntjac (41.7% of all animal captures, 0.633 captures / day), followed by sambar (*Rusa unicolor*, 20.8%, 0.317 captures / day), Malayan tapir (15.1%, 0.230 captures / day), and white-thighed langur (*Presbytis siamensis*, 5.4%, 0.082 captures / day), as reported in Tawa et al. (2022). The red muntjacs and the Malayan tapirs were recorded at all the four studied salt-licks, while

the sambar and white-thighed langur were distributed only in RBSP and recorded at three salt-licks in Tiang area. The percentage of red muntjacs, sambars, Malayan tapirs and white-thighed langur in the total animal captures at the three studied salt-licks in Tiang area was 41.0%, 27.0%, 10.6%, and 7.0%, respectively.

Table 2

The number of active cameras, captures with mammal records, days with active camera, and capture frequency. Cameras were deployed between February 2014 – February 2016

	Number of active cameras	Number of captures with mammal records	Number of days with active cameras <sup>a</sup>	Capture frequency <sup>b</sup>
Tersau	1–3	614	537	1.14
Tiang A	1–3	781	507	1.54
Taing B	1–3	685	498	1.38
Tiang C	1–2	599	221	2.71
Total	1–11	2,679	1,763	1.52
<sup>a</sup> Total days when at least one camera was active at each studied salt-lick				
<sup>b</sup> The number of captures per day with active camera				

Figure 2 compares the mineral concentrations of each salt-lick water among three salt-licks in Tiang area, which are closely located each other and the mammals in this area might easily access all of three salt-licks. Tiang C salt-lick showed significantly higher sodium concentration than Tiang A and B salt-licks (Steel-Dwass test, Tiang C-Tiang B:  $t = 3.685$ ,  $P = 0.0013$ ; Tiang C-Tiang A:  $t = 5.413$ ,  $P < 0.001$ ). Tiang C salt-lick also showed the highest calcium concentration among the three salt-licks, however, the difference was only significant between Tiang C and Tiang A (Steel-Dwass test,  $t = 4.708$ ,  $P < 0.001$ ).

The record frequency of mammals with each food-habit was compared among the three salt-licks in Tiang area (Fig. 3). The herbivores were recorded significantly more frequently at Tiang C salt-lick (590 captures, 2.67 captures / day) with the highest sodium concentration than at Tiang A and B salt-licks (Fisher's exact test,  $P < 0.001$  for both pairs of Tiang C-Tiang B and Tiang C-Tiang A), while omnivores and carnivores showed no such tendency. The omnivores were recorded significantly more frequently at Tiang A salt-lick (39 captures, 0.077 captures / day) than Tiang B and C. The carnivores were recorded most frequently at Tiang B salt-lick (16 captures, 0.032 captures / day), though there was no significant difference among these salt-licks in Tiang area (Fig. 3).

The record frequency of four herbivorous species which were recorded most frequently at the salt-licks in Tiang area, red muntjac, sambar, Malayan tapir, and white-thighed langur were also compared (Fig. 3). The red muntjacs and the sambars were recorded significantly more frequently at Tiang C salt-lick (352 captures, 1.59 captures / day, and 157 captures, 0.71 captures / day, respectively) than Tiang A and B salt-licks (Fisher's exact test,  $P < 0.001$  for both pairs of Tiang C-Tiang B and Tiang C-Tiang A), according with the difference in sodium concentration. The Malayan tapirs, however, showed different tendency from these two herbivores; they were recorded at Tiang B salt-lick significantly more frequently (163 captures, 0.327 captures / day) than Tiang A and C salt-licks (Fisher's exact test,  $P < 0.001$  and  $P = 0.006548$ , respectively). In addition, the white-thighed langurs were also significantly less



recorded at Tiang A salt-lick than at Tiang B and C salt-licks (Fisher's exact test,  $P < 0.001$  for both pairs) and there was no significant difference in the capture frequency between at Tiang B and Tiang C (Fisher's exact test,  $P = 0.1304$ ).

## Discussion

Comparison of mineral concentration between salt-lick water and stream water in this study suggested that the herbivorous mammals could supplement sodium and/or calcium by drinking water at the studied salt-licks; the concentration of sodium in salt-lick water was higher than that of stream water regardless of seasons or sites, and that of calcium was also higher in most cases. On the other hand, those of potassium and magnesium in salt-lick water were not necessarily higher than the stream water, suggesting that animals could not supplement these minerals by drinking the salt-lick water (Table 1).

Some previous studies on wet-type salt-licks also reported the higher mineral concentration (including sodium and calcium) in the salt-lick water than the control-site water in various regions (Table 3). For example, Clayton and MacDonald (1999) who studied wet-type salt-licks visited by babirusa (*Babyrusa babyrussa*) in Sulawesi Island, Indonesia reported higher concentration of sodium and calcium in the salt-lick water than control water. Matsubayshi et al. (2007a) who studied wet-type salt-licks in Borneo Island, Malaysia also reported higher mineral concentration (sodium, potassium, magnesium, and calcium) of the salt-lick water. Besides, they compared animal visit between two adjacent salt-licks at a distance of only 16 m and reported that the sambars and bearded pigs (*Sus barbatus*) visited the salt-lick with higher concentration of all four minerals more frequently than the other.

Table 3

Concentration of cations in water-drinking type salt-licks. The unit of mineral concentration is ppm. The asterisks (\*) indicate that the mineral concentration of the salt-lick was higher than that of control sites at  $P < 0.05$  (<sup>1</sup>t-test, <sup>2</sup>Mann-Whitney U test, <sup>3</sup>Wilcoxon rank-sum test)

			Na	(range)	K	(range)	Mg	(range)	Ca	(range)
Matsubayashi et al. (2007a), Borneo, Malaysia <sup>1</sup>	salt-lick	(n = 59)	801.8 ± 1173.5*	(38.7–2710.2)	14.4 ± 12.6*	(6.8–29.8)	21.4 ± 9.8*	(13.6–35.1)	83.4 ± 50.0*	(41.7–155.9)
	control	(n = 18)	6.9 ± 2.4	(4.6–8.7)	1.6 ± 0.6	(1.5–1.8)	2.7 ± 1.0	(2.3–3.1)	13.8 ± 8.5	(5.6–20.4)
Clayton and MacDonald (1999), Sulawesi, Indonesia	salt-lick	(n = 1)	259	-	1.0	-	0.1	-	90	-
	control	(n = 1)	7.1	-	< 0.1	-	6.1	-	25	-
Owen et al. (2014), Maine, USA	salt-lick	(n = 2)	12.6	(9.4–15.7)	1.6	(1.0–2.3)	1.1	(0.6–1.6)	4.5	(3.9–5.0)
	control	(n = 2)	1.4	(0.9–1.9)	0.3	(0.2–0.3)	0.7	(0.5–0.9)	4.1	(3.0–5.1)
Couturier and Barrette (1988), Quebec, Canada <sup>2</sup>	salt-lick	(n = 17)	172.5 ± 97.1*	(73–472)	-	-	-	-	-	-
	control	(n = 10)	3.6 ± 2.6	-	-	-	-	-	-	-
Bechtold (1996), British Columbia, Canada <sup>1</sup>	salt-lick	(n = 20)	107.8 ± 119.7*	-	2.7 ± 3.3*	-	10.0 ± 12.0*	-	29.3 ± 32.2	-
	control	(n = 20)	2.1 ± 2.1	-	0.5 ± 0.4	-	4.4 ± 3.7	-	17.4 ± 12.2	-
this study <sup>3</sup>	salt-lick	(n = 79)	34.5 ± 25.2*	(4.7–84.9)	9.5 ± 20.7*	(0.5–77.5)	1.5 ± 1.3	(0.2–8.7)	3.2 ± 1.5*	(0.5–12.8)
	control	(n = 79)	5.9 ± 4.3	(2.2–25.0)	2.2 ± 0.3	(1.5–2.7)	1.2 ± 0.8	(0.2–2.6)	1.5 ± 0.9	(0.2–3.0)

Some previous studies also suggested that herbivores visited salt-licks mainly for sodium supplementation. Couturier and Barrette (1988) who studied salt-lick use by moose (*Alces alces*) in Matane Wildlife Reserve, Quebec, Canada reported that among the four wet-type salt licks where they observed moose, the one that had the most visits also had the highest sodium content. Moe (1993) who studied salt-licks in Bardia National Park, Nepal showed that the concentration of minerals except sodium was not higher in the soil of dry-type salt-licks, where

animals consume mineral-rich soils, than control soils. He also showed positive correlation between the sodium concentration and visit frequency by axis deer (*Axis axis*) using the data from 12 dry-type salt-licks in the study area. Holdø et al. (2002) who studied African elephants (*Loxodonta africana*) in a Kalahari-sand habitat in Hwange National Park, Zimbabwe analysed the mineral concentration of the food plants, drinking water, and soils of dry-type salt-licks the elephants ate. They estimated that the elephants could not take minimum amount of sodium for their requirements from the food plants and drinking water while they could take enough amount of calcium and magnesium. They also reported that the salt-lick soils contained higher concentration of sodium than other soils, while the calcium and magnesium concentrations of salt-lick soils were lower than the food plants and drinking water, indicating that elephants supplement sodium, not calcium and magnesium, from salt-lick soils.

On the other hand, it is also reported that the salt-licks have an important role of supplementing not only sodium but also other minerals such as calcium for animals. Atwood and Weeks (2003), who studied the salt-lick use by white-tailed deer (*Odocoileus virginianus*) at Indiana, USA, reported that females visited salt-licks with various mineral contents more frequently than salt-licks with only sodium, pointing out that females during lactation might need minerals such as calcium and phosphorus in addition to sodium.

The calcium concentration in salt-lick water ( $3.24 \pm 1.54$  ppm, range 0.51–12.79,  $n = 79$ ) analysed in this study, however, was much lower in absolute values compared to those that reported by these previous studies (Clayton and MacDonald 1999: 90 ppm; Matsubayashi et al. 2007a:  $83.4 \pm 50.0$  ppm, Table 3). Therefore, the supplementation of sodium seems more important than that of calcium for animals that visited the salt-licks studied in this study. It cannot be denied, however, that the animals supplement some minerals other than sodium which were not measured in this study but critical for herbivore nutrition.

In this study, the herbivores (especially the red muntjacs and sambars, which was the main users of the salt-licks) most frequently visited the Tiang C salt-lick with the highest sodium concentration among the three salt-licks in Tiang area, while the omnivores and carnivores didn't show such tendency. On the other hand, the calcium concentration of the salt-lick water at Tiang C was not always higher than other salt-licks. The fact also suggests that sodium is more important than calcium in their mineral supplementation at the salt-licks. We cannot eliminate, however, the possibility that the animals supplement calcium at the salt-licks, because calcium concentration in the salt-lick water was higher than that in the stream water in most cases.

In contrast to the red muntjacs and sambars, the Malayan tapirs and the white-thighed langur, the third and fourth most frequent herbivorous visitors to the studied salt-licks in Tiang area, did not visit Tiang C with the highest sodium concentration most frequently. The Malayan tapirs visited Tiang B more frequently than Tiang A and C while sodium concentration at Tiang B was significantly lower than Tiang C. The result indicates that not all the herbivore species necessarily visited the salt-lick with higher mineral concentration more frequently. Thus, other factors which may vary with species could influence the frequency of salt-lick visit by animals in addition to the mineral concentration.

The present authors studied the behavior of wild Malayan tapirs around the same salt-licks as this study, and observed male-female interaction such as chasing and vocalization, suggesting that they used the salt-licks as a place to meet potential mating partners in addition to drink water there (Tawa et al 2021). Thus, such social interaction can affect selection of the salt-licks by tapirs. Matsubayashi et al. (2011), who studied the use of salt-licks by orangutans, also reported that multiple individuals such as two pairs of females with infants were recorded

by sensor cameras in the same frame, suggesting that the salt-licks have secondary function as a communication site for them.

Lazarus et al. (2019) conducted camera trapping surveys at the same three salt-licks in Tiang area as this study. Their results on the record frequency of red muntjac was not consistent with ours, while the results on sambar and Malayan tapir were consistent with this study. They reported that the red muntjacs were recorded significantly more frequently at Tiang A than other two salt-licks based on the research of six months (the exact survey date was not described), while in this study the red muntjacs were estimated to visit Tiang C most frequently, followed by Tiang A and Tiang B based on the research of two years. Although they did not conduct mineral analysis of salt-lick water, they discussed that the red muntjacs might preferred Tiang A because of the thick, dense underbrush and vines around Tiang A where they could quickly hide from predators. They also speculated that the sambars were observed significantly more frequently at Tiang C because they can visit there in group due to the large open area in front of the water source in Tiang C.

Although it is still not clear why their results on red muntjacs are different from ours, the difference in survey season and studied period, however, could cause the difference because the red muntjacs could seasonally change the visit frequency to the salt-licks. In addition, the physiological demand for minerals could also seasonally change by pregnancy and lactation for example. Holdø et al. (2002) reported that the females elephants spent more time than males for soil-eating at the dry-type licks. In Borneo, Matsubayashi et al. (2007b) reported that female sambars visited the wet-type salt-lick more frequently in rainy season than dry season while visit frequency of males did not seasonally change. Couturier and Barrette (1988) showed that the various age-sex (yearling or adult, male or female) classes had different seasonal patterns of salt-lick use, suggesting possible differences in mineral needs related to molting, antler grows, calf growth, lactation, or estrus.

Some previous studies indicated that the seasonal changes in visit frequency to salt-licks are also relevant to the needs for alleviation of gastrointestinal problems, as shown in Ayotte et al (2008). In the other study by the present authors, the red muntjacs and sambars visited the salt-lick significantly more frequently than the annual mean in April, the beginning of rainy season (Tawa et al. 2022). If the sprouting season in this area mainly occur in the beginning of the rainy season, it is possible that these deer species visited the salt-licks more frequently to alleviate gastrointestinal acidosis caused by their seasonal dietary change, while the concentration of bicarbonate in salt-lick water was not analysed.

Although we did not examine the effects of factors other than mineral concentration of the salt-lick water in this study, many other factors, such as topography, vegetation, predation risk and social interaction at the salt-licks could affect the pattern of animal visitation to the salt-licks. The effect of the difference in topography and vegetation, however, seemed small in our results because no remarkable differences in these factors were not observed among the studied salt-licks in Tiang area, which were located in close proximity to each other.

As for predation risk, Griffiths et al. (2020), who conducted camera trapping survey at 52 salt-licks in the northeastern Peruvian Amazon, showed that three mammals (the paca *Cuniculus paca*, Brazilian porcupine *Coendou prehensilis*, and red brocket deer *Mazama americana*) were less likely to visit salt-licks during nights with bright moon probably due to a heightened risk of predation at the salt-licks when visibility was better for predators. In this study site, the present authors recorded some carnivores: the tiger (*Panthera tigris jacksoni*) and the dhole (*Cuon alpinus*) etc. at the salt-licks using sensor cameras (Tawa et al. 2022). Although the lunar cycle seems not to be related to the difference in visit frequency of herbivorous species among three salt-licks in Tiang area because

of the same study period, it remains to be studied whether the visit frequency of prey mammal is affected by lunar cycles also in this study area.

Mineral analyses of the salt-lick water by this study showed that the wet-type salt-licks studied in BTFC could provide the sodium and/or calcium supplementation for the herbivorous mammals. Especially, the high concentration of sodium was consistent with the hypothesis that the herbivorous mammals visit the salt-licks mainly for sodium supplementation. However, the results on relationship between the mineral concentration of salt-lick water and visit frequency by mammals suggest that the factors other than sodium concentration might also influence the selection of salt-licks by each species.

## Declarations

**Acknowledgements** – We wish to place on record our sincere thanks to Pulau Banding Foundation for supporting the fieldwork and the administrative proceedings of the research permissions within Belum-Temengor Forest Complex. We also thank all the staff of Wildlife Research Center of Kyoto University for providing suitable advice.

**Funding** – This study was supported by JSPS Core-to-Core Program (S.K., JPJSCCA20170005, Wildlife Research Center of Kyoto University); JSPS Program for Leading Graduate Schools, “Leading Graduate Program in Primatology and Wildlife Science”; JSPS International Training Program (ITP-HOPE); and JSPS Institutional Program for Young Researcher Overseas Visits (AS-HOPE).

**Permits** – We conducted this work under permission from Economic Planning Unit of Malaysia (UPE: 40/200/19/3078).

**Conflicts of interest/Competing interests** – The corresponding author confirms on behalf of all authors that there have been no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

**Availability of data and material** – The datasets analysed during the current study are available from the corresponding author on reasonable request.

**Code availability** – Not applicable

**Authors' contributions** – The first author conceptualized the study, conducted project administration, considering methodology, investigation, data curation, formal analysis, visualization, and wrote the initial draft of the manuscript. The second author carried out the necessary procedures for mineral analysis of water samples and provided the resources for mineral measurement using Atomic Absorption Spectrometer. He also contributed to data curation, formal analysis, consideration of methodology, and validation, and critically reviewed the manuscript. The last author is the supervisor of the study and contributed to funding acquisition, interpretation data and critically reviewed the manuscript. All authors approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**Ethics approval** – The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as there was not sample collection from animals or humans.

Consent to participate – Not applicable

Consent for publication – Not applicable

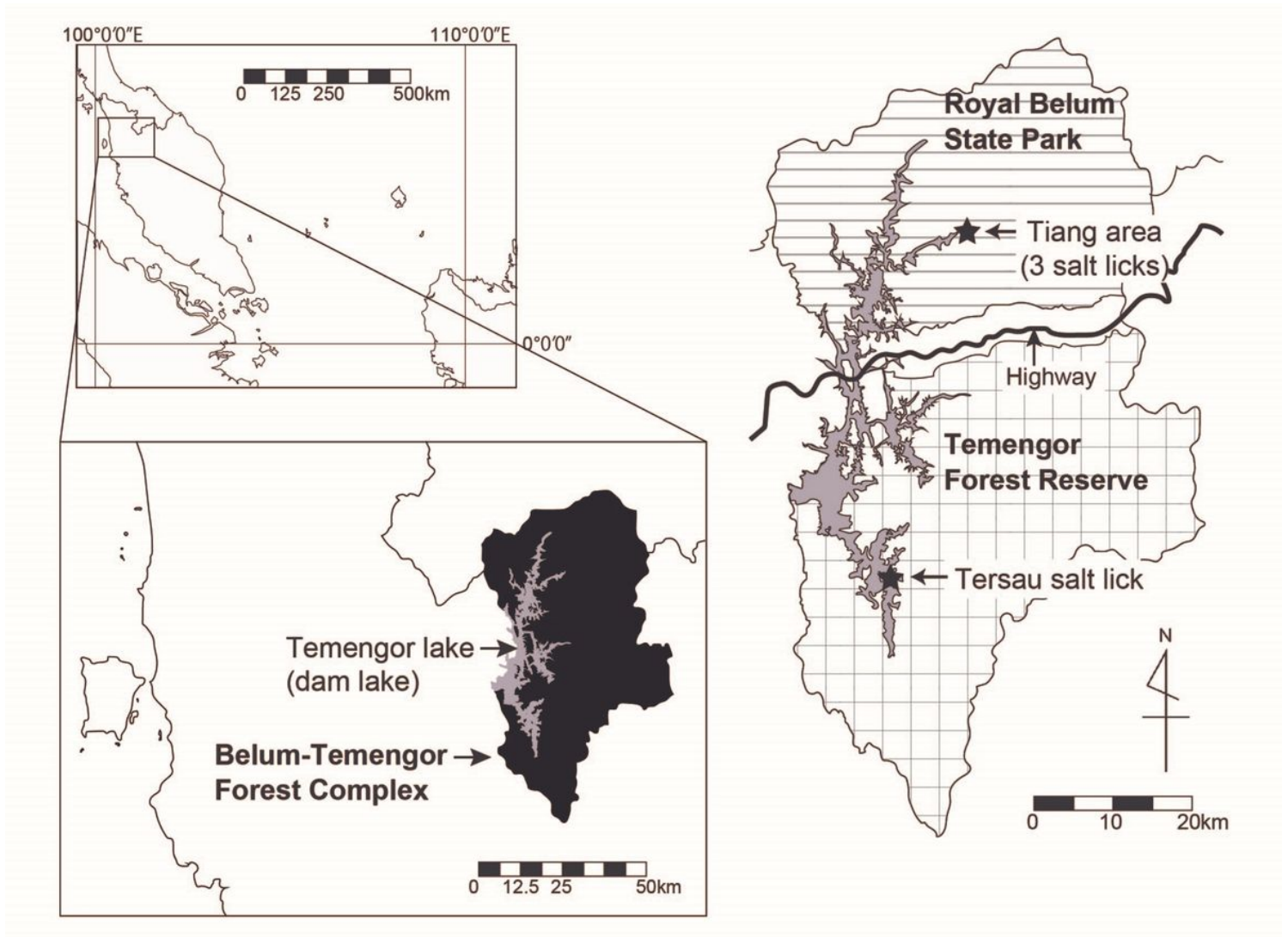
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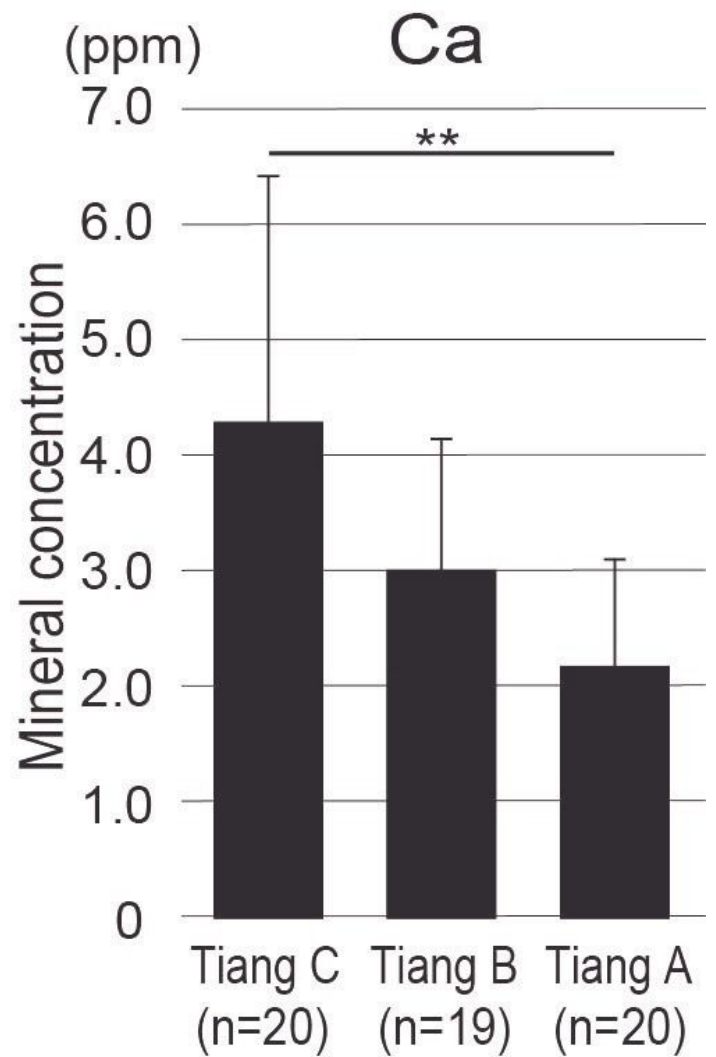
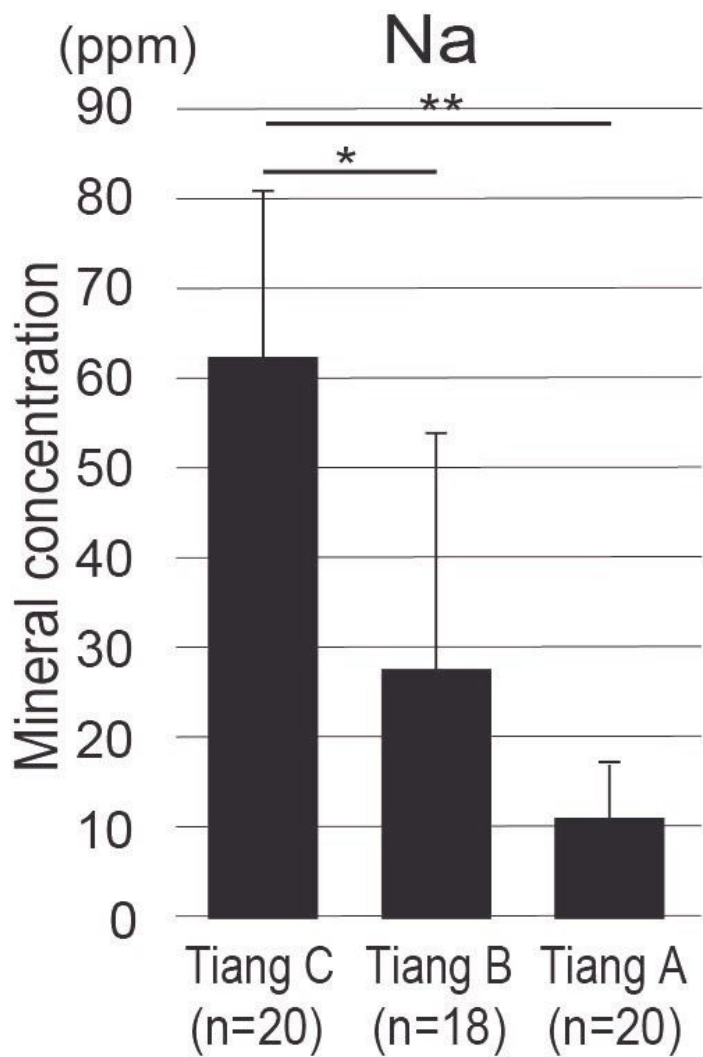
## Figures



**Figure 1**

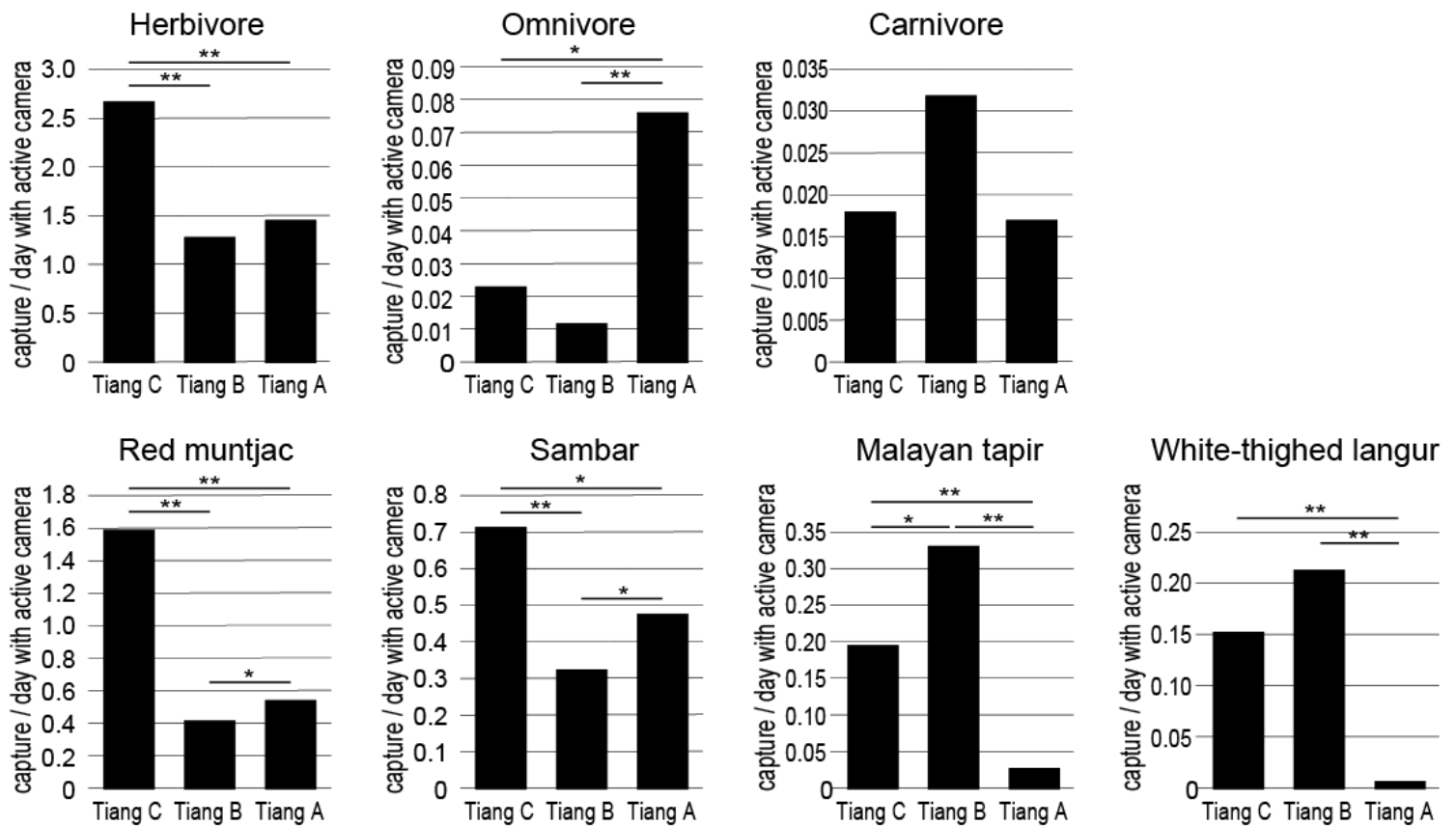
Location of Belum-Temengor Forest Complex (BTFC). The area of BTFC is about 320,000 ha which includes 17,200 ha Temengor Lake. The stars show the location of the Tersau salt-lick and Tiang area with 3 studied salt-licks, respectively





**Figure 2**

Comparison of mineral concentration in the water of three salt-licks at Tiang area in RBSP. The graphs are shown in order of the average sodium concentration. The asterisks (\*) indicate significant differences at  $P < 0.05$ ; double asterisks (\*\*) at  $P < 0.001$  (Steel-Dwass test).



**Figure 3**

Comparison of record frequency of mammals among three salt-licks at Tiang area in RBSP. The graphs are shown in order of the average sodium concentration. The asterisks (\*) indicate significant differences at  $P < 0.05$ ; double asterisks (\*\*) at  $P < 0.001$  (Fisher's exact test).