

# Animal welfare in captive Japanese macaques (*Macaca fuscata*) and pygmy slow lorises (*Nycticebus pygmeus*), and human attitudes towards animal memorial ceremonies

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## Summary

The principal objective of this study was to examine how current behavioral management plan strategies improve the lives of primates. In the captive breeding research setting for male Japanese macaques (*Macaca fuscata*) by providing vegetated-enclosures, and in conservation-zoo settings, how female pygmy slow lorises (*Nycticebus pygmeus*) fair when socially housed, and lastly how the memorial ceremonies that pay tribute to the dead animals and the humans that care of them are perceived.

In chapter 1, I introduce the concept of animal welfare that includes a brief discussion of its history and recent advancements. I discuss why it is important to take into account socioecological and stress related factors when housing primates. I establish the need for determining if multiple non-invasive methods of quantifying welfare states in primates show any differences in these indices such as behavioral (activity budgets, affiliative behaviors, stereotypic behaviors), physical (hair loss), and physiological parameters (fecal cortisol) in the relatively recent implementation of improved environments and husbandry care. Additionally, with improvements to captive environments of primates, there has also been significant advancements in animal care, especially in research facilities. However, caretakers that devote themselves for the upmost care of their captive animals may come at a cost when the animals die. For this purpose, I set out to find how animal memorial services are perceived by the people who work with them and if they bring any benefits to a management plan.

In chapter 2, I present my findings comparing activity, behaviors, and hair coats in male Japanese macaques (*Macaca fuscata*) living in vegetated vs. non-vegetated enclosures. I conducted observations in two outdoor socially housed groups at the Kyoto University Primate Research Institute (KUPRI, Inuyama, Japan). The first group, Onobora enclosure 7 (40 + 5 individuals) housed in a vegetated enclosure at the KUPRI Research Resource Station (RRS) containing natural ground cover, trees, a pond, perches and climbing structures. The second group, Takahama Group 1 (40 ± 5 individuals), housed in a concrete and non-vegetated outdoor enclosure with metal structures, swings, and some wooden platforms with shelters to protect the animals from the weather at KUPRI main campus. A total of 384 hours of behavioral data were collected for both vegetated (N = 204 hr.) and non-vegetated enclosures (N = 180 hr.). I found that the average time spent in social-play behaviors by immatures was significantly higher than mature males both in the vegetated (U=0, Z=-2.51, P=0.006) and the non-vegetated (U=0, Z=-2.51, P=0.006) enclosure. Both immature (U=1, Z=-2.3, P=0.010) and mature (U=4, Z=1.67, P=0.047) monkeys from the vegetated enclosure spent significantly more time in social-play behaviors than their non-vegetated counterparts. There was no significant difference in hair loss between immature and mature individuals in the vegetated enclosure (U=10.5, Z=0.31, P=0.756), while hair loss was significantly more extensive among mature, than immature, males in the non-vegetated enclosure (U=0, Z=2.51, P=0.012). Hair loss among males was most extreme in the head, followed by back. No statistically significant correlation between male rank and hair loss occurred in the vegetated enclosure (rs= -0.07511, n = 10, P = 0.837), but a strong correlation between rank and hair loss was observed in the non-vegetated enclosure. I showed that male Japanese macaques that live in vegetated enclosures have the closest opportunities to live a life that resembles one in the wild, improving their welfare, when compared to those living in a non-vegetated, large, outdoor enclosures. The self-sustaining vegetated enclosure, assured animals with constant feed in the form of vegetation, which might have helped in meeting satiation and keeping aggressive interactions lower than the monkeys living

in the non-vegetated enclosure which may have to compete more for food sources. I conclude that Japanese macaques should be housed in vegetated enclosures, whenever possible, to improve their welfare.

In chapter 3, I focused on the behavioral and social needs of captive female pygmy slow lorises at the Japan Monkey Center (JMC). I documented the formation of two social groups and their behavioral changes. The first group of two females were introduced together on 25 August 2017, and a group of four individuals placed into one unit together on 19 December 2017. I collected behavioral data in 10 min sampling sessions following Martin and Bateson (1984) and postural data classification following Glassman and Wells (1993). I found that female pygmy slow lorises displayed a high degree of gregariousness when transferred to the group condition (Kruskal–Wallis chi-squared = 10.71,  $df = 2$ ,  $p = 0.0047$ ). Post hoc tests showed that pygmy slow lorises were significantly more likely to spend their time at proximity under a social condition inside their nesting sites (Far – Social:  $z = -3.14$ ,  $p = 0.0051$ ). There were no significant differences between Close – Far ( $z = 2.38$ ,  $p = 0.052$ ; and between Close – Social ( $z = -0.76$ ,  $p = 1.0$ ). They spent on average around 60% of their time at arm's reach (close). There were no significant differences between postural behaviors under different housing conditions (solitary vs. social housing) ( $F(3,3020) = 0.02$ ;  $p = 0.88$ ). Post hoc tests showed that there was a significant decrease in Locomotion/Posture 1 ( $p < 0.001$ ) and a significant increase in Locomotion/Posture 3 ( $p < 0.001$ ), but no significant differences in Locomotion/Posture 2 ( $p = 0.89$ ) or in Locomotion/Posture 4 ( $p = 0.98$ ). My findings show that the formation of all-female groups in pygmy slow lorises is a feasible behavioral management strategy that promotes natural behaviors. A high degree of social behaviors displayed in the group environment challenges early reports of them being highly solitary in the wild. Daily activity budgets can change once placed under an enriched social housing condition. Both substrate complexity and enclosure size seem to mediate what postural behaviors are adopted. I conclude that pygmy slow lorises are a gregarious species that can benefit in captivity if housed more often with conspecifics of both the opposite and same sex, in large enclosures with complex substrates that also allow for a more naturalistic postural behavior repertoire and diet.

In chapter 4, I aimed to examine whether social housing helped in reducing stress by comparing fecal cortisol of 8 female pygmy slow lorises and a case of stereotypic behaviors when housed alone and when with conspecifics. The first female pair was started in October 2016, followed by a second pair in the summer of 2017, and a group of 4 females in September 2017. The collection of fecal samples continued until the summer of 2018, when male–female pairs were formed, that allowed for comparison between male–female vs female–female pairs. All samples were stored in a freezer at  $-20^{\circ}\text{C}$  until preparation and analysis. Hormones were extracted by adding 1.5 ml of 80% methanol to 0.10 g of freeze-dried, pulverized feces and vigorously shaken for 30min at room temperature ( $24^{\circ}\text{C}$ ) at 5,000 r/m. The supernatant was used for hormonal analyses by enzyme immunoassay. The extracts of cortisol metabolites in the supernatant were analyzed using a modified enzyme immunoassay (EIA) method previously used on Japanese macaques and other animals by Takeshita (2018) and Kinoshita (2011). I found that female pygmy slow lorises exhibited statistically significant lower levels of fecal cortisol once socially housed in an enriched enclosure ( $F(1,127) = 3.24$ ,  $p = 0.03$ ). The loris exhibiting stereotypic behaviors from the group of 4, had significantly higher levels of fecal cortisol than most of her cage mates in the single condition ( $F(4,123) = 3.32$ ,  $p = 0.01$ ). I found no significant differences in cortisol levels between female–female ( $n = 6$ ) housing and male–female ( $n = 6$ ) housing ( $W = 12$ ,  $z = 0.65$ ,  $p = 0.39$ ). I found that when female pygmy slow lorises are socially housed in adequate cages, their fecal cortisol levels decrease over time, demonstrating the benefits of improved welfare practices for this endangered species. A female that showed stereotypic behaviors had higher levels of fecal cortisol than most other individuals, and the behavior disappeared after she was placed in socially enriched housing, even when many of the same structures were present in the housing she was

previously living in. Our study shows that social housing with proper structures that allow the expression of natural behaviors has a positive impact on their welfare by lowering stress.

In chapter 5, I discuss the findings of an international survey on attitudes towards animal memorials. I conducted a voluntary, and anonymous, online survey with a written explanation of the nature of the survey before participation. The survey was intended for any adult that received the link who is 18 years or older, from June to August 2019. The survey was conducted through a URL link sent out through various public platforms on social media (Facebook, Instagram, Twitter), an emailing list of attendees to the International Conference of Environmental Enrichment 2019, including emailing lists of sanctuaries in Africa, Asia, and South America, as well as email listings of Primate Centers in North and South America, Europe, and Asia. The questionnaire had 23 questions that included multiple-choice, yes or no, essay, and a 3 to 5-point Likert scale with varying degrees of approval/disapproval. Differences between groups (research vs zoos, male vs female, direct vs indirect work with animals, naming or not naming animals they work with, among others) of respondents' opinions of animal memorial ceremonies, were calculated using Mann-Whitney U tests. To test if the type and frequency of human-animal interactions predict a more or less favorable opinion on animal memorial ceremonies (ranging from strongly agree to strongly disagree), I performed an ordinal logistic regression model with a Hessian matrix, with the Likert 5-point question results on the degree of agreeability on animal memorials as the outcome variable, and the types of human-animal interactions and experiences as predictor variables. Statistical significance was set at  $P < 0.05$  and analyses were conducted with RStudio version 4.1.0., with packages MASS and Likert. I had responses from 24 countries representing five continents (Asia, Africa, Europe, and North and South America) and the majority of the respondents came from the United States (21.6%), followed by Japan (18.0%), France (9%), India (8%), Taiwan (5%), United Kingdom (5%), Brazil (4%), Canada (4%), Indonesia (4%), and equally 3% for participants from Czech Republic, Portugal, and Spain. Close to half of the participants (49.2%,  $n = 64$ ) worked in research facilities, and nearly the same percentage (47.7%;  $n = 53$ ) worked in places where memorial services for deceased animals or equivalent practices were held. Out of all participants that worked at research facilities, close to 70% (67.1%,  $n = 43$ ) reported that their workplace had animal memorial ceremonies or an alternative that included pictures of animals, statues, paintings, rest areas, moments of silence, and online events. The type of respondents with the largest range of opinions on animal memorials in the 5-pt Likert scale, from strongly agree to disagree, was that of females, 36 years or older, with at least a bachelor's or master's degree, from Europe, working at zoos, who performed only a single job (i.e., not dual work, such as research/veterinary care), working directly with an animal daily, which they do name I did find a statistically significant difference between people who work as caretakers ( $U = 510$ ,  $Z = 2.14$ ,  $p = 0.02$ ), and 1.6 times more likely to agree on holding animal memorial ceremonies compared to all others. While those who have experienced extreme grief or compassion fatigue when losing animals were 1.86 times more likely to consider animal ceremonies as a positive event than those who did not experience negative emotions about the loss of an animal ( $U = 1157$ ,  $Z = 1.99$ ,  $p = 0.04$ ). The regression model revealed from that only the number of negative emotional responses related to compassion fatigue ( $p = 0.01$ ) could significantly predict an agreeable score the statement that animal memorial ceremonies are beneficial or needed. I conclude that animal ceremonies do occur around the world and that it may be indicative of a growing awareness of the closeness of the animal-human bond, despite the decrease in the extent to which many people have a chance to interact with animals or see wild animals. Most people surveyed agree with the statement that these ceremonies are needed or are beneficial, while I found that animal caretakers were significantly more agreeable to having animal memorial ceremonies than other participants engaging in other human-animal-related jobs. My results also revealed that people who work with animals that have experienced compassion fatigue are also significantly more favorable to having an animal memorial ceremony. In the regression model, I found that the number of negative emotional

responses was the only significant predictor of agreeableness to the benefits of having animal memorial ceremonies for departed animals. I suggest that the animal-human bond is gaining strength and I suspect that it will result in more institutions adopting animal memorial ceremonies to honor the dead non-human relatives while potentially alleviating the emotional fatigue for the humans that take care of them.

In chapter 6, I discuss my findings in the previous chapters in light of improving the welfare of captive primates. I focused on discussing the benefits and limitations in using non-invasive methods of quantifying behaviors and the stress differences I used such as behavioral (activity budgets, affiliative behaviors, stereotypic behaviors), physical (hair loss), and physiological parameters (fecal cortisol) in the relatively recent implementation of improved environments and husbandry care. I also discuss the findings of an international survey on attitudes towards animal memorials, for which most respondents see as a positive part of a management plan for institutions that house animals. The results showed that people who have experienced emotional feelings akin to compassion fatigue after losing an animal that they cared for was the only predictor for seeing animal memorials ceremonies are something good or needed. I conclude that my hope in documenting the success of these management practices and sharing them can have a positive impact on how we keep primates wherever they are found.