**Supplementary Results**

**Title:**

**The tail-tale of stress: An exploratory analysis of cortisol levels in the tail-hair of captive Asian elephants**

Sanjeeta Sharma Pokharel1, 2, \*, Hiroki Yoneda3,MoeYanagi2, Raman Sukumar1, 4, Kodzue Kinoshita2,\*

1Centre for Ecological Sciences, Indian Institute of Science, Bangalore-560012, India.

2Wildlife Research Center, Kyoto University, Kyoto, Japan.

3Kyoto City Zoo, Kyoto, Japan.

4Institute for Advanced Study, Kyoto University, Kyoto, Japan.

Correspondence and requests for materials should be addressed to SSP and KK (email: pokharelsanjeeta@gmail.com and kinoshita.kodzue.8v@kyoto-u.ac.jp)

**Detailed results of hC levels in each study elephant:**

**Mito (a 48-year old female/ KCZ)** The mean monthly tail-hair growth rate was 15.56 (4.19) mm for Mito (individual hair samples were up to 40.0 cm long). The tail hC levels in Mito were extracted by both grinding and manually mincing the samples, thus there were 22 powdered sub-samples representing 22 previous months from the month of sample collection (April, 2018) and 14 minced sub-samples representing 24 previous months from the month of sample collection (May, 2018; refer to Table 1).

*Powdered samples.* The overall mean hC level (in powdered samples) in Mito was 58.55 (106.01) ng/g. The hC levels ranged from 4.53 ng/g to 485.12 ng/g. There were five distinct behavioural records (marked as a, b, c, d and e) reported for Mito (Fig. 2; Table 2). As per the records, during July-2016 (a), she was observed being reluctant to enter her enclosure and the hC level measured for this period (based on the hypothesis that each tail-hair segment corresponds to previous months) was found to be 6.58 ng/g. From hair segments corresponding to August to September 2016 (b), the hC levels were found to increase from 5.92 to 100.45 ng/g and during these periods, Mito continued to show reluctance in entering her enclosure, showed signs of anaemia, the bleeding toe (trimmed and treated) and interaction (indirect) was initiated with four new elephants (she displayed slight aggression). Hair segments corresponding to November-December 2016 (c) showed an increase in hC levels from 101.57 to 205.27 ng/g and this was the period when she suffered from the colic infection. Subsequently, after the severe colic infection, hair segments corresponding to January-March (2017; d) had the tenfold increase in the hC levels from 45.91 to 485.12 ng/g and as per the records, interaction of Mito with other four new elephants was also initiated during this period. The hC levels of hair segments corresponding to April-2017 (e) decreased to 60.09 ng/g and during this period, the protected contact trials were initiated between Mito and four new elephants. The hC levels after this segment gradually declined from 22.78 ng/g (May-2017) to 9.84 ng/g (April-2018; see Fig. 2). There were no unique biological events recorded during these periods.

*Minced samples.* The overall hC levels assessed in Mito were relatively lower (mean = 10.88 (7.68) ng/g) in manually minced segments than in pulverized samples (mean = 58.55 (106.01) ng/g; Refer to Supplementary Fig. 4). The hC levels ranged from 2.6 ng/g to 33.39 ng/g. There were two events corresponding to the hC levels (c, and d & e; refer to Fig. 2 and Fig. 3). The hC levels in the hair segments relating to the months of November-December 2016 (c) were 14.41 ng/g associated with colic infection along with anorexia in Mito and 14.40 ng/g in the months of March-April 2017 (d & e) when the interaction between Mito and four new elephants were initiated (Table 1). Beside these, there were a few peaks of hC levels observed during the months of June-July (2017; hC level = 12.01 ng/g), January-February (2018; hC level = 6.44 ng/g) and May (2018; hC level = 33.39 ng/g; Fig. 3, Table 2).

**Tonkun (a 10-year old female/ KCZ)** The mean monthly tail-hair growth rate in Tonkun was 10.83 (0.83) mm (individual hair samples were up to 5.7 cm long; Table 1). Therefore, there were 5 segments (pulverized) from Tonkun corresponding to five previous months. The overall mean hC level was 53.62 (65.96) ng/g and the level ranged from 10.06 ng/g to 166.72 ng/g. There was one distinct biological event reported for Tonkun during November-2017 (f) where she showed the signs of estrous (during early November) and also suffered from the skin defects on the septum of nostril. During this period, the hC level in the corresponding segment was the highest (166.72 ng/g; Fig. 2, Table 2). There were no other distinct events recorded for Tonkun during these five months.

**Bunnyun (a 7-year old female/ KCZ)** The mean monthly tail-hair growth rate in Bunnyun was 13.61 (1.27) mm (individual hair samples were up to 8.5 cm long; Table 1). Based on this monthly growth rate and length of tail-hair samples, the tail-hair from Bunnyun was divided into 6 hair segments (pulverized) representing six previous months. Out of all five individuals of KCZ, Bunnyun had the lowest levels of hC (Fig. 2). The overall mean hC level was 5.03 (7.58) ng/g. The hC levels ranged from 1.25 ng/g to 20.45 ng/g. There was one distinct biological event (g) recorded during November (2017) where Tonkamu (a 6 year old male) continuously followed Bunnyun and Kampart. During this period, Bunnyun showed the highest level of hC (20.45 ng/g; Fig. 2, Table 2). Other than the following behaviour by Tonkamu, there were no other distinct behaviours observed in Bunnyun during these six months.

**Kampart (a 8-year old female/ KCZ)** The mean monthly tail-hair growth rate in Kampart was 12.78 (0.96) mm (individual hair samples were 6.5 cm long; Table 1). There were five hair segments (pulverized) from Kampart corresponding to five previous months from the date of sample collection. The overall mean hC level was 96.41 (43.22) ng/g. The hC levels ranged from 42.04 ng/g to 151.81 ng/g. There were three unique behavioural events recorded for Kampart (marked as ‘h’, ‘i’ and ‘j’ in Fig. 2 and Table 2). During December (2017) to January (2018), Kampart suffered from oral sores and peri-anal lesions reflecting the highest hC levels, *i.e*. 127.10 ng/g to 151.81 ng/g. The hC levels during January-February (2018) lowered from 151.81 ng/g to 81.30 ng/g, when all the five elephants from KCZ were trained to share the ground together. During March (2018), hC level was 79.82 ng/g as she suffered from skin abrasion and slight swelling on the left forelimb. All these levels were quite higher than the mean tail hC levels in Kampart.

**Tonkamu (a 6-year old male/ KCZ)** The mean monthly tail-hair growth rate in Tonkamu was 20.00 (1.92) mm (individual hair samples were up to 23.0 cm long; Table 1). There were 11 hair segments (pulverized) representing 11 previous months. The overall mean hC level was 36.27 (39.47) ng/g. The hC levels ranged from 7.15 ng/g to 131.21 ng/g. There were two unique behavioural records (marked as ‘k’ and ‘l’) reported for Tonkamu which corresponded with peak hC levels (Fig. 2 and Table 2). During October-November 2017 (k), Tonkamu showed ‘following’ behaviour towards Bunnyun and Kampart and the hair segments corresponding to this period had hC levels ranging from 131.21 ng/g to 25.57 ng/g. The period between January-February 2018 (l), hC levels increased from 13.90 ng/g to 23.96 ng/g as Tonkamu suffered from swelling and infection in his nostril.

**Zuze (a 28-year old female/ KOZ)** The mean monthly tail-hair growth rate in Zuze was 19.40 (0.015) mm (only two hair strands were marked and measured for daily growth rate; individual hair strands were up to 37 cm; Table 1). There were 12 hair segments (minced) from Zuze corresponding to 12 previous months. The overall mean hC level was 9.28 (6.31) ng/g and the level ranged from 3.43 ng/g to 23 ng/g. There were two unique behavioural records associating with two peak values (marked as ‘m’ and ‘n’ in Fig. 3 and Table 2). The hC levels significantly lowered from 12.07 ng/g to 7.85 ng/g during March-2017 (combined months November-2016 to March-2017) to April-June 2017, this was the period when Zuze’s calf was brought back to the KOZ. Interestingly, the hC levels corresponding to months July- September 2017 sharply increased from 8.33 ng/g to 15.70 ng/g and this was when her calf was taken back to the previous zoo. The peak from September-2017 continued to increase from 15.70 ng/g to 16.87 ng/g (October 2017) and finally, to 23.00 ng/g (the highest value observed for Zuze in the month of November 2017; Fig. 3). This peak drastically declined to 6.37 ng/g in the month of December-2017. There was a slight surge in hC level observed in the month of February 2018 (6.06 ng/g), however there were no associated behavioural records.