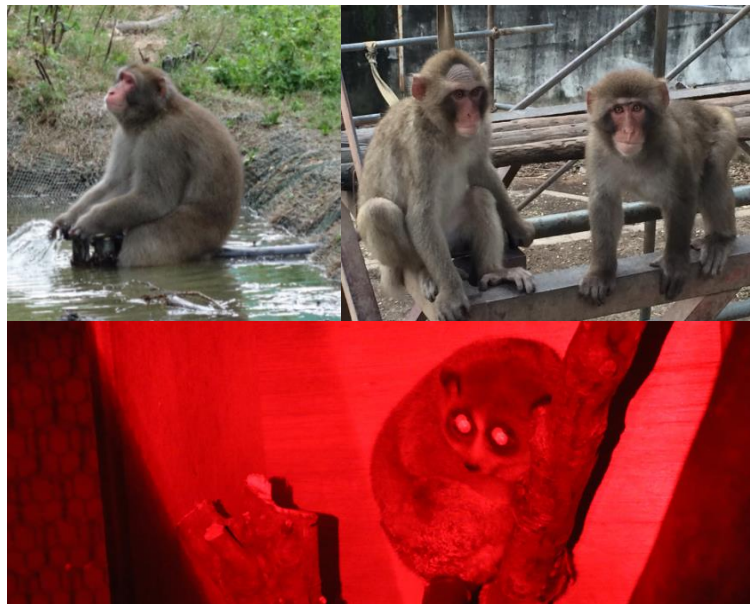


Animal welfare in captive Japanese macaques (*Macaca fuscata*)  
and pygmy slow lorises (*Nycticebus pygmeus*), and human  
attitudes towards animal memorial ceremonies



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## Abstract

Animal welfare should be maximized when primates are kept in captive conditions. Multiple non-invasive methods were adopted in order to address the impact of recent refinements of captive conditions on the welfare of two primate species. In addition, the increasing awareness of the importance of animal welfare and human-animal bonds has led to a growing number of places where memorial ceremonies are adopted when animals die, and I determined people's perceptions of these ceremonies.

In this study, using activity budget averages of wild populations of macaques, 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 non-vegetated, large, outdoor enclosures. Behaviors associated with optimal living standards, such as playing and foraging were higher in the vegetated enclosure, while stereotypic and aggressive behaviors were significantly higher for monkeys living in the conventional non-naturalistic enclosure. Moreover, I scored alopecia prevalence in both enclosures and found that hair loss was minimal to non-existent among males in the vegetated enclosure. Individuals in the vegetated enclosure allocated their time across activity patterns in a proportion more similar to that of their wild counterparts and spent time feeding more than any other activity, as it is often observed across most primate taxa. 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, have fewer spaces to escape aggression, and may be more difficult to reach homeostasis, with stereotypic behaviors occurring by the constraints of their enclosure. I focused later on the behavioral and social needs of captive female pygmy slow lorises, which have been historically described as semi-solitary primates in the wild, and found a high degree of social behaviors displayed once in the group environment, and choosing to be close, and nesting together most of the time, even with ample space with the option to avoid others. I also noted daily activity budgets and postural behaviors changing once placed under an enriched social housing condition, with an increase in postural modes associated with more resting time. I then set out to find if the behavioral changes observed once socially housed would be reflected by the physiological findings (fecal cortisol). I used an enzyme immunoassay to analyze fecal cortisol before and after social housing and found there was a significant decrease in stress once their housing status (single to social) and environment (enriched caging) were improved. Moreover, one of the females engaged in stereotypic behavior, pacing and self-squeezing her body through a small crevice in her cage, had fecal cortisol levels higher than most of her cohort, which is the first case reporting stereotypical behaviors and physiological associations in this species.

Finally, I discuss the findings of an international survey on attitudes towards animal memorials, which most respondents see as a positive part of a management plan for institutions that house animals that will, like all animals, ultimately die. The results showed that people who have experienced emotional feelings akin to compassion fatigue after losing an animal that they cared for were the only predictor for seeing animal memorials ceremonies are something good or needed.

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# **Chapter 1**

## *General Introduction*

### 1.1. *What is animal welfare?*

Animal welfare encompasses many, if not all, biological functioning and behavioral aspects of animals found in captivity. It is usually defined as the physical and mental state of an animal in relation to the conditions in which it lives and dies (WOAH, 2022). The Guide for the Care and Use of Laboratory Animals (2011) establishes guidelines for the institutional animal care and use committee (IACUC) to use as mandates for responsible and humane care and use of animals, but the definition of animal welfare itself is referenced as a contested concept (Haynes, 2011). Words such as “general well-being”, “safe”, “comfortable”, and “unpleasant” are common in describing animal welfare. These words can be subjective and at times a challenge to determine in non-human animals (non-verbal communication), for which animal ethics and welfare may interplay in the search of what is a good welfare state. Fraser and others (1997) suggested that research on “animal welfare” began because of ethical concerns from the public over the quality of life of animals and that three potential problems should be taken into account to address ethical concerns over the well-being of animals. The first problem that may arise is that 1) unpleasant subjective sensations may occur in animals who have adaptations that are no longer useful in the new environment, but there may not be a significant change in biological functioning as a result. For example, a slow loris might be fed adequate amounts of gum in captivity yet have the urge to gouge a tree bark to obtain it as it would naturally do in the wild. 2) Functional problems may develop if an animal is faced with environmental challenges for which it has not evolved, although these issues may not have a substantial impact on subjective experiences for the animal. Captive conditions may induce hair loss in macaques used for research, but they may appear to not notice or mind the problem. 3) Animals have evolved with adaptations that are appropriate for the types of environmental obstacles they confront, but if those adaptations are insufficient, challenges could

still occur. Hence, an ape's behavioral adaptations in a captive social group can be insufficient, leading to an environment high in aggressive and low to no affiliative interactions, such that the animal both feels and functions poorly in a group setting.

Animal welfare is distinct from animal ethics, where philosophers hypothesize on subjective feelings or focus on individuals' responses, rather than empirical evidence on behavioral and physiological adaptations. Nonetheless, both disciplines have been merging recently as they have been aiming for the same goal, improved quality of life for captive animals. The science of animal welfare has become more in line with some ethicists' methods and vice-versa. Many scientists are working to comprehend the ethically significant subjective experiences that animals have, and have identified and attempted to elucidate the interaction of normative and empirical factors in the assessment of animal welfare (Fraser, 1999).

### *1.2 Animal welfare and the type of human-animal interaction*

Moreover, differences in the definitions and importance of animal welfare may be mediated by different types of human-animal relationships and interactions. For veterinarians, animal welfare might chiefly focus on the animal's nourishment, health, and freedom from pain, fear, and distress, while a behavioral ecologist may concentrate on species-specific behaviors, and finding evidence (time engaged in a certain behavior, physiological parameters, energy consumption, etc.) that validates what has been observed and can interpret what these behaviors might imply regarding their welfare. Likewise, a farmer or an animal breeder might only look at the body and the physical environment, its fitness, and if an animal is healthy and producing, then its welfare is in a good state (Blood, 1984; Broom, 1991; Duncan, 1996). However, most people agree that animals have feelings (fear, satisfaction, frustration, etc.) (Bekoff, 2000), and it has been suggested

that feelings are the only components of animal welfare and that they developed to safeguard the animal's basic requirements (Duncan, 2002; Hewson, 2003). For purposes of this work, my main strategy for measuring welfare is from the ecological perspective, with empirical behavioral and physiological measurements (chapters 2-4) of welfare; however, in chapter 5, I discuss ethical concerns that arise in humans when an animal they work with dies.

### 1.3 *Standards in Animal Welfare*

Most advancements in animal welfare, especially for animals kept for research purposes, have occurred only since the latter half of the past century. In 1947 the founder of the Universities Federation for Animal Welfare, Major Charles Humes, published the *Handbook on the Care and Management of Laboratory Animals* which included certain fundamental guidelines for animal testing from 1871 by the British Association for the Advancement of Science. This resulted in the Cruelty to Animals Act 1876, which was only recently replaced by the Animals (Scientific Procedures) Act 1986 after a Royal Commission. However, it has been almost 200 years since people have been promoting animal welfare. Physiologist Marshall Hall highlighted the moral conundrum that the scientific world was experiencing about animal experimentation as early as 1835. He outlined that the least sentient animals should be used for scientific studies on animals, they should be retained for future experiments, they should have a clear goal, and that previous invasive research should not be repeated (Hall, 1835). A more recent historical advancement, animal welfare was done by the zoologist William Russell who started a systematic study of laboratory techniques and their ethical aspect. His work led to the now famous work *The Principles of Humane Experimental Technique*, which was published in 1959, and defined humane procedures under the headings of *replacement*, *reduction*, and *refinement*, now commonly known as the three “Rs” (Russell, 1959). Other guidelines have become popular, such as the five freedoms,

(freedom to exhibit natural behaviors and freedom from hunger, pain, stress, and discomfort) developed as a response to Brambell's report (1965) for the humane treatment of farm animals (ASPCA, 2022). Similarly, *the five domains in animal welfare*, a structured, systematic, and thorough paradigm for evaluating welfare hazards and improvements in sentient animals. The model has undergone frequent changes since its creation in 1994 to take into account improvements in the scientific and philosophical understanding of animal welfare. The model is divided into five sections, or domains, that concentrate attention on particular elements or circumstances that may have an impact on an animal's welfare. They are based on how providing animals with an optimal environment, health, nutrition, and the facilitation of natural behavior affects or maintains mental health, the fifth domain (Mellor and Reid, 1994; Kells, 2022). It is more common to see this model used when researchers are investigating the welfare state in farms, zoos, and sanctuaries. However, the three R's have remained very popular in the research setting around the world, and as we improve welfare standards, more updated versions arise, with an additional "R" for *responsibility*, or an additional "R" for *remembrance* suggested for departed animals (Iloff, 2002). Good housing design and access to conspecifics can provide a good level of welfare for captive primates because it benefits the animals by optimizing the five domains of welfare (also the "R" for *refinements*).

#### 1.4. *Housing Primates in captivity*

Primates are found in 90 countries around the world, but the Neotropics (171 species), mainland Africa (111 species), Madagascar (103 species), and Asia (119 species) are the four regions where primates are found naturally. However, only four countries (Brazil, Madagascar, Indonesia, and the Democratic Republic of the Congo) are home to two-thirds of all species (Estrada et al., 2017), for which many of these primates have evolved to specific types of

ecological needs. Therefore, one important component of enhancing animal welfare is allowing primates to reside in enclosures that reflect the natural habitat that influenced their evolution. It is especially important in macaque species, as the majority of non-human primates kept in research facilities are macaques, and it is estimated that around 100-200,000 monkeys are used worldwide every year (Weatherall, 2006). Non-human primates remain key animal models for specific types of research (Phillips et al., 2014) because of their close phylogenetic relationship and physiological similarities to humans (Lankau et al., 2014). Given that macaques make up more than 79% of the research monkeys used in Western nations and more than 70% of the research monkeys used in Asia (Hagelin, 2004), it is critical to understand how recent advancements in environmental captive conditions affect behavioral and physiological parameters. Differences found in recent advancements on how we better keep primates in captivity not only have welfare implications but could also have implications for long-term data research where “captive primates” are lumped together and might be expected to react and produce the same data they did decades before, even though rearing and living conditions have drastically changed.

Providing adequate living spaces for primates is a common practice at zoos and sanctuaries. However, the challenge to provide them with good enclosures comes when they arrive unexpectedly, such as in confiscated animals from the illegal trade. According to Estrada and others (2017) between 2005 to 2014, there were around 450,000 live primates traded globally, in addition to 11,000 individuals traded as body parts. While between 2007-2008, along the Colombia, Perú, and Brazil border region, 4,000 wild night monkeys (*Aotus nancymaae*, *Aotus vociferans*, and *Aotus nigriceps*) were sold to one single biomedical facility (Maldonado et al., 2009). In 2022, there was a public outcry because 359 cynomolgus macaques (*Macaca fascicularis*) imported to the United States from Cambodia were carrying a pathogen, *Burkholderia pseudomallei*, (Taetzsch

et al., 2022) which causes nonspecific clinical signs, for which primates may find themselves in prolonged quarantine or euthanized.

Even when animals are rescued, they may remain in captivity indefinitely, as laws and regulations make it close to impossible for repatriation, while in other cases release back into the wild becomes a bigger problem for wild populations. Evidence of outbreeding and introgression caused by the reintroduction of rescued animals of different species and subspecies into natural populations, such as Bornean orangutan populations, underlines the caution that must be taken when utilizing these types of initiatives (Banes et al., 2016). As it seems we will continue housing primates in zoos, use them for research, or protect them from anthropogenic activities in sanctuaries, providing primates with good living standards in captivity will remain an undeniable need for the foreseeable future.

## 1.5. *Primate Social Systems and Captive Conditions*

### 1.5.1. *Housing macaques*

The type of housing a primate is kept in is usually dictated by what we observe of them in the wild and their social systems. Macaques have the widest distribution of any nonhuman primate, occurring in much of southern and eastern Asia and in parts of North Africa (Bercovitch and Huffman, 1999). They are found in all types of environments, from barren deserts to tropical rainforests, to highly human-populated areas. They live in multi-male multi-female social groups in the wild, with clear dimorphism as males are much larger than females once they reach adulthood. Macaques are omnivorous, with diets consisting mainly of vegetation, seeds, and fruits (Fooden, 1980; Wolfheim, 1983; Nakagawa et al., 2010). Excluding humans, Japanese macaques (*Macaca fuscata*) are the most northern naturally existing primate species on Earth (Enari, 2021).



In the wild or free-ranging provisioned areas, they may move to find more nutritious food sources during the year (Itani and Nokuda, 1954), and their activity can be affected by climate and season, changing and adapting their behaviors based on their environment (Hanya et al., 2018). These attributes require enclosures with good welfare standards to be designed with enough ample space to house large social groups, enough vegetation to satisfy most of their nutritional requirements, and a variety of substrates that allow for seasonal changes in behavior. Perhaps because of their great ability to adapt to many types of environments and human accessibility to them, they have been used frequently in research. While much of the work on improving the welfare of primates is based on macaques, not many studies have shown the differences seen with the recent construction of well-designed self-sustaining enclosures.

### 1.5.2. *Housing slow lorises*

While much is known about the social systems and needs of macaques, pygmy slow lorises are nocturnal, small, and less researched for which much of the details of their social systems are still clearly unknown. They have a more limited distribution than macaques, and more than half of their diet is reliant on tree gums found in S.E. Asia (Cabana and Plowman, 2014). Unlike macaques used in research, they make their way into captivity largely because they are captured for the illegal wildlife trade (Nijman et al., 2014). Although not as many are reported in captivity compared to macaques, most are probably living under deplorable conditions, since there are no regulations in place to protect their welfare in the illegal trade. Other threats include various anthropogenic activities and vary from deforestation, the illegal pet trade, and hunting for use of body parts for traditional medicine (Ratajszczak, 1998; Nahallage et al., 2008; Starr et al., 2010; Nekaris and Starr, 2015; Musing et al., 2016). For this reason, most arrivals of slow lorises into captivity come unexpected or unannounced, leaving many government and non-profit organizations that take care

of confiscated animals unprepared and not well equipped to house them. Because they remain one of the least studied primates in the wild, much of their needs in captivity remain under-reported by the scientific community. Recent studies have contributed to improving their lives in captivity, such as more natural diets (Cabana and Nekaris, 2014), general guidelines for enclosure size and design (Fitch-Snyder and Schulze, 2001), and how to meet their social needs and what type of interactions when socially housing them in captivity (Yamanashi et al., 2021). Therefore, recent findings that can add to the growing scientific findings on improvements for the captive lives of this primate should be shared.

#### *1.6. Creating close human-animal bonds and the cost of caring*

Human-animal interactions can have both negative and positive outcomes for the welfare of an animal, but recently, strong human-animal bonds have been encouraged for those who work with or live alongside them. According to previous studies (Clayton et al., 2009; Kustska, 2009), zoos can help visitors develop compassion for the care of the zoo animals and, in turn, for their wild counterparts and the ecosystems in which they reside, as their positive interactions with animals in zoo settings catalyze this empathy (Godinez and Fernandez, 2019). Modern zoo exhibits promote interactions between their zoo visitors and, for example, chimpanzees may choose to play with projections or video games that visitors can interact with (Yamanashi et al., 2022). Zoos with naturalistic enclosures are seen as more favorable by visitors, as they spend more time observing the animals (Nakamichi, 2007), which in turn may create more periodic visits. Repeat zoo visitors will most likely be involved more in outreach and conservation activities, more than the one-time visitor, and they may also visit their preferred animals or the ones they have created bonds with. Likewise, in research settings, strong human-animal bonds are encouraged between caretakers and their primates, as it has proven to decrease stress and promote general well-being in primates

(Waite et al., 2002). These practices of high-quality animal care, which includes identifying and reducing distress, is important for optimal scientific validity (Coleman, 2011; Bliss-Moreau et al., 2013). However, creating these strong bonds can come at a cost (Thurston et al., 2021), when considering the moral and psychological effects of caring for animals that die. Institutions, animals, caretakers, and the public perception can benefit when grief associated with the loss of animals is acknowledged and supported (Coleman, 2011).

### 1.7. *Outline of the Study*

The main goals of this work were to scientifically study the impact of environmental and behavioral enhancement of two species of captive primates, and to assess how memorial ceremonies that honor deceased animals are perceived by the humans that take care of them in order to evaluate the extent to which human/animal bonds can have an impact on animal (including human) welfare.

**Chapter 2** compares the behavior and activity budgets of macaques living in two kinds of enclosures, vegetated vs. (traditional) non-vegetated. In addition, some signs of potential stress, such as hair loss, stereotypic activities and degree of aggressive behaviors were recorded. **Chapters 3 and 4** report on the behavioral and physiological changes of female pygmy slow lorises who were confiscated from the illegal pet market and moved to single cages at the Japan Monkey Centre (Inuyama, Japan). Whether moving the slow lorises from single housing to socially enriched housing improved their welfare by reducing stress and promoting species-specific behaviors was examined by recording measures of sociality combined with analysis of fecal glucocorticoid concentrations. **Chapter 5** examines how animal memorial ceremonies, which are done in many research institutions, are perceived by the people that work with animals and how

they may differ depending on the type of human-animal interaction. Whether animal memorial ceremonies can benefit primates housed in captivity, as well as their caretakers is also discussed in Chapter 5. **Chapter 6** integrates the information presented in the previous chapters and summarizes how the findings relate to the health and well-being of animals in captivity, along with the humans that work with them. While the foundations for my research were based upon the scientific study of animal welfare, my studies also merge into the science of ethics by yielding recommendations and suggestions that should enhance and improve the health and well-being of primates.

## **Chapter 2.**

*Costs and benefits of living in a vegetated, compared with non-vegetated, enclosure in male Japanese macaques (Macaca fuscata)*

## 2.1. Abstract

Improving captive environments for primates has been an important tool to enhance animal welfare. One method has been to provide primates with naturalistic vegetated enclosures to promote species-specific behaviors, enhancing interactions with their social and natural environment, such as an increase in feeding, foraging, and positive social behaviors such as play. To investigate the benefits in which living in naturalistic environments promote general animal well-being, we observed immature and adult males living in two outdoor housed groups of Japanese macaques (*Macaca fuscata*) in the Kyoto University Primate Research Institute (KUPRI), Inuyama, Japan, from June of 2015 until June 2016. One enclosure was naturally vegetated and the other was not. We recorded male activity budgets, affiliative (groom, play) behaviors, and rates of agonistic interactions. To examine health status, we recorded and compared coat conditions for both groups. We found that males in the vegetated enclosure spent more time in social play than males in the non-vegetated enclosure, while males in the non-vegetated enclosure displayed more stereotypic behaviors and agonistic interactions. We recorded better coat conditions in the vegetated enclosure males while rates of social grooming or self-grooming were no different between males in the two enclosures. The males in the vegetated enclosure did not have activity budgets more similar to their wild counterparts; but they spent more time in feeding-related activities and less time resting, which was more similar to their wild counterparts than males in the non-vegetated enclosure. Our findings suggest that individuals housed in naturalistic environments have significantly greater behavioral and physical markers of wellbeing than those housed in unnatural, large outdoor enclosures. Although we found that males in both types of enclosures overall had similar time budgets to males in the wild, the detailed behavioral and health results suggest that the welfare benefits to males were greater in the vegetated enclosure, compared

with non-vegetated enclosures. Efforts to mimic more natural environments should promote the well-being of primates.

## 2.2. Introduction

For decades, efforts have been made to improve the housing and care of non-human primates (NHP) in laboratories and other captive environments (e.g. Chamove et al., 1982; Lawrence, 1987; Boinski et al., 1999; Baker et al., 2000; Waitt and Buchannan-Smith, 2001; Fontenot et al., 2006). In particular, improvements in animal welfare have been made in the creation of more complex and enriched housing (O'Neill et al., 1991; Redshaw and Mallison, 1991; Barnard and Hurst, 1996; Reinhardt, 2002; Kerridge, 2005; Barber, 2009; Broom, 2014). An important parameter that has stood out in welfare studies is behavioral stress. While many studies and reviews have been done to validate methods of evaluating animal welfare in captive conditions, few studies have evaluated individual wellbeing in relatively natural, highly enriched, and vegetated environments compared to less enriched housing conditions (Chamove and Rohrhuber, 1989; Chang et al., 1999; Hosey, 2005; Kurtycz et al., 2014). Because there are many potential stressors that cannot be controlled in captivity, providing a complex captive environment for social living primates is imperative to promote positive interactions between animals, to increase natural behaviors, and to improve animal care and welfare (Anderson and Chamove, 1984; Byrne and Suomi, 1991; Price, 1992; Britt, 1998; Hardy et al., 2004; Birkett et al., 2011). Singly caged rhesus macaques (*Macaca mulatta*) showed lower levels of physiological stress when their cages were placed outside than those housed indoors with the same caged conditions but with limited access to natural sensory stimuli and just inanimate enrichment devices or socially paired, suggesting that environments outside the cage (environmental variation) influences physiological stress (Schapiro et al., 1993). DuFour et al. (2011) found that both capuchins (*Cebus apella*) and

squirrel monkeys (*Saimiri sciureus*) were negatively affected by movements to a new environment, while providing access to outdoor enclosures in rhesus macaques has been shown to reduce self-injurious and other unnatural behaviors (Fontenot et al., 2006). The environment includes both social and natural surroundings and both are critical for individual well-being (Beisner and Isbell, 2008; Sambrook and Buchannan-Smith, 1997).

In a range of captive situations, including zoos and laboratory facilities, it is common to observe individuals display stereotypic behaviors (Vandeleest et al., 2011), but they seem to decrease in highly enriched environments. These behaviors include stereotypic or repetitive behaviors that appear to have no obvious goal or function - such as pacing, swaying, head-bobbing or circling. Fontenot and others (2006) reported that outdoor housing (non-vegetated corrals with access to the outside) in adult rhesus macaques, regardless of whether individuals were housed socially, decreased display of stereotypies and non-wounding self-injurious behaviors. Although the outdoor housing was not naturally vegetated, access to the outside seems to have had a role in decreasing some stereotypic behaviors. In addition, rhesus macaques reared in outdoor housing with their mothers displayed less self-abuse than those reared by their mothers indoors (Rommeck et al., 2009). Jaman and Huffman (2008) found that Japanese macaques living in a vegetated enclosure had activity budgets more similar to their wild counterparts than to a non-vegetated group, which engaged in a specific stereotypic behavior of circular group pacing. Complex substrates in the form of earth, straw, or other similar bedding allows species-typical nest building behavior, provides more opportunities to manipulate objects, and increases foraging activities, all of which can reduce boredom and the frequency of stereotypic behaviors (Chamove et al., 1982; Fraser et al., 1991; Morgan and Tomborg, 2007). The fact that unwanted behaviors in captive primates living in outdoor enclosures may be observed less frequently compared to less enriched



enclosures stresses the important role in which captive environmental conditions improve animal welfare.

Not only the absence of stereotypic or unwanted behaviors, but the presence of behaviors associated with good animal well-being, such as play, can also serve as an indicator of animal welfare in captive primates. Animals play less when in stressful situations (Loy, 1970; Leyhausen, 1979; Rasa, 1984), and the contribution of the play-welfare relationship is now widely acknowledged in applied ethology and animal welfare science (Boissy et al., 2007; Oliveira et al., 2010). Burghardt (2005) defined play as behaviors that have no apparent function, spontaneous, atypical, and repeatable and initiated in the absence of stress. Play has long been identified as a potential welfare indicator because it often disappears when animals are under social and environmental challenges and because it is thought to be accompanied by a pleasurable emotional experience (Helda and Spinka, 2011; Nahallage and Huffman, 2012). Most animal play is reduced by detrimental environmental conditions, but under favorable conditions it can become more prominent in the behavioral repertoire of infants and juveniles in particular (Helda and Spinka, 2011). Thus, an increase in play behaviors observed in a more naturalistic-vegetated enclosure compared to a less enriched one, might serve as a good indicator of less stress and healthier living conditions (Nahallage et al., 2016).

A combination of behavioral activities, body condition, and physiological measures will produce a more complete picture of animal welfare (Bassett and Buchanan-Smith, 2007; Brand et al., 2016). Thus, it is crucial to investigate visual indicators of general well-being in order to evaluate animal welfare as part of a captive behavioral management plan. Species-specific behaviors in captive primates can be the first visual markers to assess an animal's psychological well-being. NHP engaged in social grooming in captivity, as in the wild, reflect a certain level of

healthy group cohesion. Feeding-related behaviors, such as foraging on the available vegetation, reveal the ability of an enclosure to provide opportunities for animals to engage in more natural feeding behaviors. In many cases, to enhance animal welfare, enrichment devices include foraging tasks (Hardy et al., 2004; Hocking et al., 2015). Frequent and complex play behavior, as a sign of less stressful conditions, can be influenced by the availability of items to manipulate in the environment.

Hair loss is used to gauge body condition as a visual cue to animal health and well-being in captive settings (Beisner and Isbell, 2009; Kroeker et al., 2016; Brand, 2016). Many factors affect individual coat conditions of primates, such as early rearing, genetic background, diseases and immune-competence (Novak et al., 2016). However, hair loss can be drastically exacerbated as a consequence of social and environmental factors in unnatural captive settings (Novak, 2009; Novak et al., 2016). For example, providing a grass substrate vs. a gravel one simultaneously promotes foraging behaviors and increases time spent grooming to levels that are comparable to wild populations (Beisner and Isbell, 2008). Primates in captivity with extensive leisure time may spend much more time self-grooming or grooming others, which can cause hair loss. Vegetated enclosures have been found to play a role in reducing the incidence of hair loss and thus improve individual wellbeing, possibly because it balances individual activity budgets (Venatesan et al., 2004; Beisner and Isbell, 2009; Zhang, 2009). More specifically, these studies suggest that when genetic or disease factors are not attributed to hair loss, primates show better coat conditions in vegetated than in non-vegetated enclosures (Zhang, 2009).

My study aims to identify the degree to which more enriched vegetated-enclosures promote general animal well-being in captive Japanese macaques, as well as providing more information on current behavioral management methods and its potential in showing to what degree these

markers differ depending on the quality of the captive environment. NHP living in a highly enriched environment (hereafter vegetated enclosure), which have the ability to express a greater variety of behaviors, are predicted to: 1) spend more time in relaxed-affiliative behaviors (e.g. social grooming, play), 2) spend less time engaging in stereotypic behaviors, 3) display less agonistic behaviors, 4) have better coat condition than animals living in the non-vegetated enclosure, and 5) have time budgets that resemble their wild counterparts. Such findings can be useful for monitoring stress in captive, and non-captive primate populations, as well as for promoting non-invasive ways for assessing welfare.

## **2.3. Methods and Materials**

### *2.3.1. Study site*

I conducted observations in two outdoor socially housed groups of Japanese macaques (*Macaca fuscata*) at the Kyoto University Primate Research Institute (KUPRI, Inuyama, Japan). The first group, Onobora Enclosure 7 (40 + 5 individuals) is housed in a vegetated enclosure (Figure 1A) at the KUPRI Research Resource Station (RRS) containing natural ground cover, trees, a pond, perches and climbing structures. Enclosure 7 is divided in two sections and animals are shifted once a year to the other section in order to allow for re-growth of vegetation. During our study, the group was housed in the 3,900 m<sup>2</sup> section 1 between January and May 2015, and in section 2 (5,900 m<sup>2</sup>) from June 2015 to June 2016. The second group, Takahama Group 1 (40 ± 5 individuals), is housed in a 960 m<sup>2</sup> 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 (Figure 1B). These two groups were selected for the study based on similar age-sex class composition, group size and rearing histories.

Both study groups were provisioned by husbandry staff with commercial monkey chow twice a day, evenly dispersed to minimize feeding competition, between 9:00-11:00, and 13:00-17:00. In addition, both groups were given enrichment feeding during the afternoon that consists of sweet potatoes or wheat grain alternatively every other day. Bamboo branches, fruit tree branches, and special paper foraging treats were also provided every other week in the Takahama Group 1 (non-vegetated enclosure). Subjects included only males because some females have their offspring removed, which could have an effect on stress hormone profiles and time budgets. The male focal subjects were chosen for similarity of age and rank in the two groups. I divided the males into mature (older than 5 years old) and immature (2 to 5 years old) with 5 males in each age group, for a total of 10 males in each enclosure. I established rank during our observations by monitoring dominant-subordinate interactions, especially during provisioned feeding, with 1 being the highest ranking through 5 the lowest ranking for both mature and immatures.

### *2.3.2. Behavioral data collection*

I collected data from January 2015 to March 2015, and from September 2015 to June 2016. Ten min continuous focal animal samples (Altmann, 1974) were recorded on each subject. During the 10 min observations, if the focal animal was lost for less than 60 seconds, observations were continued after re-sighting the subject. Otherwise, a different focal animal was assigned and followed instead. A total of 384 hours of behavioral data were collected for both vegetated (N = 204 hr.) and non-vegetated enclosures (N = 180 hr.). Observations were conducted inside both enclosures, except during holidays, where observations were made from an outside observation tower on the wall of the non-vegetated enclosure for safety reasons. Observations were conducted during two time periods; one in the morning (9:00-12:00) and one in the afternoon (14:00-17:00). An equal number of focal samples were collected in each time period of the day for each focal

subject to facilitate comparisons of behavioral data across time of day. Observations were done in both enclosures weekly, for each focal animal, for an average of 6 hours weekly for each enclosure.

Behavioral data were collected following the ethogram of Jaman and Huffman (2008). Behaviors recorded were object manipulation, feeding, foraging, licking, resting, moving, grooming, and playing (Table 1). Grooming was separated into self-grooming (i.e., a monkey grooming its own coat) and grooming others (i.e., social grooming with other conspecifics) for further analysis in relation to hair loss (see below). Similar behaviors were grouped into four respective categories for comparison of activity budgets: feeding, grooming, resting and moving. I also recorded ad libitum observations on the occurrence (events, bouts) of stereotypic behaviors for descriptive purposes, but they were not included in statistical analyses (Table 1). Observations were stopped when clinical intervention required capturing animals or when events occurred that would interrupt normal daily activity. For comparison with activity budgets in wild Japanese macaques, averages were calculated from three previous studies on free-ranging populations (Soumah and Yokota, 1991: Takasakiyama; Hanya, 2004: Yakushima).

### *2.3.3. Assessment of hair loss*

Coat condition, i.e., hair loss, was noted twice a month for each focal subject by JSA. Assessing hair loss is somewhat subjective, but has been applied in prior studies evaluating potential health status (Zhang, 2011; Runeson et al., 2011). The first is based on a 3-point scoring method for head and back alopecia (HS0/BS0: no hair loss, HS1/BS1: some hair loss, HS2/BS2: bald areas) (Zhang, 2011). The second, is a 4-point scoring system, where hair missing by body area can be quantified into body percentages (score 0: no hair loss; score 1: 1-25% hair missing; score 2: >25% hair missing; score 3: >50% hair missing; score 4: >75% hair missing to complete hair loss/baldness), where body area is divided into 12 sections; head, shoulders, dorsal torso,

ventral torso, ventral and dorsal forelimbs, and ventral and dorsal left and right limb (Runeson et al., 2011). Body sections were assigned weights roughly corresponding to their relative surface areas. The sections were then multiplied by their corresponding weights and summed to produce a total body alopecia rating ranging from 0 to 1.0.

#### 2.3.4. *Data analyses*

I used the Mann-Whitney U test for group comparisons. I examined the relationship between male rank and hair loss in both enclosures using a Spearman's Rank Order Correlation test. Comparisons with wild populations were performed using a Kruskal-Wallis Analysis of Variance Test. For all tests statistical significance was set at  $P < 0.05$  and two-tailed results are reported.

## 2.4. Results

### 2.4.1. *Behavioral differences*

Enclosure type and age class behavioral differences. 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 (Figure 2). Stereotypic behaviors were never observed among individuals in the vegetated enclosure. They were numerous, however, in the non-vegetated enclosure with pacing being the most prevalent, occurring daily. Among the seven other stereotypic behaviors observed, perch-hanging (Figure 3) was by far the most prevalent (71%) followed by head-bobbing (16%). Twirling, hair eating, hair pulling, self-clasping and feces-rolling were rarely observed (Table 2).

All 10 mature and immature males exhibited perch-hanging behavior for a proportion of their total resting time; with mature doing so about twice as often (Figure 4). These males spent an average of 17% of all their resting time in this position. The focal animals that engaged more in this behavior did not rest more than others and rank did not seem to be an indicative factor for perch-hanging behavior. There was a significantly higher incidence of the number of agonistic events recorded in the non-vegetated, compared to the vegetated enclosure ( $U=2$ ,  $Z=2.09$ ,  $P=0.018$ ).

#### 2.4.2. *Coat condition*

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 ( $r_s = -0.07511$ ,  $n = 10$ ,  $P = 0.837$ ), but a strong correlation between rank and hair loss was observed in the non-vegetated enclosure (Figure 5).

#### 2.4.3. *Activity budgets by age group*

Immature and mature males housed in each enclosure had significantly different time budgets (Figure 6). In the vegetated enclosure, immature monkeys spent significantly more time feeding ( $U=2.5$ ,  $Z=-1.98$ ,  $P=0.047$ ) and moving ( $U=0$ ,  $Z=-2.51$ ,  $P=0.012$ ) than their mature counterparts, that spent more time resting ( $U=0$ ,  $Z=2.51$ ,  $P=0.012$ ). There was no significant difference in average time spent grooming between these age groups ( $U=6$ ,  $Z=-1.25$ ,  $P=0.207$ ). Similarly, in the non-vegetated enclosure, immature individuals spent more time feeding ( $U=0$ ,  $Z=-2.51$ ,  $P=0.012$ ) and mature animals spent more time resting ( $U=0$ ,  $Z=2.51$ ,  $P=0.012$ ), while

there were no significant differences in time spent grooming ( $U=9.5$ ,  $Z=0.52$ ,  $P=0.595$ ). Because of statistically significant differences between age classes within enclosures for all activity states except for grooming, I analyzed potential differences between enclosures for the two age classes separately.

When comparing vegetated to non-vegetated enclosures, immature individuals spent significantly more time feeding ( $U=2$ ,  $Z=2.1$ ,  $P=0.036$ ) in the vegetated enclosure, but there was no significant difference between enclosures in time spent resting ( $U=3.5$ ,  $Z=-1.78$ ,  $P=0.075$ ) or moving ( $U=7$ ,  $Z=-1.04$ ,  $P=0.298$ ). When combining both age classes, there was no significant difference between animals living in the vegetated and non-vegetated enclosures in time spent grooming ( $U=44$ ,  $Z=0.46$ ,  $P=0.673$ ).

When comparing the overall activity budgets of males in the two types of enclosures with the two free-ranging population reports, the results indicate that there is no statistically significant difference in the time budgets of wild macaques with either vegetated ( $H = 0.171$ ,  $P = 0.966$ ) or non-vegetated ( $H = 0.264$ ,  $P = 0.966$ ) captive ones (Table 3). Nonetheless, animals in the vegetated enclosure and in the wild spend about 40-50% of their time feeding, while those in the non-vegetated enclosure feed for half that amount of time (Figure 6). Contrariwise, animals in the non-vegetated enclosure spent nearly half the day resting, while those in the wild and in the vegetated enclosure rest for about one-quarter to one-third of the day.

## **2.5. Discussion**

### *2.5.1. Overall differences*

The differences documented in males housed in vegetated versus non-vegetated enclosures were consistent with four out of five of our predictions. Males in the vegetated enclosure exhibited



higher rates of affiliative behaviors, such as play, than males living in the conventional concrete-outdoor enclosure. On the other hand, males living in the non-vegetated enclosure spent more time exhibiting stereotypic behaviors (pacing, twirling perch hanging, and others) and higher rates of agonistic behaviors. The coat condition of males living in the vegetated enclosure, especially mature ones, resembled that of free-ranging individuals. Significant hair loss and poor coat condition was prevalent exclusively in mature males in the non-vegetated enclosure. Although the time budgets of males living in the vegetated enclosure were not significantly closer to their free-ranging counterparts than the non-vegetated enclosure males overall, males in the vegetated enclosure exhibited less stereotypical behavior, more play, and less aggression than those in the non-vegetated enclosure. Therefore, time budget analysis might be overly simplistic for identifying similarities between the wild and captive conditions relevant to animal welfare.

### *2.5.2 Differences in affiliative behaviors*

Grooming (i.e. social grooming or self-grooming) frequency was not higher in the vegetated than the non-vegetated enclosure, conflicting with Prediction 1 because social grooming may be a sign of affiliative-relaxed behaviors. The discrepancy could be explained in part because only males were observed and male Japanese macaques tend to spend less time grooming than females (Watanuki and Nakayama, 1993). Nonetheless, our results did support previous studies done on environmental factors influencing play (e.g. Sommer and Mendoza-Granados, 1995), where enriched (vegetated) environments promote play in immatures. In previous studies, playing seems to decrease in rhesus macaques, or completely diminish in other primates, under unfavorable environmental conditions (Loy, 1970; Leyhausen, 1979; Rasa, 1984; Nahallage and Huffman, 2008), and in species such as bonobos, play behavior is directly related to the housing environment (Tacconi and Palagi, 2009). Thus, when these behaviors are expressed, it could be associated with

less stress or positive social interactions. Free-ranging male immature gray langurs (*Presbytis entellus*) played nearly twice as often and in larger numbers in favorable vegetated environmental conditions, than in non-vegetated environments (Sommer and Mendoza-Granados, 1995). Play is also beneficial as it promotes the development of important species-typical social behaviors. In other primates such as chimpanzees and bonobos, by engaging in play, the immature individual enhances their social status, decreases tension, or attenuates aggression, and thus may experience less stress (Palagi et al., 2006). The greater time spent playing by immature males in the vegetated enclosure in our study likely had an immediate positive effect on managing stressful situations as well as one that influences their well-being past the developmental stage into adulthood (Martin and Caro, 1985; Byers and Walker, 1995; Watson, 1998; Burghardt, 2005; Pellis and Pellis, 2009).

### 2.5.3. Stereotypic behaviors

Males in the non-vegetated enclosure engaged more in stereotypies, as expected (Prediction 2). Only one individual male from the vegetated enclosure was documented hair pulling in one instance, but all other stereotypic behaviors were observed only in the non-vegetated enclosure. The stereotypic behavior previously documented by Jaman and Huffman (2008) in Japanese macaques living in a non-vegetated enclosure was that animals spent time in synchronized pacing around the periphery of the enclosure, a “whirlpool” like behavior where all animals from the enclosure unidirectionally walked the periphery of the enclosure. During this study, an unusual stereotypy, “perch hanging” was recorded only in the non-vegetated enclosure, although both types of enclosures have tubes and perching equally distributed around the enclosure. The complexity and abundance of natural substrate in the vegetated enclosure probably provides more opportunities for animals to express their natural behaviors, and thus decreases the chances of stereotypic behaviors like this from developing in captive conditions. Perch hanging could reflect

a peculiar resting posture and not a stereotypy, that is adopted by animals in this enclosure and resembles a ‘culture-like’ behavior. Nonetheless, lower-ranking animals or animals that rested more did not engage in this behavior more than higher-ranking animals or animals that rested less. This suggests that the behavior might have developed as an adaptation to unfavorable conditions or a form of avoidance. In addition, ‘perch-hanging’ (see Fig. 2) is oftentimes perpendicular to the support beam, whereas when animals rest in the wild, the posture is generally parallel to a tree limb with the underside resting on the substrate (e.g., koalas, *Phascolarctos cinereus*; leopards, *Panthera pardus*; FBB, pers. obs.) Although stereotypic behaviors may not always cause harm, they can be indicators of suboptimal environments, either past or present (Mason, 1993) and should be further investigated with the aim of improving animal welfare.

Suboptimal environments can adversely affect animals’ natural behaviors. Such environments include confined spaces in captivity, which inhibit individuals from displaying normal species-specific ranging behavior, e.g., lacking a vertical dimension for arboreal species, or too little space to keep a safe distance from aggressive or dominant conspecifics (Fam et al., 2012). The greater time spent foraging or engaged in other affiliative behaviors in a more natural environment, leave less time to express such stereotypy or pacing behavior. In one comparative study (Marriner and Drickamer, 1994) of rates of stereotypies in eight species of captive primates, omnivorous species showed significantly more stereotypies than did frugivores or folivores. The authors interpret this difference as a consequence of greater food foraging and handling time for omnivores that is limited in captivity. Males that move more than others in the non-vegetated enclosure might be spending more time moving to compensate for the limited food foraging and handling time they would spend in the wild.

#### 2.5.4. Aggressive interactions

Similar to northern plains gray langurs (*Semnopithecus entellus*) (Little and Sommer 2002) and in support of Prediction 3, I recorded less aggression in males living in the vegetated, than those in the non-vegetated enclosure. Males in the vegetated enclosure had constant access to natural plant foods in their enclosure, and subordinate males could probably feed on vegetation while out of sight of dominant males. In contrast, males in the non-vegetated enclosure depended solely on monkey chow. Among yellow baboons (*Papio cynocephalus*) in the wild dominant males tend to monopolize high-quality food patches (Post et al., 1980), which could increase aggressive interactions. Population density might also be contributing to differences in activity budgets as reported in two captive macaque species (Eaton et al., 1981; Bercovitch and Lebron, 1991). However, in captive gorillas living in naturalistic enclosures, Stoinski et al. (2001) found that the quality rather than quantity of space was a critical factor in their well-being. Moreover, provisioning in camouflaged areas reduced aggression in captive gorillas (Blaney and Walls, 2004). In our study, aggression occurred most often during provisioning time in both vegetated and non-vegetated enclosures, but only males in the vegetated enclosure also had the option to feed on natural vegetation. Therefore, the limited and concentrated food supply in the non-vegetated enclosure is a restricted high-quality resource that probably exacerbates food competition among males.

#### 2.5.5. *Hair loss*

I found better coat conditions in males of the vegetated enclosure than males in the non-vegetated enclosure, consistent with Prediction 4. Shedding of fur (seasonal hair loss) occurs in several wild animal species (Ling, 1970), and in Japanese macaques, it occurs naturally every year in the late spring or early summer (Inagaki and Nigi, 1988), but it is rare that free-ranging, wild mammals exhibit hair loss exposing the skin as extensively or frequently as that seen in captive

mammals (Vessey and Morrison, 1970; Isbell, 1995), which suggests that some aspect of the captive environment contribute to abnormal hair loss. Japanese macaques in the vegetated enclosure had similar hair coats to those of wild conspecifics (JSA, MAH, pers. obs.), which have the best hair condition with almost no hair loss, compared to their non-vegetated enclosure conspecifics (Zhang, 2011). These results support other findings that found that ground substrate had the strongest main effect on hair loss in captive rhesus macaques (Beisner and Isbell, 2009). Venatesan et al. (2004) found that a vegetated environment may help prevent hair loss in captive juvenile macaques. More foraging opportunities and less aggression seem to prevent or ameliorate hair loss in captive macaques.

The greatest loss of hair occurred in mature males in the non-vegetated enclosure and it was correlated to rank, with the higher the rank, the more the hair loss. Because age is also associated with dominance rank, hair loss seems to affect older and higher-ranking mature males more than immature males of lower ranks. Nonetheless, all immature monkeys had some hair loss in the head area when using the categorical method (Zhang, 2011), which may be explained by allogrooming from mothers or dominant animals. Hair loss was also more extreme on the back of the head followed by the upper and lower back areas of mature males in the non-vegetated enclosure. These areas of hair loss were areas that are more difficult to self-groom, which may indicate that grooming from other conspecifics, rather than a lack of foraging and other natural behaviors, is more likely the cause. High-ranking female rhesus macaques living in gravel substrate enclosures exhibited more hair loss than high-ranking individuals living on grass substrates (Schino et al., 2000). Our findings support the idea that although hair loss is a multifactorial condition (Judge and DeWaal, 1997; Reinhardt, 2005; Davis and Suomi, 2006);

environmental conditions and socially stressful situations seem to play an important role in ameliorating or exacerbating hair loss.

#### 2.5.6. *Captive vs wild behaviors*

Male Japanese macaques living in vegetated enclosures were found to have time budgets that resemble their wild counterparts more so than the monkeys in the concrete enclosure, which is consistent with Prediction 5. Various studies have demonstrated that an individual's behavioral repertoire is directly affected by the environment they live in, and more complex environments seem to improve general wellbeing and care of captive primates (Hardy et al., 2004; Jaman and Huffman, 2008; Birkett et al., 2011). My findings resemble those of Jaman and Huffman (2008) in that males in the vegetated enclosure spent more time feeding than did males in the non-vegetated enclosures, but differed in that their activity budgets were not more similar to the wild than found among monkeys living in the non-vegetated enclosure. One possible reason for the discrepancy between the two studies is that Jaman and Huffman (2008) included both males and females, while I only looked at males.

Foraging is a complex behavior that consists of appetitive and consummatory phases (Lindburg, 1998), and seems to be a highly motivated behavior (Neuringer, 1969; Rushen et al., 1993). Animals in their natural habitat spend a large portion of their day foraging and feeding (Herbers, 1981; Soumah and Yokota, 1991; Hanya, 2004; Merkle, 2005); and I found that males in the vegetated enclosure more closely resembled their counterparts living in natural habitats than did males living in the non-vegetated enclosure as regards feeding time. Reduced opportunities for foraging in captivity may be inherently stressful (Morgan and Tromborg, 2007), which aligns with my findings that suggest that males in the non-vegetated enclosure had less opportunities to

engage in their natural behaviors than those in the vegetated enclosure. Having more foraging opportunities seems to promote the expression of more species-specific behaviors, which may decrease food competition and increase the chances of more affiliative interactions.

## **2.6. Conclusion**

I conclude that living in a vegetated environment provides male Japanese macaques the closest opportunities to live a life that resembles the wild, improving their welfare, when compared to those living in a non-vegetated, large, outdoor enclosure. Behaviors associated with optimal living standards, such as playing and foraging have an important role in reducing stress and they seem to be more prevalent in highly enriched enclosures. Because living in an enriching environment is more beneficial, animals spend less time in stereotypic and aggressive behaviors than animals living in conventional non-naturalistic environments. Hair loss is an issue for NHP in captivity, but hair loss was minimal to non-existent among males in the vegetated enclosure. Individuals in the highly enriched, vegetated enclosure allocated their time across activity patterns, although not significantly, in a proportion more similar to that of their wild counterparts and spent time feeding more than any other activity. More opportunities for feeding behaviors to be expressed might help in satiation and decreasing other unwanted behaviors for primates in captivity. Moreover, the vegetated enclosure is self-sustaining, assuring individuals will benefit from the vegetation indefinitely by seasonally rotating them between two sections of the larger enclosure, allowing time for the vegetation to grow back. Our findings suggest that, to the extent possible, primates in non-vegetated environments should have their condition improved in order to provide a healthier living area.

## 2.7. Figures and Tables



Figure 1. Study group enclosures, A) Onobora Group 7 vegetated enclosures (Section 7-1, Left; Section 7-2, Right), B) Takahama Group 1 non-vegetated enclosure (left side). Photos courtesy of Kyoto University, Primate Research Institute.



Activity	Behavior	Description
<b>Rest</b>	Rest	starts when no other behavior is observed regardless of the posture
	Object manipulation	picking up objects such as stones and sticks and manipulating them
<b>Feed</b>	Forage	uninterrupted search for food on ground or vegetation, often but not always, ended by ingestion of food or water
<b>Move</b>	Move	terrestrial or arboreal locomotion, lasting a minimum of 5 seconds, except when foraging or following another animal.
	Play	jumping, running or hanging on, mounting, or grasping another individual in a non-threatening context
<b>Groom</b>	Social-groom	picking out, scratching, or removing debris from hair
	Self-groom	picking out, scratching, or removing debris from its own hair
<b>*Aggression</b>	Supplanting, grabbing, hitting, chasing, grabbing, threat bark, etc.	Physical contact, gesture, or vocalization typically directed to a subordinate individual
<b>*Stereotypic Behaviors</b>	Head-bob, hair-eating, hair-pulling, feces-rolling, self-clasping, pacing, twirling	(See Table 2)

Table 1. The ethogram used in this study. \* These activities were recorded *ad libitum*, thus were not included in the statistical analysis of time budgets.

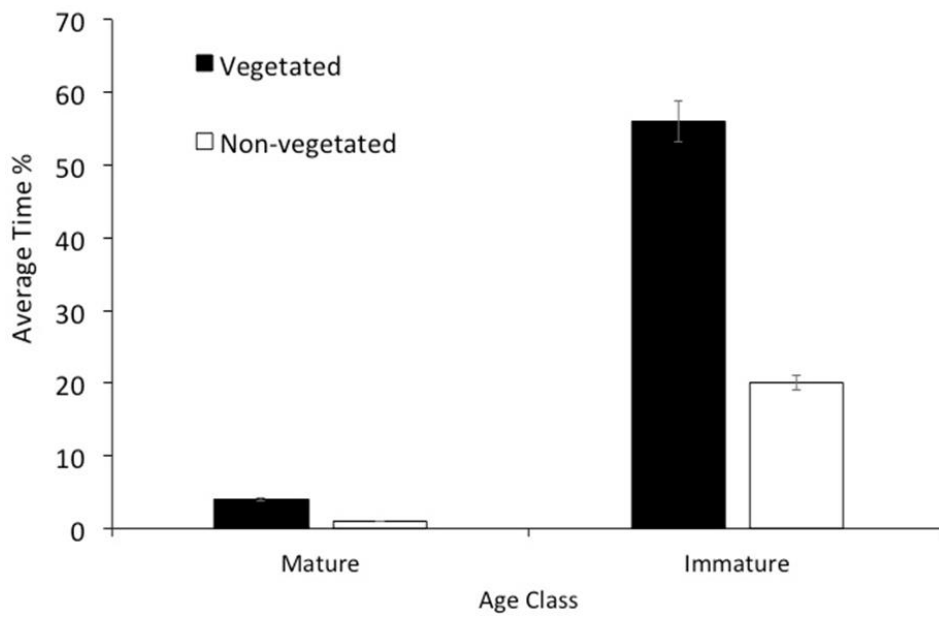


Figure 2. Average percent time spent in social-play by age group in both enclosures.



Figure 3. Monkeys perch-hanging in the non-vegetated enclosure



Figure 4. Average percent time spent perch-hanging by age class in the non-vegetated enclosure.

Behavior	Frequency	Description
<b>Perch-hanging</b>	80 (71.42)	hanging by the torso where upper and lower body dangle under the perching substrate
<b>Head-bob</b>	18 (16.07)	moving head side to side or up and down with no apparent function or receiver
<b>Twirl</b>	5 (4.46)	spinning in the same location in one leg
<b>Hair eating</b>	4 (3.57)	removing hair from own body or of another monkey and ingesting it
<b>Hair pulling</b>	2 (1.78)	pulling hair from own body or of another monkey
<b>Self-clasp</b>	2 (1.78)	clasping strongly a body part such as limbs, tails, and other extremities
<b>Feces-rolling</b>	1 (0.89)	rolling feces on the floor or walls of enclosure

Table 2. Stereotypic behaviors recorded in the non-vegetated enclosure

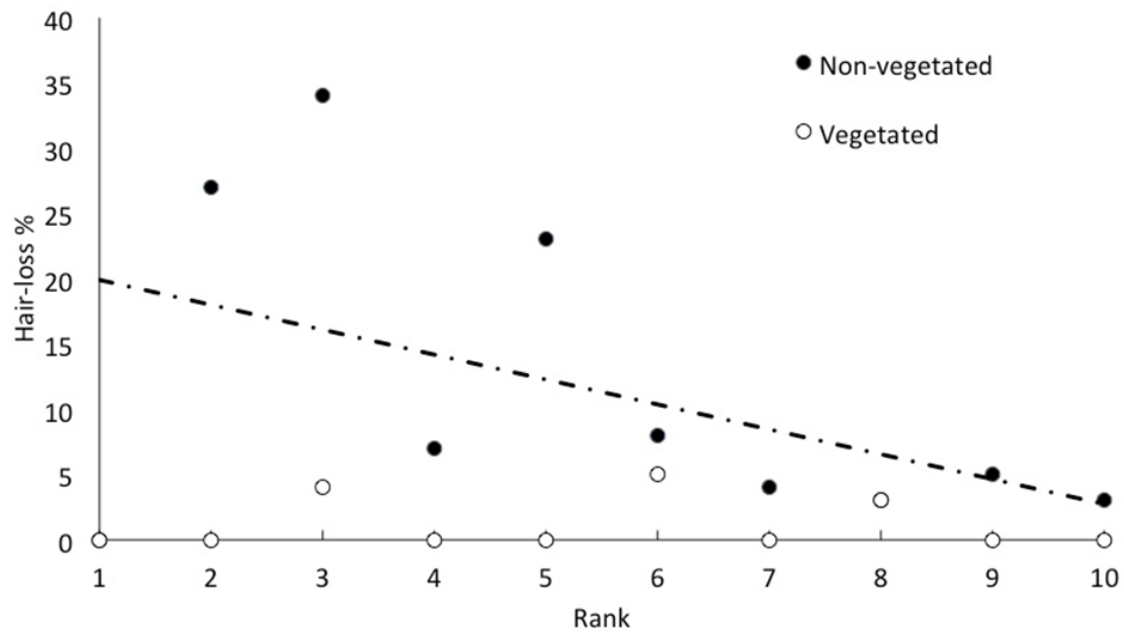


Figure 5. Relationship between hair-loss and rank in the non-vegetated enclosure.

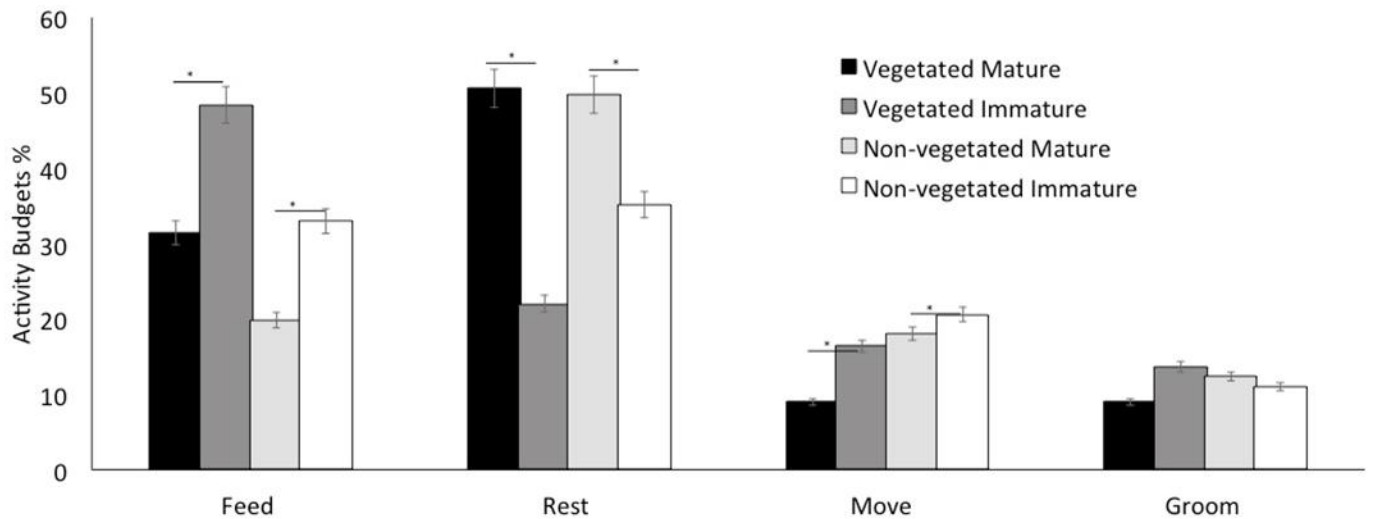


Figure 6. Average percent time spent in different activities by age class by age class in the vegetated and non-vegetated enclosure.

Study site	Duration (year)	Rest	Activity (%) feed	Move	Groom	References
Vegetated	1	26	51	18	5	this study
Non-vegetated	1	45	22	24	9	this study
Averages of the free-ranging groups below		34	41	14	11	
Takasakiyama (provisioned)	1	36	44	12	8	Soumah & Yokota (1991)
Yakushima (non-provisioned)	1	32	38	16	14	Hanya (2004)

Table 3. Comparison of the activity budgets of captive and free-ranging populations

## **Chapter 3.**

*Behavioral changes of solitary housed female pygmy slow lorises (Nycticebus pygmeaus) after introduction into group enclosures*

### 3.1. Abstract

Pygmy slow lorises (*Nycticebus pygmaeus*) are threatened with extinction in the wild. Their nocturnal lifestyle and small size make them difficult to study in their natural habitat, but increasing evidence suggests that they are more social than previously thought. Our study was designed to assess the sociability of pygmy slow lorises by transferring six adult females from solo cages into environmentally enriched group home cages at the Japan Monkey Centre's Slow Loris Conservation Centre. Two females were paired to create one group, while the other four were placed together in a second group. We compared their social interactions, activity budgets, and postural behaviors before and after social housing was initiated. We found that all-female slow loris groups had a high degree of sociality, preferred to stay close to each other, nested together every night, and spent less time in locomotion and more time grooming than when living alone. These results suggest that female pygmy slow lorises actively seek companions when available. The captive housing of all-female groups of lorises could lead to better husbandry practices and improved animal welfare by allowing them to have conspecific companions. We conclude that isosexual groups of pygmy slow lorises should be preferred over single housing when possible.

### 3.2. Introduction

Slow lorises (*Nycticebus spp.*) are a taxon of nine nocturnal species that live in Southeast Asia (Starr et al., 2011; Nekaris and Starr, 2015). Although lorises have been categorized as “Endangered” on the IUCN Red List (Blair et al., 2020), and are included in Appendix I of CITES, a listing that prohibits international trade in specimens, Japan has been a major destination for illegal pet lorises (Musing et al., 2016). The illegal smuggling of lorises into Japan, and their rescue by authorities, poses a challenge to animal welfare advocates seeking to provide the best environment possible for them. To improve the conditions of animals found in Japan, recent studies on the behavioral management strategies for male pygmy slow lorises in captivity have reported that social grooming and affiliative contact are important in their social interactions and appear to improve their well-being (Yamanashi et al., 2021).

In previous studies, pygmy slow lorises have been considered to be among the most solitary of the nocturnal primates (Petter and Hladik, 1970; Barret, 1985; Bearder, 1987). However, recent studies of both wild and captive populations have reported that they are more social than initially thought (Yamanashi et al., 2021; Nekaris, 2001). Due to the difficulty of observing and following nocturnal prosimians, details of their social system and the degree of sociality have been inferred by home range size and overlap (Bearder and Martin, 1980; Pimley et al., 2005). Some of these species were thought to maintain contact only during the mating season and between mother and offspring, with such interactions accounting for only about 2% of their entire activity budget (Radhakrishna, 2005; Nekaris, 2006). Recent radio-tracking studies of Malay slow lorises (*Nycticebus coucang coucang*) in the wild have shown that they form social networks with adults



involved in affiliative interactions for up to 8% of their activity budgets (Wiens 2002). Animals have been seen allogrooming, playing, and traveling together, as well as forming sleeping associations of two to three adults. In Sri Lanka, the closely related slender lorises (*Loris tardigradus*) spent 44% of their nightly activity together with conspecifics, and up to nine individuals occupied the same nest (Nekaris, 2006). Captive studies have also noted some degree of sociality in the slender loris (Goonan, 1993). In pygmy slow lorises, interactions arising from home range overlap have been shown to account for up to 5% of their social interactions in the wild (Starr and Nekaris, 2020). A recent re-evaluation of the evolution of social organization in Lorisiformes has shown that pygmy slow loris have the largest home range size with a multi-female type of mating system (Pointdexter et al., 2017). These findings emphasize the need for improvements to captive space in order to promote their natural tendency to have social interactions, and calls for the reconsideration of social management routines for pygmy slow lorises in captivity, as most pygmy slow lorises are still housed alone (Fisher et al., 2003).

Most literature regarding the social management of captive primates has focused on monkeys and apes, with less information reported on the strepsirrhines. They are found in captivity mainly in research facilities, followed by captive breeding colonies, and zoos, with the Duke Lemur Center (DLC) conducting close to 95% of all noninvasive research (Fernández Lázaro et al., 2017). Strepsirrhines have been considered an important suborder in understanding primate evolution, as they are considered basal primates (Agnani et al., 2021), yet except for their diurnal lemurs, much of their captive conditions are under-reported. This suborder of primates exhibits a unique set of behaviors such as torpor found in tropical environments seen in bush babies in Africa, lorises of Asia, and lemurs of Madagascar (Blanco et al., 2018). Many are nectar, exudates, and pollen feeders, providing good models for angio-sperm co-evolution (Muchlinski and Perry, 2011).

Their small size and low basal oxygen consumption also provide insights into life history and energy expenditure studies (Simmen et al., 2021). These factors that make strepsirrhines a unique group within primates also pose challenges in captivity, and what we know of them in the wild might be different and difficult to replicate in captivity. Black lemurs (*Eulemur macaco*) are mainly found in pairs in the wild, and their seasonal food scarcity leads to high food competition for which single or pair living might seem more suitable in captivity to prevent aggression, but when housed in pairs in captivity, they exhibit very low reproductive rates (Price and Stoinski, 2007). However, with the addition of nonreproductive males to the groups, reproductive rates increased by 100% (Hearn et al., 1996). Early pilot studies (Ehrlich, 1977) housing wild caught nocturnal greater galagos (*Galago crassicaudatus*) in groups of 4 (1 male and 3 females) in indoor (12' × 14') enclosures found high rates of aggression, with females being removed from the group due to injuries. Animals spent most of their time ignoring and avoiding each other when approached, agonistic interactions did not significantly decrease over time, and solicitations for play or groom were between 10 and 50% successful. In a sequential analysis of captive lesser galagos (*G. senegalensis*), Bercovitch (1978) reported that female–female aggression was not systematically followed by an escalation in threats, but most often occurred after one of the females exited a nest box, while Nash and Flinn (1978) documented more intrasexual, than intersexual, aggression in the same captive colony upon formation. As opposed to galagos, slow lorises living under the same caged conditions for the same amount of time, with equal 1 male 3 female group formations, spent most of their waking time in proximity to others, often 3–4 animals, agonistic interactions significantly decreased over time, and all forms of play or groom solicitations were 90% successful (Ehrlich, 1977).

Strepsirrhines vary in size and dietary needs, and some are diurnal while others are nocturnal; therefore, their captive housing has to be tailored to each species. Previous housing conditions for pygmy slow lorises included mixed species and enriched enclosures, but due to their endangered status as of 2019, the 50-pygmy slow lorises left in North America are now housed by the Species Survival Plan, and the DLC does not house any lorises of any species (DLC website, 2021). There are earlier reports on captive loris (*Tardigradus nordicus*) housing conditions (Schulze and Meier, 1995), varying from singly to group housed, while more recent studies on Javan slow lorises (*Nycticebus javanicus*) describe housing them in uni-male multi-female groups (Putri et al., 2015). Reports on the housing conditions and social behaviors of pygmy slow lorises under group-housed conditions remain underreported in *Nycticebus*.

As with all primates in captivity, efforts to improve cage space and complexity should be implemented, and previous work has detailed the importance of structures such as branches, nesting, feeding areas, and cage space for captive pygmy slow lorises (Fitch-Snyder and Schulze, 2001; Fuller et al., 2013). Pygmy slow lorises belong to the subfamily Lorinae that cannot leap (Sellers, 1996) but have adapted to an arboreal lifestyle as slow climbing specialist (Das and Nekaris, 2020). Providing substrates that promote these positional species-specific behaviors should be a captive behavioral and conservation management priority. Pygmy slow lorises are particularly susceptible to stress in non-natural environments and have the highest infant mortality rates of all prosimian primates in accredited zoos (Fuller et al., 2014). Positional behaviors are directly correlated to their environmental adaptations and social needs. Bridging and horizontal or vertical suspension from one to all limbs are needed for movements in the canopy, and positional clinging suspension with their hind legs is needed for mating behaviors, while cryptic slow vertical or horizontal postural modes are associated with predator avoidance or feeding behaviors (Nekaris,

2001; Goonan, 1993; Das and Nekaris, 2020; Glassman and Wells, 1994). Previous studies on positional and locomotor behaviors in lorises have been focused on anatomical evolutionary perspectives, substrate preference, and their ecological arboreal adaptations. However, none have focused on a purely quantifiable method for assessing welfare. As improvements in captive conditions include more space and complexity of substrates, their postural and locomotor behaviors should reflect that change.

One method used for assessing the welfare of captive animals is to investigate their activity budgets, especially the time devoted to conspecific interactions (Jaman and Huffman, 2008; Howell and Cheyne, 2018). Activity budgets of captive pygmy slow lorises have been restricted to singly housed males (Cabana and Plowman, 2014; Fuller, 2014), and although, recently, activity budgets of the Bengal slow lorises have been documented in the wild (Al-Razi et al., 2020), to our knowledge, activity budgets of pygmy slow lorises housed in all-female groups have not been reported. Understanding changes in behaviors and how animals spend their time may be indicative of an improvement of their welfare state. Re-fining housing conditions by the creation of isosexual groups over solitary housing in primates has been widely used as a behavioral management strategy to reduce stress in captivity (Reinhardt et al., 1991; Baker and Bloomsmith, 2012), and more recent work in the formation of male–male pygmy slow loris pairings (Yamanashi et al., 2021) has been shown to be a viable behavioral management strategy in the reduction in physiological stress while demonstrating their choice to engage in affiliative behaviors and sharing of nesting sites.

My study examined how the isosexual housing of one all-female pygmy slow loris pair and one multi-female group influenced individual behavior by comparing solitary and group activity.

My goal was to investigate whether or not behavioral changes were indicative of an improvement in captive housing in the female pygmy slow lorises.

The specific objective of this study was to provide new information about the gregariousness of pygmy slow lorises as they adapt to larger enclosures with conspecifics. I predicted that: (1) females in a same-sex group would affiliate with each other, becoming more gregarious, rather than restrict themselves to maintaining a solitary lifestyle; (2) changes in daily activity budgets would occur after the transfer to a more complex environment; and (3) postural and locomotor behaviors would be more varied living in an environment with a variety of climbing structures compared to solitary quarters without more natural substrates.

### **3.3. Materials and Methods**

#### *3.3.1. Study Site*

This study was conducted in the Japan Monkey Centre (JMC), Inuyama, Japan. One of the key goals of JMC is to promote the survival and wellbeing of endangered primate species. Between 2006 and 2007, JMC accepted more than 20 pygmy slow lorises that had been confiscated at airports in Japan over the last decade. Given increasing information from the field suggesting that lorises are more gregarious than originally thought, JMC saw the need to update their husbandry practices to improve the lives of the lorises housed. Previous reports of socially housing lorises in captivity were reported with both successful, for up to several years, and unsuccessful attempts. Most of these studies were based on solitary or male–female pairs, with no information on isosexual pairings (Fitch-Snyder and Schulze, 2001; Fuller et al., 2013; Cabana and Nekaris, 2015). To house the confiscated lorises, JMC built the “Slow Loris Conservation Centre” (SLCC) in 2015

to maintain multiple animals in a single unit. The primary goals of SLCC are to enhance the lives of illegally caught lorises by mimicking to the extent possible their natural habitat and to engage in conservation education and science activities (Yamanashi et al., 2021).

Housing is based on a reverse-lighting cycle, with red-film lights in each room during the night cycle, to minimize light pollution (Starr et al., 2012; Ariana et al., 2020). The reverse light–dark cycles have a gradual change over time, with lighting levels at 0.0 to 0.18 lux during the dark phase. The cages were regularly provided with natural branches of various sizes and girths. Wooden nest boxes were provided both in the single and social housing conditions, with branches from natural foliage for hiding places. Enrichment devices such as insect dispensers, wooden devices filled with tree gum, and various feeding stations were placed inside the new larger-enriched enclosures (Yamanashi et al., 2021). The diets were mainly composed of natural exudates (gum), various insects, and supplementary vegetables (Cabana and Plowman, 2014; Cabana and Nekaris, 2015). All animals were kept singly housed before the transfer to group housing (see 2.2.).

### *3.3.2. Study Subject Testing Environment*

Six adult females brought to the SLCC in 2016 were the focus of this study. All females appeared to be in good health after initial physical examinations but were placed in individual quarantine units as part of standard operating procedures of the JMC. Some animals may have had social interactions with conspecifics for a few years after their confiscation, but it is unclear which individuals had social housing experience and which did not (Yamanashi et al., 2021).

Females were housed in solitary cages facing each other to promote visual, olfactory, and vocal contact, enabling investigators to monitor subjects for signs of either compatibility or antagonism before pairing, but no signs of aggression or incompatibility were observed. After a

period of habituation of several weeks, the cages were placed closer to each other, with enough space to not allow physical contact, in front of their new enriched enclosures for the initial part of the observations in the single caged condition. The quarantine units were steel cages (satellite cages) ( $610 \times 70 \times 820$  mm;  $0.3 \text{ m}^3$ ), with a mesh top, bottom, and sides, a wooden nest-box, and a few vertical and diagonal branches. The group formation process was as follows: after an average of 90 days in solitary housing, for the first pair of lorises in the first group, and 150 days for the second group of four lorises, the six females were relocated to a large enriched enclosure containing tree branches, perches, and six wooden nest-boxes. The first group of two females were introduced together into a large enclosure of two separate compartments connected by multiple tunnels ( $128 \text{ cm} \times 125 \text{ cm} \times 207 \text{ cm}$  each compartment) on 25 August 2017, after a few weeks of small one-hour monitored introductions. The group of four individuals were placed into one unit ( $239 \text{ cm} \times 282 \text{ cm} \times 249 \text{ cm}$ ) together on 19 December 2017, without small introductions, as the largest enclosure was not constructed, to allow for the quick emergency separations of one individual from others. All observations in the post-social-housing conditions were made once the groups were formed without monitored separations. The potentially aggressive activities that I observed occurred in the first few introduction trials for the one pair of females. They engaged in minor hair clasps and leg grabs, and it was unclear if the initial chasing bouts were antagonistic or playful. The group of 4 also had a few events of clasping, chasing, and one bite not requiring veterinary intervention on the introduction day, but none were seen after that and they all shared the same nest from day 2. As I started our formal observations once the groups were established, and no aggression occurred during my observations, I did not report it in our findings. The period after the introduction is referred to as the social housing condition.

### 3.3.3. *Data Collection*

Behavioral data were collected in 10 min sampling sessions following Martin and Bateson (1984) and are described in Table 1. Postural data classification followed Glassman and Wells (1993) and is described in Table 2.

Daily 10 min focal recording sessions for each female were made with a night vision video camera during the night-cycle between 13:00 and 16:00 from June 2017 to March 2018, with an average of 3.14 ( $\pm$  1.21; standard deviation SD) observations per individual weekly. The same methods were used before and after the group formation. Observations for the pair group before the social housing started were conducted from June through September (3 months) and from September to February (5 months) post-social-housing, while for the group of 4, pre-social-housing was performed from November through December (2 months) and December through February (3 months) in the social-housing condition. I conducted instantaneous sampling (Martin and Bateson 1993) at one-minute intervals during the 10 min focal sample. Social gregariousness or solitariness was assessed based upon proximity. I divided proximity scores into three categories: far (opposite side of enclosure), close (within 2 m), and social (arms-reach). Positional behaviors were divided into 4 groups for statistical purposes (see Table 2). I recorded behavior using the night-mode video cameras. I collected a total of 2,740 min of behavioral data, with an average of 30 min per week (solitary:  $\pm$  56.21; SD-social:  $\pm$  118.77; SD) of data per subject.

#### 3.3.4. *Statistical Analyses*

I used the Kruskal–Wallis rank sum test to evaluate the effect of housing condition on proximity, and then performed a post hoc Dunn test with Bonferroni correction. I used analysis of variance (ANOVA) to test for differences in 1) activity and 2) positional behavior between different periods. “Activity” was used as the dependent variable and “period” as the independent variable. Where main effects were significant, I used the post hoc Tukey’s HSD test for pair-wise



comparisons. All datasets were checked for normality with the Kolmogorov–Smirnov test and for homogeneity of variance using Levene’s test ( $p > 0.05$ ). Statistical significance was set at  $p < 0.05$  and two-tailed results were reported. *P*-adjusted values were used for multi-pair comparisons where ANOVA was conducted with nonhomogeneous data. All statistical analyses were conducted with RStudio version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria).

### 3.4. Results

#### 3.4.1. Proximity

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) (Figure 1).

#### 3.4.2. Positional Behaviors

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 ( $p = 0.98$ ) (Figure 3).

### 3.5. Discussion

### 3.5.1. *Sociality Among Female Pygmy Slow Lorises*

The group housing of adult male pygmy slow lorises as a behavioral management strategy has been shown to improve their well-being (Yamanashi et al., 2021). As with their male counterparts in the previous study (Yamanashi et al., 2021), the newly formed female social groups showed little to no aggression, even though twice as many animals (4) were introduced into the same amount of space per group. Our study provides further evidence that pygmy slow lorises are not only gregarious animals, but also not antagonistic toward conspecifics. Female–female dyads were quicker to form affiliative relationships when compared to our male–male pairs (Yamanashi et al., 2021), providing a stronger case for female pairings as a viable behavioral management strategy to improve loris well-being in captivity. Dyads slept together in the same wooden nest-boxes, rather than alone, and they spent about 10% of their time grooming each other.

It has been shown that lorises in captivity (*Nycticebus coucang*) prefer to sleep in dark areas of their enclosures, especially in wooden nest-boxes when natural foliage is not readily available (Chinn, 1980), and our findings show that females preferred affiliative interactions in the nesting area over more ample and solitary spaces. At the SLCC, all groupings of males and females interacted in similar ways when housed with same-sex conspecifics. Both sexes tended to sleep with others, not alone, and both readily engaged in allogrooming. However, one difference is that females, unlike males, did not change nesting sites after the first day of introduction, while males slept in different nests over time (Yamanashi et al., 2021). This resembles findings in wild populations of slow pygmy lorises in Cambodia, where females returned to the same sleeping sites while males did not (Starr and Nekaris, 2020). In captivity, *Nycticebus coucang* females housed together have been reported to actively seek each other for grooming and affiliative interactions (Ehrlich, 1977), and our females also behaved similarly. Our findings also resemble those found

in female macaques housed in laboratories (Cassidy et al., 2020); when continuously paired over intermittently paired, females show a high degree of affiliation. It may not be possible to provide social housing to all animals, but whenever possible, all-female social housing should be tried in slow pygmy lorises over single housing to improve their captive care.

### 3.5.2. *Activity Budget*

Our findings supported our predictions that daily behavioral activity changed once the female pygmy slow lorises moved to their socially enriched enclosure, with a decrease in movement and an increase in grooming. Social grooming has been reported to help maintain group cohesion, reduce intergroup hostility (Cheney, 1992), assist in establishing and maintaining dominant–subordinate relations, and informing nonkin alliances (Seyfarth, 1977; Chapais et al., 1991). The female pygmy slow lorises in our study started allogrooming after group formations, suggesting that they are a relatively gregarious species. One would not expect solitary species to interact in this manner. For example, koalas (*Phascolarctos cinereus*) are a solitary species, and when females are housed together, they rarely, if ever, sit in the same tree (Bercovitch, pers. obs.). On the other hand, self-grooming significantly increased among female pygmy slow lorises housed together and is often considered a sign of increased stress (Maestriperieri et al., 1992). However, self-grooming is context-dependent, and for *Nycticebus* spp., self-grooming plays important and complex roles. Pygmy slow lorises rely heavily upon olfactory cues for mate selection, competition, and territorial behaviors. Lorises use the chemical compounds in their saliva mixed with secretion from their brachial glands as a protection from predators, as well as ectoparasites, and their glands become active as young as 6 weeks old (Hagey et al., 2007). In the wild, self-grooming may function to reduce ectoparasites in various primates. Fisher et al. (2003) studied countermarking among singly housed captive pygmy slow lorises at the San Diego Zoo and demonstrated the

importance of chemical signaling in their behavioral repertoire. It has been hypothesized from recent findings that lorises undergoing solitary torpor or infant parking may self-groom, covering themselves or their infants with protective venomous saliva (Nekaris et al., 2013). This appears to be a naturally occurring behavior that may not necessarily indicate stress or poor welfare in captivity. Ehlich and Musicant (1977) reported that Sunda slow lorises (*Nycticebus coucang*), living socially in one-male-multi-female groups of wild-caught individuals in captivity, had grooming and self-grooming at the top of their activities in social behaviors, with 5% of their time spent in social grooming and 8% in self-grooming under social conditions. Grooming was also a common behavior noted during bouts of play. In the same species (*Nycticebus coucang*), some studies have reported that males self-groomed more than females (Hagey et al., 2007; Duncan, 1982), while other studies (Tenaza et al., 1969) have documented the opposite trend. In summary, while allogrooming is an indicator of sociality, self-grooming need not be an indicator of elevated stress and could even be adaptive for pygmy slow loris chemical communication.

Translocation to the larger social enclosure resulted in a significant decrease in moving time, which seemed to be compensated for by increases in both self and social grooming. Although not statistically significant, the increased time spent eating could have resulted from access to enrichment devices with insects and tree gum that were not available in their small cages, due to a lack of space. I suggest that the larger enriched enclosures with feeding devices such as tree logs with gum may have provided more opportunities for greater diversity in food resources, and more natural feeding postures (Williams et al., 2015), which should enhance animal welfare. Given the high level of sociality that appeared when placed with conspecifics, the principal finding of our study is that female pygmy slow lorises probably benefit from living in an enriched large enclosure with other females because it provides an avenue to express their natural behaviors. As reported

by Yamanashi et al. (2021), male lorises opted to feed, travel, and nest together, suggesting that both sexes are more gregarious than often assumed. In the wild, both male and female pygmy slow lorises have been found to have a large home range relative to their small body size (Starr and Nekaris, 2020). The pygmy slow lorises engaged in social behaviors such as feeding together, as well as social grooming. After an initial exploration of their new enclosure, time was most likely better spent in social behaviors rather than locomotion. The complexity of the social-housing condition of both enclosures provided more behavioral opportunities. Leisure time, social grooming, and resting with other females in the wooden nest-box seem like plausible explanations for a significant decrease in moving time.

### 3.5.3. *Postural and Locomotor Behaviors*

Although there were some differences in the setup of the pair and four group enclosures, both had access to a dozen climbing structures, vines, perching, platforms, and other structures where they could suspend themselves from. In general, female pygmy slow lorises decreased the time spent moving when in the larger enclosure, as well as increasing the times spent stationary. I suggest that this combination of findings mimics the natural postural and locomotor behavior of the species in their natural habitat. Our findings resemble those reported for socially housed slow lorises (*Nycticebus coucang*) that spent more time sitting as the most important postural behavior in social interactions when in proximity to several animals, and our pygmy slow lorises behaved the same while self-grooming and allogrooming (Ehrlich, 1977). In our study, a likely possibility is that sitting increased because the animals were grooming more, and thus spending less time in quadrupedal locomotion. The lack of significant differences in other postures between solo and social housing could have resulted from the relatively enhanced conditions of the solo enclosures, where animals could still cling to branches and move about on multiple substrates. The larger and

more complex social enclosure was essentially a larger, and more complex, scale of the heavily enriched small solitary cages. Although not significant, one postural mode behavioral difference that I did record was in “bridge” behavior. Instead of jumping or leaping from tree to tree, a common postural accommodation for moving across various substrates among lorises is “bridge” (Glassman and Wells, 1984). This activity is used by an individual to pull a branch toward it in the canopy and elongate his body to travel across the canopy from branch to branch without having to go down to the ground, exposing itself to predation. The enriched small enclosure might not have had enough room above the horizontal bridge for them to express this behavior as much. Contrastingly, the significant decrease in climbing up or down, or walking on all fours around the enclosure, was most likely due to their increase in time inside the one wooden nest-box shared by all. A decrease in some locomotive postures may be indicative of a decrease in stress-related behaviors such as pacing (one female engaged in stereotypic pacing before being socially housed), while an increase in postures such as sitting may be related to more relaxed states and affiliative interactions that could be associated with compatibility with cage mates and an increase in affiliative interactions. A behavioral management plan that includes scanning postures of an animal that is hard to observe because of their size and lighting conditions could give us insight into their welfare state.

### **3.6. Conclusions**

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.

### **3.7. Figures and Tables**

<b>Behaviors</b>	<b>Description</b>
<b>Rest</b>	The body is immobile and not engaging in any activity
<b>Feed</b>	Consuming any animal or provisioned food or mastication
<b>Move</b>	Any mobile activity in any direction
<b>Self-Groom</b>	Licking or combing with teeth its coat
<b>Social-Groom</b>	Actively licking or combing with teeth a conspecific's coat or receiving the same

Table 1. Behavioral ethogram. Based on Nekaris (2001) and adapted to our study.

<b>Postural Mode</b>	<b>Posture</b>	<b>Description</b>
<b>Locomotion Posture 1</b>	Climb up	climbing up using all limbs
	Climb down	climbing down using all limbs
	Quadrupedal walk Cling	walking on all four limbs hanging with more than two limbs
<b>Locomotion Posture 2</b>	Quadrupedal stand	standing with all four legs
	Bipedal stand	standing with hind legs
	Bipedal hang	hanging face down with hind legs
<b>Positional Posture 3</b>	Sit	sitting or lying down
	Sleep ball	curled up in a ball
<b>Positional Posture 4</b>	Bridge	extending all limbs to grab or move between two substrates
	Other	unclear posture or transitional position

Table 2. Postural modes and locomotor postures. Postural behaviors adapted from Glassman and Wells (1993).



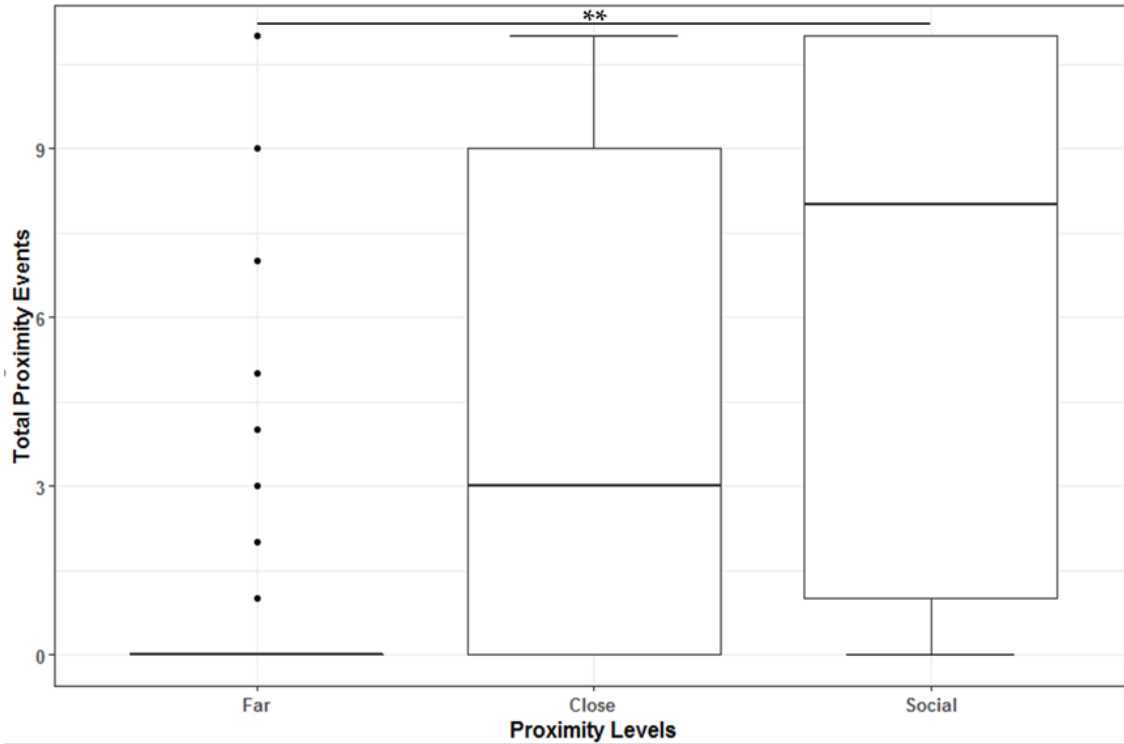


Figure 1. Boxplot representing all observations in the three types of proximity, where the boxes represent the lower and upper quartiles, whiskers represent the minimum and maximum observations, and horizontal line represents the median. Statistical significance codes: \*\* 0.01.

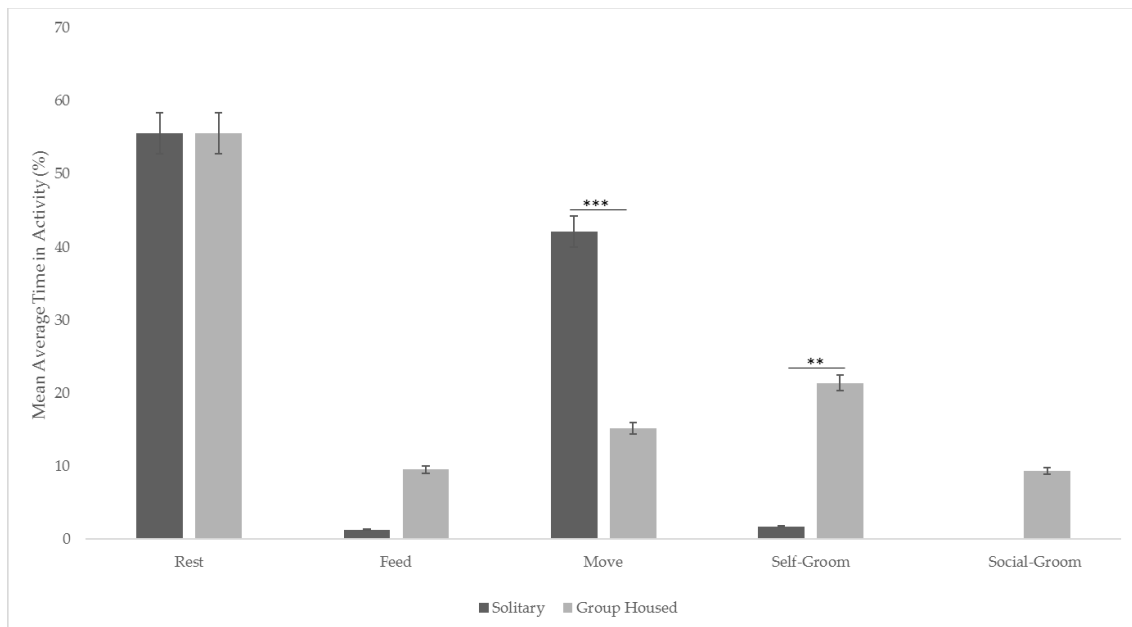


Figure 2. Activity budgets before and after group formations with bars representing standard error SE and Significant. Statistical significance codes: \*\*\* 0.001 \*\* 0.01.

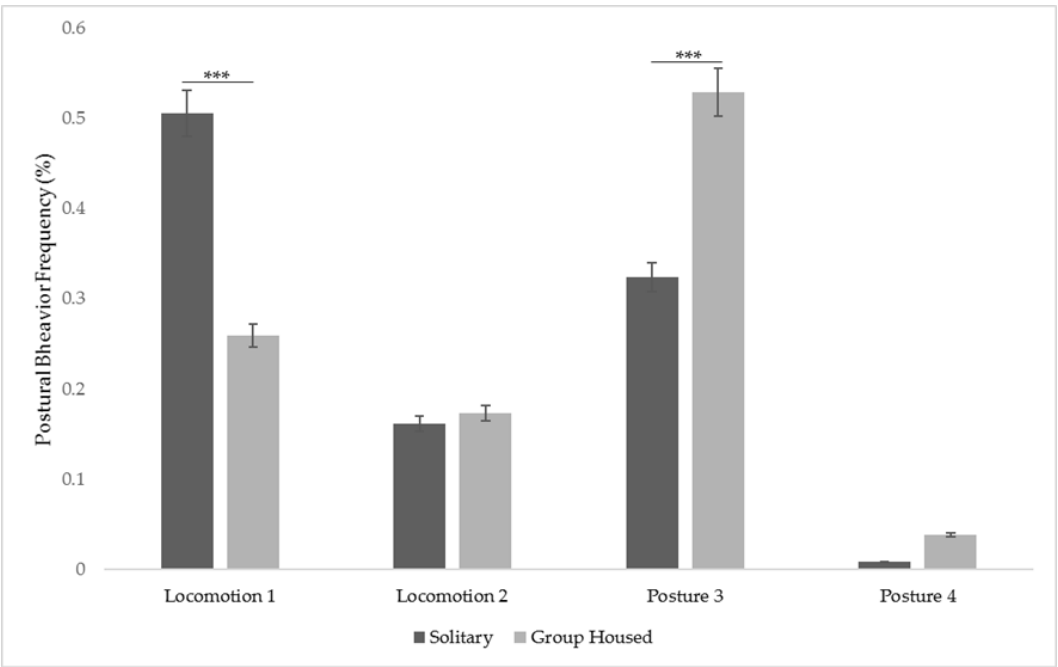


Figure 3. Locomotor postures and postural modes before and after group formations with bars representing standard error SE. Statistical significance code: \*\*\* 0.001.

## Chapter 4

Reducing stress and stereotypic behaviors in captive  
female pygmy slow lorises (*Nycticebus pygmeaus*)

#### 4.1. Abstract

Improving captive conditions of pygmy slow lorises (*Nycticebus pygmeaus*) poses many challenges because detailed aspects of their lives in the wild are incomplete. This hinders efforts to replicate sustainable environments for them. To improve their well-being in captivity, 8 rescued female pygmy slow lorises at the Japan Monkey Center (JMC) were socially housed in two types of groups following their solitary housing: two pairs and one group of four individuals. They spent much of their time in affiliative behaviors, as well as sharing sleeping sites after placement in a social group. The purpose of my study was to examine whether social housing helped in reducing stress by comparing fecal glucocorticoids and stereotypic behaviors when housed alone and when with conspecifics. Overall, the levels of fecal glucocorticoids were significantly lower when socially housed, than when kept alone. One individual exhibited stereotypic behavior when housed alone, but this behavior disappeared after social housing. These findings support recent evidence that pygmy slow lorises are social animals and will benefit from group housing in captivity. I conclude that social housing of slow pygmy lorises improves their well-being by reducing stress levels, and that the group housing of loris in captivity can provide dividends for the conservation of this endangered nocturnal primate because lorises intended for release should find it easier to adapt to natural conditions.

## 4.2. Introduction

### 4.2.1. Challenges with lorises

Pygmy slow lorises (*N. pygmaeus*) are nocturnal primates endemic to Southeast Asia. They are exudativorous, give birth to twins, have venomous bites, hibernate during the winter months, and are the smallest and fastest of all the loris (Nisbett, 1993; Cabana and Plowman, 2014; Madani and Nekaris, 2014; Ruf et al., 2015; Gardiner et al., 2018). Both Sumatran orangutans (*Pongo pygmaeus abelii*) (Utami and Van Hooff, 1997) and Bornean orangutans (*P. pygmaeus wurmbii*) (Makur et al., 2022) have been observed eating slow lorises, but most of their threats are anthropogenic, including deforestation, hunting for the illegal pet trade, and their use in traditional medicine (Ratajszczak, 1998; Nahallage et al., 2008; Starr et al., 2010; Iseborn, 2011; Feng, 2012, Nekaris and Starr, 2015; Musing et al., 2016). As a result of their drastic population decline pygmy slow lorises are categorized as “Endangered” by the IUCN Red List (Blair et al., 2020). Field surveys in Cambodia revealed that between 2001-2008, populations considerably decreased. Those populations distributed along the Mekong River find themselves in what has been termed a “wildlife trade hotspot”, where this practice poses a disproportionately large threat to the species (Davies, 2005). Malaysia, Vietnam, Indonesia and China as the major exporters of wild-caught live animals, and the European Union and Japan are the most significant importers (Nijman, 2010). In a coordinated international effort to combat the illegal wildlife trade, new laws, enforcement, and interdiction have resulted in an increase in the number of live wild animals seized and housed by government organizations (Fuller, 2013; Maddison, 2019; UNODC, 2020). Several legal and logistical factors make it difficult for confiscated animals to be released back into the wild, and a CITES report found that 86% of confiscated live animals are subsequently sent to captive facilities, of which many are unregulated prone to poor conditions and exploitation (Zhou et al., 2016; CITES

2017; Rivera et al., 2021). In Japan, 400 individuals were confiscated between 2000 and 2013 (Musing et al., 2016). Unfortunately, many confiscated lorises are still found in less than optimal conditions in captivity (Yamanashi et al., 2021).

#### 4.2.2. *Lorises in the wild*

In the wild, lorises have rarely been recorded to form groups, but recent data suggests they are more social than initially reported. Observations of multiple individuals of the Mysore slender loris (*Loris lydekkerianus lydekkerianus*) show that they often share sleeping sites (Nekaris, 2006), and adult pygmy slow loris (*N. pygmaeus*) females sleep with their young or other sub-adult females (Starr et al., 2011; Starr and Nekaris, 2020). Long-term field data in West Java of slow lorises (*N. javanicus*) show that they too often share sleeping sites with conspecifics (Nekaris, 2014), and home-ranges overlap (Starr and Nekaris, 2020). Released female pygmy slow lorises in Vietnam have been shown to be tolerant of each other and overlap in some areas of their home range (Pointdexter et al., 2017).

#### 4.2.3. *Benefits of social housing in captivity*

For most primates in captivity, social housing is the default housing status, and it is an important requirement that should be met whenever possible (AWA, 1985). When social housing and adequately complex living spaces are provided to primates, most animal welfare concerns can be alleviated (Lutz and Novak, 2005; Hannibal et al., 2017). Social housing has been well documented to decrease stereotypic behaviors, prevent chronic diseases related to stress, and promote general well-being across various primate species, including pygmy slow lorises (Bernstein, 1991; Leonardi et al., 2010; Baker et al., 2012; Yamanashi et al., 2021). As animals move into an enriched space with conspecifics, a myriad of behaviors can be expressed, potentially

modulating the adverse effects of circulating cortisol through the resultant positive social interactions among conspecifics (Bercovitch and Ziegler, 2002).

#### 4.2.4. *Costs and benefits of social housing*

Nonetheless, for primates living in captivity, group living has both costs and benefits, under stressful circumstances it can be detrimental to health, while access to conspecifics usually has the opposite effect. Positive interactions with other individuals where close social bonds are formed can help buffer HPA axis activity, lowering fecal cortisol levels (Cohen and Willis, 1985). Similar relationships between stress and affiliative behaviors have been reported in various primate species. Strong social bonds between male Barbary macaques (*M. sylvanus*) help reduce environmental stress (Young et al., 2014), female yellow baboon grooming networks (*Papio cynocephalus*) help alleviate stress in the wild (Witting et al., 2008), and affiliative interactions help reduce stress in chimpanzees (*Pan troglodytes*) from daily stressors (Witting et al., 2016), while bonobos responding to other conspecifics distress has the same effect (Clay and de Waal, 2013). Nonetheless, social housing can lead to aggression and have adverse effects by increasing stress, even when group formations mimic those found in the wild. Early reports of socially housed slow lorises (*N. coucang*) in breeding colonies found that the amount of space affected locomotion, rates of social and self-grooming, and the frequency of fights between adult and juvenile slow lorises that required removal or intervention (Dasbach et al., 1983).

#### 4.2.5. *Success rates of social housing*

When attempting social housing, a primate's social system and organization must be taken into account to maximize success (Thompson, 2015), as not all pairs and group formations are equally successful or stress free. A retrospective study comparing the success rates of pairing owl

monkeys in captivity (Williams et al., 2017) showed an overall 82% success rate, with female-female pairs having a 62% success rate compared to a 40% success rate with male-male pairs. While rhesus macaques (*M. mulatta*) paired in laboratory settings have been reported to have an inverse success rate, with male-male pairing success at 95%, compared to 88% for female-female pairs. Previous studies have reviewed success and failures in housing lorises in captivity in European and American zoos (Dixson, 2012; Elvidge, 2013; Fuller et al., 2014), but none have discussed the stress reducing potential of female-female pairs compared to male-male or breeding pairs.

#### 4.2.6. Social housing to reduce stress and stereotypic behaviors

Although stereotypic behaviors in lorises have been reported (Moore et al., 2015), few have focused on the physiological responses and their implications for welfare (Yamanashi et al., 2021). When animals are faced with a physical or mental stressor, they rely on a complex interplay between several biological systems (behavioral, autonomic, neuroendocrine, and/or immunological) to elicit responses to cope with various kinds of stressors (Palme et al., 2005). An association between levels of fecal cortisol and higher rates of stereotypic behaviors has been studied across various species such as mink (*Mustela vison*) (Malmkvist et al., 2011) and panda (*Ailuropoda melanoleuca*) (Liu et al., 2006). Tigers (*Panthera tigris*) and leopards (*Panthera pardus*) show higher fecal cortisol levels exhibiting stereotypic behaviors in zoos under stressful conditions (Vaz et al., 2017), while horses have lower fecal cortisol levels in compromised welfare conditions (Pawluksi et al., 2017). Female squirrel monkeys (*Saimiri sciurius*) housed in pairs had significantly lower plasma cortisol levels than squirrel monkeys housed individually, but lower cortisol levels than those housed in larger social groups (Gonzales et al., 1982). Since many biological systems are involved in such behavioral responses, with potential HPA axis responses



varying depending on intrinsic and extrinsic factors, with methods (hair, plasma, discharge) also varying on stability (Novak et al., 2013), rescued wild animals without clear rearing or health histories pose challenges in interpreting behavioral adaptations concerning their physiological responses. Therefore, a combination of behavioral and physiological responses as an attempt to improve their welfare state should be investigated and interpreted on a case-by-case basis.

The main purpose of this study was to examine the relationship between stress hormones, housing condition, and behavior among adult female slow lorises confiscated from illegal markets. A previous study revealed that females were more social than expected, and that their behaviors significantly changed once paired; these changes should be reflected in their hormonal profiles (Alejandro et al., 2021). Additionally, one of our subjects displayed stereotypical behavior (see Methods), allowing us to assess the impact of housing condition on hormones and stereotypies. Here, I hypothesized that: 1) successful female pairings should be accompanied by reduced levels of fecal cortisol, 2) the hormonal responses when pairing adult female lorises would differ depending on the type of pairing, 3) behaviors observed when singly housed, such as pacing, should be associated to higher levels of stress, 4) the female that exhibited stereotypic behaviors should exhibit higher levels of fecal cortisol than the rest of her cohort. Subsequently, when female-female pairs are disbanded and placed into female-male pairs, 5) female-female pairs should have higher levels of fecal cortisol than the female - male pairs.

### **4.3. Materials and Methods**

#### *4.3.1. Study Sites and Subjects*

The study took place at the Slow Loris Conservation Center (SLCC) in the Japan Monkey Center (JMC) where pygmy slow lorises confiscated at various airports in Japan between 2006

and 2007 were brought. The purpose of establishing the SLCC was to improve the welfare of slow lorises, conduct outreach education and awareness for visitors, and provide an opportunity for slow loris research. The confiscated animals were moved in 2015 after the enclosures were refurbished for slow lorises. The enclosures were with provided with adequate lighting, one nest box per individual, branches, tree trunks to mimic natural conditions, and enrichment devices. Diet for the animals consisted mainly of gum, insects, and vegetables (Cabana and Plowman, 2014; Cabana and Nekaris, 2015). Our eight study subjects were housed in small quarantine cages, (50 × 50 × 75 cm; 0.3m<sup>3</sup>) before being moved to the larger permanent enclosures (Table 1). The permanent enclosures ranged from two compartments of around 1.5 m<sup>3</sup> (0.91 x 0.92 x 1.78 m) with multiple tunnels in the smallest one, a medium-sized enclosure consisting of two compartments with multiple tunnels (128 cm × 125 cm × 207 cm each compartment), and the largest enclosure with open space for social grouping (239 cm × 282 cm × 249 cm), where the pairs and groups were formed (Figure 1). The study subjects were placed into two pair groups and one foursome group after being removed from their quarantine cages. Establishment of the first female pair was accomplished 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 during the process until the summer of 2018, when male-female pairs were formed.

The collection of fecal samples (see below), as well as behavioral data, continued throughout the study. Details of the pairing process, the history of SLCC, the behavioral and physiological responses of male pygmy lorises, and the behavioral changes for the female pairings have been reported in detail elsewhere (Yamanashi et al., 2020; Alejandro et al., 2021). This study conformed to “The Guide for Animal Research Ethics” of the JMC, legal requirements of Japan, and the ASP Principle for Ethical Treatment of Non-Human Primates. This study was approved

by the ethics committee of JMC (#2015001, #2016001, #2017003) and the Institutional Review Board of the Primate Research Institute, Kyoto University (# 2018-159).

#### 4.3.2. Behavioral data collection

Behavioral data were collected in 10 min continuous focal sampling sessions following Martin and Bateson (1993). Postural behaviors were classified following Glassman and Wells (1984). Daily 10 min focal recording sessions for each female were made with a night vision video camera during the night cycle between 13:00 and 16:00 from June 2017 to March 2018, with an average of 3.14 (1.21SD) observations per individual weekly. The same methods were used before and after group formations. Pair group observations before social housing commenced, were conducted from June through September (3 months) and from September to February (5 months) post-social-housing, while for the group of 4, pre-social-housing was performed from November through December (2 months) and December through February (3 months) in the social-housing condition. For behavioral data collection, I conducted instantaneous sampling at one-minute intervals during the 10 min focal sampling, recorded their postures, and assessed sociality based on proximity (Alejandro et al., 2021). Other behaviors, such as stereotypies, were collected *ad libitum*. Additionally, one of our pygmy slow lorises, Sakura, exhibited a repetitive pacing behavior in the singly housed condition that was followed by self-squeezing through the nest where she remained stuck, accompanied by loud vocalizations, until she removed herself from the opening in the nest, repeating this behavior several times (Figure 2). Although all other pygmy slow lorises were housed with similar nest boxes, perching, and cage size in the singly housed condition, no others were observed engaging in this behavior. The emergence and potential reasons for this behavior were limited as Sakura was confiscated at an airport with no data on her health or life history.

### 4.3.3. *Physiological Stress*

#### 4.3.3.1. *Fecal sample preparation*

Fecal samples were collected during light cycles by the husbandry staff (2016-2018) and JA (2017) during observation hours for subsequent analysis by EIA. All samples were stored in a freezer at -20°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°C) at 5,000 r/m. The supernatant was used for hormonal analyses by enzyme immunoassay.

#### 4.3.3.2. *Cortisol EIA*

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). Briefly, the fecal extracts were diluted 98-100 fold with EIA buffer (0.15-M NaCl, 0.04-M Na<sub>2</sub>HPO<sub>4</sub>, 0.1% bovine serum albumin, pH 7.2) and duplicates of 50 µl aliquots of this solution were added to 96-well plates bound with goat anti-rabbit IgG (H&L) Affinity Pure (A16112, Invitrogen MD USA). To obtain the standard curves, 0.024–50 ng/ml of cortisol (H4001; Sigma-Aldrich Co.) diluted with EIA buffer were also dispensed into the wells in duplicates. Immediately after the addition of 50µl of cortisol antiserum (x40000, FKA404E; Cosmo Bio Co., Ltd., Tokyo, Japan) and an equal volume of horseradish peroxidase-conjugated cortisol (x80000, FKA403; Cosmo Bio Co., Ltd., Tokyo, Japan), the plates were incubated in the dark overnight at 4°C. The following day, plates were washed to separate free from standard, control, and sample-bound wells, and 50 µl of substrate buffer was added to all wells, and then incubated in a dark sealed shaking incubator (300 rpm) at 37°C for 25 min. After

color development, the reaction was stopped by 50  $\mu$ l of 6N H<sub>2</sub>SO<sub>4</sub> and read at 450 nm absorbance. The values were expressed as the means of duplicate determinations, corrected for extraction recovery, and indicated as ng/mg of dry fecal weight.

#### 4.3.3.3. Assay validation

To validate the cortisol assay for fecal samples of slow lorises, a recovery test was conducted by adding a known amount of cortisol standard to a subset of sample extracts (Keay *et al.*, 2006). The mean recoveries of the high, medium and low cortisol spikes from the fecal extracts were 100.9 %, 88.5 %, and 106.2 %, respectively. Parallel displacement curves were obtained by comparing serial dilutions of pooled fecal extracts and cortisol standard preparation. The pooled fecal extracts consisted of the three loris housing conditions; singly housed, pairs, and the group of four. The slope generated by three pools did not significantly differ from the cortisol standard curve ( $F(2, 63) = 0.2, p = 0.82$ ). The average 90 % binding concentration of 10 assays was 0.0133 ng/mL (0.665 pg/well). All results were obtained within assay confidence range levels. Inter-assay variabilities were 18.9% (high) and 17.2% (low), and intra-assay variabilities 13.2 % (high) and 11.8% (low). Data with a coefficient of variation values that were higher than 10% were re-assayed for the analysis.

#### 4.3.4. Statistical Analysis

All statistical analyses were conducted with RStudio version 4.02.0.0 (R Core Team, 2020). I used repeated measures of ANOVA to test for differences in hormonal profiles between 1) pre- and post-social housing, and 2) individual cortisol levels in the single housing compared with the slow loris engaged in stereotypic behaviors. As our hormonal data were not normally distributed, they were log transformed (Sollberger and Ehlert, 2016) to remove skewness. The findings were

reported as log (ng/dry fecal g). Hormonal profiles were used as the dependent variable and housing condition (single vs social) as the grouping variables. P-adjusted values were used for multi-pair comparisons where ANOVA was conducted. Where main effects were significant, I used the post hoc Tukey's HSD test for pair-wise comparisons. All datasets were checked for normality with the Shapiro-Wilk test and homogeneity of variance using Levene's test ( $p > 0.05$ ).

To explore associations between activities and postural behaviors with our fecal glucocorticoid findings in the singly housed condition, I conducted a linear mixed-effects model, with fecal cortisol as the dependent variable, while activity and positional behaviors were the independent variables, with dates, time, and animal subjects as random factors (lme4 and drop-1 function). Activities and cortisol were compared when fecal samples were collected, with data points excluded from each subject when fecal samples and behavioral data were not matched ( $> 24$ hr). To compare fecal cortisol levels of socially housed females' vs subsequent male-female pairs, I used a non-parametric Wilcoxon-sign rank test. Statistical significance was set at  $p < 0.05$  and two-tailed results were reported.

#### **4.5. Results**

Female pygmy slow lorises exhibited statistically significant lower levels of fecal cortisol once socially housed in an enriched enclosure ( $F(1,127) = 3.24, p = 0.03$ ; Shapiro Wilk (SW) normality test:  $W = 0.99, z = 1.23, p = 0.21$ ). The first pair of females in the smaller housing condition was the only pair with results opposite to our predictions as they showed a significant difference between single and social housing, with a significant increase in fecal cortisol ( $F(1,98) = 4.25, p = 0.04$ ). The second pairing that was attempted, with Mizuki and Nanten, in a bigger enclosure allowing for 1hr introductions. A statistically significant decrease in fecal cortisol

between single and social housing ( $F(1,119)=12.94, p < 0.001$ ). Post-hoc tests showed a significant difference between the singly housed ( $n=28$ ), the initial 1hr introduction phase ( $n=37$ )  $z = 4.68, p < 0.0001$  (Figure 3) and the fully social condition ( $n = 56$ )  $z = 2.28, p = 0.03$ . While the 4 female group showed a decrease in fecal cortisol over time, the differences were not statistically significant in the socially housed condition ( $F(1,126) = 0.14, p = 0.07$ ) (SW normality test:  $W = 0.99, z = 0.35, p = 0.723$ ). The loris exhibiting stereotypic behaviors from the group of 4, had statistically significantly higher levels of fecal cortisol than 2 of her cage mates in the single condition ( $F(4,123) = 3.32, p = 0.01$ ) (Sakura ( $n = 16$ )-Momiji ( $n = 16$ )  $z = 2.17, p=0.03$ , Sakura ( $n = 16$ )-Tsubaki ( $n = 12$ )  $z = 2.58, p=0.01$ ), but not with Roji ( $n = 13$ )  $z = 1.81, p=0.07$ ) (Figure 4). There was a statistically significant association between moving and cortisol, with higher levels of fecal cortisol and time spent moving ( $SE = 0.35, df = 47.47, p = 0.01$ ). However, the opposite effect was observed with statistically significantly lower fecal cortisol levels with time spent resting ( $SE = 0.36, df = 51.8, p=0.009$ ). There were no significant associations between fecal cortisol and self-grooming ( $SE = 1.49, df = 67.32, p = 0.67$ ), feeding ( $SE = 2.30, df = 68.89, p=0.72$ ), or any of the four postural behaviors (See Table 2). No significant differences in cortisol levels between female-female ( $n = 6$ ) housing and male-female ( $n = 6$ ) housing ( $W = 12, z = 0.65, p = 0.39$ ) were observed.

## 4.6. Discussion

### 4.6.1. *Physiological response coincides with affiliative interactions*

Regarding prediction 1, fecal cortisol levels decreased in most females after changing from a solitary quarantine enclosure to a socially housed environment; a pattern also reported among male pygmy slow lorises (Yamanashi et al., 2021). Nest sharing, affiliative behaviors, and lower

levels of stress continued for over 30 days. After the first night, they never slept alone, and such quick affiliation and less time moving could be one of the main causes for a decrease in fecal cortisol levels when socially housed.

#### 4.6.2. *Pair formation and stress response*

Prediction 2 was met, as physiological responses varied according to the type of pairing. The first pair attempt was housed in the smallest enclosure and did not show a significant decrease in cortisol after pairing, and there was no long-term success (> 60 days). They were later separated because, although no aggressive interactions were recorded, one female sustained an injury that needed medical care. Our findings align with the recommendations of Fitch-Snyder and others (2008), that a minimum cage size of 2.5m x 2.5m x 2.5m with enough furnishing should safely house 1-3 lorises. The smallest cage was close to the minimum requirement for two lorises, suggesting that perhaps the minimum is not suitable and should be increased for more than one animal.

For the second female pair, I was able to document the slow introduction pairing in a large enclosure, where animals could be separated easily through tunnels. The second pair in our study, Mizuki and Nanten, showed a significant difference in fecal cortisol levels between all different housing statuses; single, 1-hour introduction, and fully socially housed. From the weeks spent on each housing status, 1-hour pairing showed the lowest levels of fecal cortisol. Intermittent pairing, daily separation and re-pairing of captive caged primates (Hannibal *et al.*, 2018), are commonly used in macaques when full contact social housing is not possible. As our results showed lower levels of fecal cortisol over single housing, it seems it could be feasible for the species when full-time pairings cannot be achieved. Crab-eating macaques (*M. fascicularis*) are intermittently paired



or given contact with others through grooming-contact bars due to research constraints (Lee et al., 2012). In such cases they seem to benefit from affiliative interactions, hence a better welfare state, compared to housing in solitary conditions.

#### 4.6.3. *Physiological response to behaviors*

Regarding prediction 3, that behaviors expressed when solitarily housed would be reflected in our physiological findings was accomplished. The time spent moving and engaged in stereotypic behaviors was associated with higher levels of fecal cortisol, while the opposite was noted for resting. Although not significantly different, there was a trend for postural modes associated with pacing in a circle (locomotion) inside the cage to be associated with higher levels of stress. Our results are consistent with other studies comparing environmental housing and fecal cortisol associations in spider monkeys (*Ateles geoffroyi yucatanensis*), with the natural environments having the lowest levels (Rangel-Negrin et al., 2009). Emphasizing the importance of resting, Ebinghaus and others (2020) found that cows with more resting spaces have lower levels of fecal cortisol than those with less. The range of behaviors that can be expressed by captive animals is dependent of the amount and type of space provided (Daschbach et al., 1983). Generally, for primates, limited space such as quarantine cages are apparent by their behaviors and can be validated by physiological measures.

Regarding prediction 4, Sakura, the female exhibiting stereotypic behaviors when singly housed, had significantly higher levels of cortisol than most of the other females housed singly. This is consistent with studies of *M. mulatta* showing that neuropsychiatric disorders, also found in humans, can be associated with increased or decreased HPA system activity (Novak, 1988; 2013). Our results provide insights into the findings on stereotypic behaviors reported in

confiscated lorises. Long-termed housed Bengal slow lorises (*N. bengalensis*) in Thailand showed significantly more stereotypic behaviors. Khudamrongsawat and others (2018) hypothesized that cage space and cage interior substrate complexity might resolve this issue, and our findings provide support for this claim. Sakura ceased to exhibit stereotypic behaviors once moved into enriched social housing. Similarly, in a survey on the prevalence and predictors of stereotypic behaviors in prosimians, a 15% prevalence of stereotypic behaviors were reported in *Nycticebus spp.*, where the only predictor for its occurrence was the amount of therapy enrichment given (Tarou et al., 2005). Moore et al. (2015) showed that the prevalence of stereotypic behaviors is based on social pair composition (solitary, same-sex, and sex-pairs). Out of 99 Indonesian lorises (including *N. javanicus*, n = 41; *N. coucang*, n = 42; *N. menagensis*, n = 7), singly housed animals were found to display the highest percentage of stereotypies (67%) compared to male-female groups (33%), and same-sex groups (7%). Since our case of stereotypic behavior had one of the highest levels of fecal cortisol, and same-sex pairings seem to have the least number of stereotypies, the same-sex pairing strategy seems to be a feasible procedure for decreasing unwanted behaviors and to lower stress in female pygmy slow lorises.

#### 4.6.4. *Female-female vs male-female pairs*

Regarding prediction 5, when I compared fecal cortisol levels between the female-female and the subsequent male-female pairs, no significant differences were found between them. Long-term pair success of captive primates might be accomplished by selecting animals to pair by age, sex, and weight (Byrum and St.Clair, 1998; Crockett et al., 1994), but success rates varies between species (Jorgensen et al., 2017; Truelove et al., 2015), and within species depending on the institution (Divincenti and Wyatt, 2011). New methods for selecting compatible primates to pair based on their behavioral responses (Capitanio et al., 2017; MacAllister et al., 2020), rather than

age or sex (Crocket et al., 1994), seems to be a promising approach for pairing confiscated surplus slow lorises. Male-male and female-female pairing success rate did not differ (Yamanashi et al., 2021) and our findings showed no difference in stress between the two. I conclude that same-sex pairings are no more stressful than male-female pairing. Both types can be used to reduce the number of singly housed individuals.

#### 4.6.5. *Challenges with confiscated lorises*

Unfortunately, I had no life history, health or detailed management records on any of our lorises from the moment they were confiscated to the time they arrived at JMC. My findings on stress responses cannot be solely attributed to the type of housing they were provided with, it is possible that many lorises were kept in poor conditions for years before being confiscated, which may result in un-pairable and un-releasable animals. There are reports of lorises arriving to rescue centers in deplorable conditions; where healthy adult slow lorises (*Nycticebus spp.*) spent 48.2% of their active period performing stereotypies and 28.6% of the slow lorises died within the first 6 months (Fuller et al., 2018). It should be a priority when caring for animals that are un-releasable, or those that require long periods of rehabilitation before release, to find ways to keep their stressors to a minimum. As the long-term success rate of releasing pygmy slow lorises back to the wild is not known, and releasing them to forests might have adverse effect on local populations (Campera et al., 2020), such practices might turn out not be an option for all rescued pygmy slow lorises. In a few of the reintroduced pygmy slow lorises (*N. pygmaeus*) in Vietnam, two disappeared, two were killed by predators, the tracking devices of the remaining three were lost, and one of them reportedly died of hypothermia. In a second attempt, out of 10 lorises, three died, four lost their radio collars, and one had to be re-caught. Only two were in good condition after two months living in the wild (Streicher et al., 2003; Osterberg and Nekaris, 2015; Kenyon et al.,

2014; Pointdexter et al., 2017). Until we can find a sustainable way to release pygmy slow lorises back into the wild and monitor their success, our efforts should be equally placed on improving captive conditions for their welfare and survival.

#### **4.7. Conclusion**

This study 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. Social housing may also have positive implications for their conservation before and during their release at *in situ* sanctuaries, increasing the number of animals that can be saved and rehabilitated by maximizing cage space without sacrificing their living standards. I encourage future studies to report and share their success rates on socially housing pygmy slow lorises to serve as a resource for those working in the captive management of this endangered species.

#### 4.8. Figures and Tables



Figure 1. We used three different types of enclosure for housing the slow loris groups: (A) Enclosure used for the first pairing attempt of Mimoza-Kaede, in 2016. (B) Enclosure used for the second pair, Nanten and Mizuki in 2017, (C) enclosure for a group of 4 females; Roji, Tsubaki, Momiji, and Sakura in 2017-2018.



Figure 2. Sakura, the only female engaged in self-squeezing through the nest in the singly housed condition, squeezing through a 6cm opening in the nest box. The picture depicts her hind legs pushing into the nest from below.

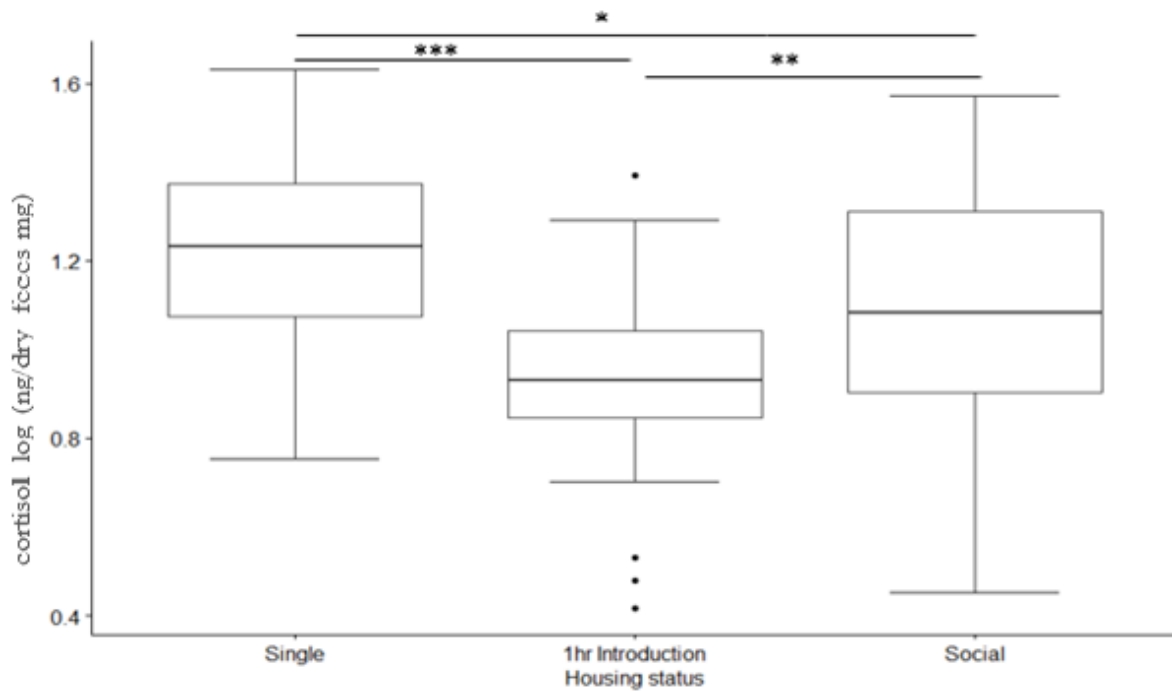


Figure 3. Boxplot represents fecal cortisol log (ng/ml) in the three types of pairing process of the second pair, Mizuki and Nanten, where the boxes represent the lower and upper quartiles, whiskers represent the minimum and maximum, and the horizontal line the median, with significant codes set as \*0.05, \*\* 0.01, \*\*\*0.001

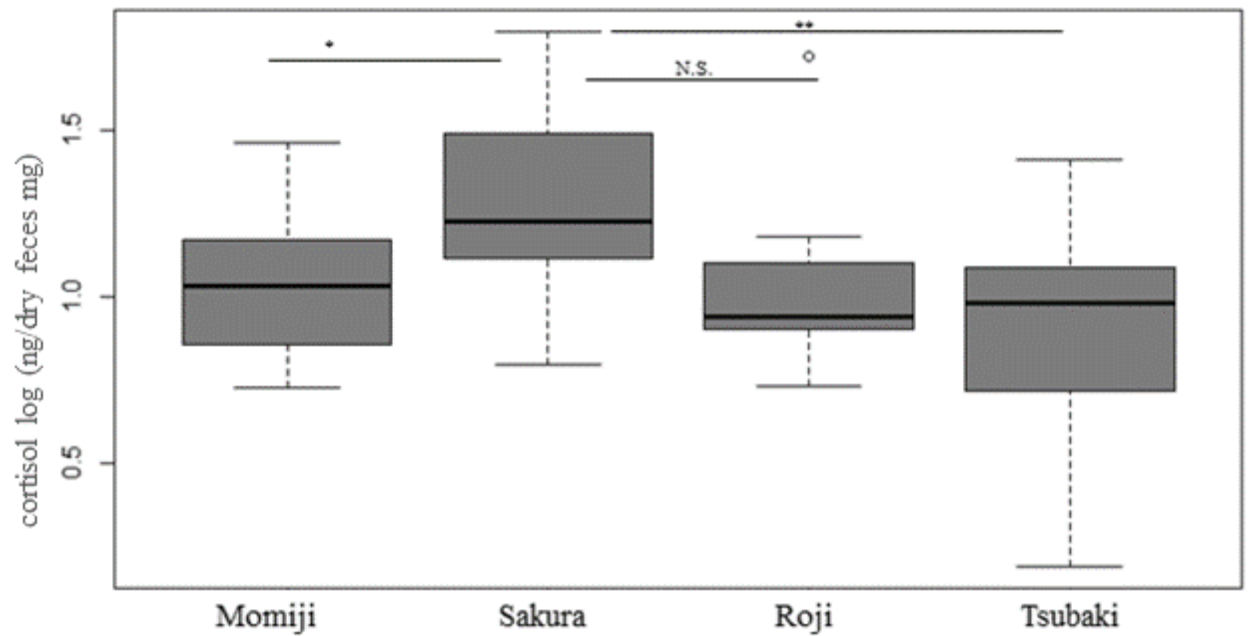


Figure 4. Boxplot represents fecal cortisol log (ng/dry feces mg) comparing fecal cortisol of all 4 females in the singly housed condition compared with Sakura, which engaged in stereotypic behaviors. Boxes represent the lower and upper quartiles, whiskers represent the minimum and maximum, and the horizontal line is the median. Statistically significant codes were set as follows: \*0.05, \*\* 0.01

Name	Former Cage size	Fecal samples (single)	Behavior data (days)	No of lorises	Group cage size	Group	Fecal samples (social)	Behavior data (days)
<b>Mimoza</b>	0.3 m <sup>3</sup>	37	NA	2	1.5m <sup>3</sup> (0.91 x 0.92 x 1.78 m)	A	23	NA
<b>Kaede</b>	0.3 m <sup>3</sup>	30	NA					
<b>Nanten</b>	0.3 m <sup>3</sup>	33 (15)	32	2	3 m <sup>3</sup> (1.28 x 1.25 x 2.07 m) x 2 rooms	B	56	46
<b>Mizuki</b>	0.3 m <sup>3</sup>	32 (13)	28					
<b>Momiji</b>	0.3 m <sup>3</sup>	13 (0)	13	4	16 m <sup>3</sup> (2.39 x 2.82 x 249 m)	C	71	58
<b>Sakura</b>	0.3 m <sup>3</sup>	16 (0)	16					
<b>Roji</b>	0.3 m <sup>3</sup>	11 (0)	11					
<b>Tsubaki</b>	0.3 m <sup>3</sup>	16 (0)	16					

Table 1. Data collected by subject before and after pairing.



Cort~Behav	Estimate	SE	DF	T-value	P-value
Intercept	10.82	2.36	13.33	4.57	0.000489 ***
Move	0.90	0.35	47.47	2.57	0.013354 *
Intercept	20.69	2.85	18.29	7.24	8.8e-07 ***
Rest	-0.95	0.35	51.82	-2.68	0.00968 **
Intercept	14.61	2.32	6.47	6.29	0.000554 ***
Feed	0.80	2.30	68.89	0.34	0.728617
Intercept	14.60	2.32	6.50	6.29	0.000547 ***
Self-G	0.62	1.49	67.32	0.42	0.676152

Cort~Posture	Estimate	SE	DF	T-value	P-value
Intercept	11.10	2.78	15.97	3.99	0.00105 **
Pos1	0.67	0.34	61.12	1.92	0.05905
Intercept	14.29	2.74	8.85	5.20	0.000592 ***
Pos2	0.26	0.80	62.04	0.32	0.746780
Intercept	17.02	2.45	9.20	6.94	6.06e-05 ***
Pos3	-0.63	0.33	60.64	-1.90	0.061
Intercept	14.71	2.25	6.13	6.53	0.000558 ***
Pos4	0.25	1.06	66.60	0.23	0.812145

Table 2. Results from linear mixed effects model (lme4 using drop-1 function) on associations between fecal cortisol on activity and postural behaviors. Associations between fecal cortisol and behaviors (above) and postures (below) using Linear mixed model fit by REML. T-tests use Satterthwaite's method with significant codes set at: 0 “\*\*\*” 0.001 “\*\*” 0.01 “\*” 0.05 “.”.

## **Chapter 5.**

*Human attitudes towards animal memorials: An  
international survey*

## 5.1. Abstract

There are many benefits to positive human-animal interactions in the care of animals in farm, zoo, and research settings alike. In research settings, positive interactions between animal and caregiver not only improve or insure the welfare of the animal, but also the welfare and safety of the caregiver. For example, in primates and other species, animals are more likely to stay calm and cooperate with caregivers that they trust more than ones they do not. Furthermore, these interactions can improve research both directly and indirectly. Animals under pain or stress may be less stoic in front of caregivers they trust, hence providing better care that would lead to better and more consistent results. Similar findings have been found in positive husbandry practices in both farm and zoo settings. Nonetheless, these positive human-animal interactions may come at an emotional cost. Staff may find themselves conflicted when animals under their care fall ill or may be euthanized for various reasons. Animal care staff may find themselves particularly conflicted if they have formed a special bond with an animal that they dually care for but also is part of an invasive study. Staff may feel even more conflicted if they do not understand the benefits the ongoing research in which their animals are involved with, if it does not demonstrate any advancements on both human and animal health. These conflicting interests may lead to anxiety, uneasiness or sleeplessness of the staff. This conflict has been termed as compassion fatigue, and it is also observed with caregivers outside the research setting (i.e. sanctuaries, zoos, farms, etc.). As our concept of animal care and improvement in welfare practices have evolved over time, one practice that has increased over the past decades is providing the facilities that house animals with a memorial service. These ceremonies not only address the conflict staff may feel as a cost for caring, it provides a place to pay tribute to an animal, and space for staff to share their feelings of grief. These types of ceremonies are increasing all over the world, and our work by means of

surveys aims to elucidate where are these ceremonies taking place, which institutions have recently adopted these ceremonies (increased since last publications), what are the perceptions of participants, and do they feel these types of practices demonstrate or promote better welfare. Specifically, I would like to know how memorial services are perceived within institutions between staff that have closer bonds to animals compared to those that do not. I hope my findings will contribute to the emerging literature on memorial services for departed animals and what they signify to people that work alongside them.

## 5.2. Introduction

### 5.2.1. *Evolving human-animal interactions*

Human beings are animals who have evolved numerous inter-specific relationships and bonds with other, non-human animals, henceforth referred to as simply “animals”. Some animals have become pets, while others produce or become our food, contribute to work, are used as medical research substitutes for people, are deployed in law enforcement and the military, are highlighted for tourism and nature experiences, and some strike terror and fear in many. Human-animal interactions vary greatly across the world and over time. From animals seen mainly as food sources in the early hunter-gatherer days, archeological findings suggest humans domesticated animals over 9,000 years ago without the need to kill them (honey bees) for food resources (Roffet-Salque et al., 2015), around the same time humans developed agriculture. Archeological findings also suggest that in the upper Paleolithic, humans domesticated dogs (ca. 15-30,000 years ago) for utilitarian reasons, although they may have also cared for animals that they had emotional attachments with (Janssens et al., 2018). As human societies evolved to include more complex needs and cultural traditions, the dependence and interactions with animals co-evolved. From domesticating elephants in India 8,000 years ago (Choudhury, 1989), to using wild animals for sport in the Roman Empire gladiator arenas, animals have played both partner and adversary roles in our history. Examples such as jousting with horses in the 14<sup>th</sup> to 16<sup>th</sup> centuries in Europe, to establishing the first zoo in Austria in 1752, people have developed complex relationships with other animals. In the last century, interactions with animals have allowed humans to find cures for many diseases by conducting laboratory research (Leader and Stark, 1987). Studying animal behavior has increased our knowledge into new insights into evolutionary perspectives (Budaev et al., 2019), and educational programs in zoos, aquariums, and sanctuaries help protect species from

extinction (Che-Castaldo et al., 2018). Fruit flies, dogs, and primates were the first to help humans reach previously unimaginable heights in space, by taking their place in the first rockets launched. Undoubtedly, as our human-animal interactions become more complex, so do the human-animal bonds that have been created.

Human-animal interactions and bonds are diverse, and their strength may depend on the type of relationship and the amount of time spent together. It can be assumed that in prehistoric times people in charge of domesticating and training elephants in India had a different type of interaction and bond with these animals than a soldier riding atop an elephant in 218 BC when Hannibal crossed the Alps (Mahaney et al., 2016). In modern times, where animals are kept in sanctuaries, zoos, aquariums, research facilities, homes and farms, the reasons why and how these animals are kept vary. The conditions animals are kept in and the attitude humans have towards their animals can also reflect these differences. Zookeepers might confront moral consequences for keeping a wild animal in a small enclosure, staff at a sanctuary might feel conflicted about releasing or breeding some animals over others, and animal care staff at a biomedical research facility who have bonded with an animal that will be euthanized for research purposes might feel moral ambivalence towards their duties (Herzog, 2002). Although research has shown that establishing positive interactions between care staff and the animals in their care benefits both the caregiver and the animal, as well as science (Reinhardt, 1997; Waitt et al., 2002), there are also emotional costs in caring for animals (Coleman, 2011). Primates are more likely to stay calm and be more cooperative, especially when accompanied by daily efforts and training (Laule et al., 2003). Positive human-animal relationships can be enriching for the animals and improve their welfare (Claxton, 2011) by reducing stress and fearful behavior in primates, as well as exerting a positive effect on productivity in farm animals (Bloomsmith et al., 1994; Breuer, 2000; Basset et

al., 2003). Bonds created while walking a dog are associated with lower complication risks among human patients with cardiovascular disease (McCune et al., 2019). While human-animal therapy such as solely pet ownership can benefit the owner's health, data suggests it is mainly subjective feelings ("placebo effect") that people have toward animal ownership that creates these benefits for pet owners (Cherniack and Cherniack, 2014). Nonetheless, these attitudes toward pet ownership may also inadvertently create a stronger human-animal bond. The positive or negative reaction farm animals may have toward their caretakers and the benefit they have is dependent on the relationship with the human handler (Boivin et al., 1998; Hemsworth and Barnett, 1992). A high-quality human-animal relationship requires positive human contact, which involves regular, intense, and long-term time investments in contact between humans and the animals that are under their care (Waiblinger et al., 2006). It is because of these time investments people have with the animals they work with, that for those who create strong bonds, whether domestic or even with wild animals in non-captive settings (Yerbury and Lukey, 2021), the loss of that animal could have more of an impact. In research facilities, staff become attached to animals and, because of the emotional investment, the loss of the animal may feel similar to that of a loved one (Herzog, 2002; Coleman, 2011). Animal caregivers may give their animals names and play with them, yet some caregivers encounter an unavoidable ethical dilemma. For example, under experimental conditions, when an animal is destined to be euthanized, a caregiver can still bond with the animal and provide limited positive interactions, despite knowledge of the future of the animal. Horse trainers and owners can be working with racehorses, providing the best care possible, but knowing that the training and treatment of racehorses are quite artificial or detrimental to their health (Campbell, 2021). The emotional stress that may become overwhelming to human-healthcare has been termed compassion fatigue (Cocker and Joss, 2016; Showalter, 2010; van Mol et al., 2015), and more

recently in places where laboratory animals are housed, this cost of caring that can evolve to compassion fatigue has been validated as a serious issue in human-animal caregiving (Engel et al., 2020; Lafollette et al., 2020; Newsome et al., 2020; Pavan et al., 2020). These emotional responses can range from a feeling of anger, sadness, frustration, confusion, helplessness, conflicted feelings, loss of appetite, and guilt, among others (Figley and Roop, 2006). Although they have been recently well-reported and documented for humans that work with animals (Thurston et al., 2021), potential activities to mitigate compassion fatigue for people who work with animals have not been explored. These conflicting moral dilemmas in the evolution of human relationships with animals are likely one of the reasons that have brought about the practice of animal memorials but the effects and attitudes towards these practices have not been well reported.

### *5.2.2. The types of human-animal interactions and animal memorial ceremonies*

In recent decades the organizing of animal memorials has increased in several countries to pay homage to the animals sacrificed for the sake of human-related activities and advancements (Arluke, 1988). There are memorials for animals used in world wars, in the food industry, and in biomedical research facilities, among others (Lynch, 2001). In Asia, many countries pay their respects to animals used in various types of institutions to help animal care workers with the grief resulting from the bonds established between animals and their caregivers (Iliff, 2002). Probably one of the most well-known animal memorials is the statue of Balto, the dog, in Central Park, New York. He was a lead sled dog who helped control a diphtheria outbreak in the remote outpost of Nome, Alaska in 1925 by heading a team of dogs through freezing temperatures and blizzard conditions to save a group of children from contracting the disease (Macivor, 1954). At the Primate Research Institute Kyoto University in Japan, yearly animal memorial services have been taking place since 1973 (Asquith, 1983). Asquith (1983) attended the animal memorial for the first



time in 1982 as she was told that Japanese have a different relationship with animals and their death. She reported the attendance to the “sarukyou” or monkey memorial service as a Buddhist ceremony, held in front of the “saruzuka” or monkey memorial stone where a priest from a local temple offered prayers of appreciation and apologies for the lives of primates lost during the year. The first participant to hand flowers and pay respects was a chimpanzee accompanied by its keeper. The ceremony was then followed by drinking rice wine and eating snacks around the memorial stone for a few hours. However, in recent times, there is no priest and participants are not expected to light incense for the dead, drink rice wine, and there is no chimpanzee attendance. At Osaka University, primatologists in Japan conducted a similar tradition during Spring. The first animal memorial service reported in the *New World* took place in Canada at the University of Guelph in 1993 (Iliff, 2002). Unlike their Japanese counterparts, the Canadian ceremony was secular, and the main purpose was to acknowledge the animals that are used, rather than any spiritual or religious belief (Taylor and Davis, 1993). Differences between countries on how to deal with death and pay respect to animals are unsurprising, but differences also exist between institutions within the same country. Nishikawa and Morishita (2012) found that institutions within the same country, Japan, had different ways of conducting memorial services, with some secular and others more agnostic, few were mandatory and others optional, and most had some form of rituals involved while others just presented a plaque without a ceremony. More importantly, the researchers found that 70% of their respondents that worked with animals felt guilty for using animals in research, but 40% of them found they were able to manage their conflict through animal memorials (Nishikawa and Morishita, 2012).

Differences on how humans interact with animals are not limited to the type of relationship they have or any cultural differences, but also gender differences. Fogle and Abrahamson (1990)

found that animal deaths caused by illness and euthanasia provoke significant short- and long-term emotional reactions in a substantial proportion of veterinarians, especially women. Herzog (2007) points out that men support animal research, hunt animals for recreation, and engage in animal cruelty more than women. In contrast, women nearly always outnumber men in hoarding large numbers of cats or dogs in their homes, which has dire consequences for both animal welfare and public health. Since gender differences have been observed in how humans interact with animals, I sought to see if there were any differences in the attitude toward animal memorials.

### *5.2.3. Main purpose of the survey*

Because of differences within institutions and countries, and more importantly, individual differences in the types of interactions people may or may not have with the animals they care for or work with, and the emotional burden when an animal is lost that some may feel stronger than others, I investigated how animal memorial ceremonies are conducted around the world and how they are perceived by the people that take care of the animals. I predict that:

1. People working where animals are housed, who self-report having established strong bonds with animals, or given names to animals at their workplace, will value these types of ceremonies more than those who do not do so.
2. Gender differences in perspectives regarding animal ceremonies will reflect gender differences in attitudes towards animals in general.
3. Continent, or country, wide attitudes about animal ceremonies will reflect continent, or country, wide attitudes about death.

4. People who engage in invasive research will be more inclined to have a positive viewpoint about animal ceremonies, possibly due to emotional feelings related to guilt.

5. Respondents who have self-reported having compassion fatigue and emotions previously described by Figley and Roop (2006) when they lost an animal will agree more that animal ceremonies are beneficial or needed.

### 5.3. Methods

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 held at Kyoto University. These included sanctuaries in Africa, Asia, and South America, as well as email listings of Primate Centers in North and South America, Europe, and Asia. Our online questionnaire had 23 questions (Table 1) 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: see Table 1), 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. For essay questions, I grouped the answers by frequency and reported them by order of most occurrences. I also asked respondents to contribute their opinions in writing regarding their answers to questions and their attitudes about animal memorials in general.

#### 5.4. Results

Our sample included 111 adults ranging between 18 and 74 years of age. Nearly  $\frac{3}{4}$  (73.8%) of respondents had an advanced college degree, and 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 (Figure 1). Close to half of our 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.

Some participants explained that their institutions perform annual ceremonies, while in other cases e-mails are sent or websites are set up containing information about the deceased animals. Respondents described animal ceremonies including incense, exhibitions of photos, honorary plaques, flowers, and footprints of the animals (Figure 2). The majority of the respondents indicated that the ceremonies were secular. In response to the question, “if your job

was related to animals, and if the job or institution did not conduct memorial services, do you think they are necessary?”, 45.0% (n=50) replied yes, 36.9% (n=41) replied no, and 18.0% (n=20) replied something else. Comments that I received included: “[I] felt memorial services can help with teamwork, but not sure if necessary” and “memorial services are not necessary, but it [sic] could be implemented”. For those whose institutions have ceremonies, 35.1% (n=39) indicated that it is only a ceremony for the animals; 21.6% (n=24) indicated that this is for the animals and the employees; and 8.1% (n=9) indicated that the ceremony is for the animals, the employees and religious (spiritual), while 35.1% (n=39) responded as other.

To examine the possible attachment or bonding that may exist between people and animals under their care, or whom they work with, participants were asked if they have a special bond with a particular animal, and 72.9% (n = 81) replied yes, while 17.1% (n=19) wrote no. When asked if they name the animals they work with, 69.4% (n = 77) responded yes, while 24.3% (n=27) replied no, and 6.3% (n=7) provided another response (e.g. they already had a name). In response to the question about whether or not they had ever felt extreme grief over the loss of an animal, 59.5% (n=66) indicated yes, 20.7% (n=23) indicated somewhat, 13.5% (n=15) indicated no, 6.3% (n = 7) had other replies, such as “I have never lost an animal with which I have worked”, “it is more of a feeling of astonishment and discomfort”, and “I have felt pain and sorrow”. Individuals who experienced emotional pain after the loss of an animal with which they have worked were asked for more details about the negative effects that they had experienced. Participants were given a list of negative emotions and were able to select multiple replies. Results revealed that 37.6% felt sorrow, 16.1% suffered from guilt, 2.4% experienced frustration, 10.6% claimed impotency, 6.9% were conflicted, 6.9% had a loss of appetite, 6.9% were angry, and 2.8% endured confusion. Among the emotions written on the questionnaire that were not on our list were acceptance,

emptiness, depression, despair, loss of energy, loss of will to live, and a desire to avoid repeated situations.

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 (see Figure 3). I found no statistically significant differences in opinions on animal memorials between genders ( $U=1256$ ,  $Z=-0.70$ ,  $p=0.40$ ), whether their work environment involved either invasive or non-invasive research ( $U=3539$ ,  $Z=0.515$ ,  $p=0.6$ ), whether they name their animals or not ( $U=1239$ ,  $Z=-0.003$ ,  $p=0.98$ ), or even whether or not their institution housed animals ( $U=1155$ ,  $Z=0.803$ ,  $p=0.404$ ). 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 ( $U=1157$ ,  $Z=1.99$ ,  $p=0.04$ ) (See Figure 4). 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 (See Table 2).

When respondents were asked to write what they thought were the positive aspects of holding an animal memorial ceremony at their place of work, the most frequent reason given was that it was a place and time for people to overcome grief ( $n=21$ ), with comments such as “it is a good place to clarify their feelings”, “it may help people to cope”, and “comforting when close to

the animal”. The second most frequent reason was that it is a good way to put the loss of animals under their care into perspective (n=17). Comments included, “it is important to recognize that I have a responsibility”, “it is not good to deal with animals crudely even after they pass away”, and “it also shows (e.g. to visitors) that the animals that are used for peoples' amusement or research are respected and mourned for”, and “forced awareness of the suffering inflicted to the animals”. The third primary reason provided was an appreciation for the animals (n=13), with individual respondents writing “It is a good time to appreciate the sacrifices made by animals under our care”, “convey thankfulness”, and “deserve the utmost honor and respect for what they give us”. Eleven people noted that animal ceremonies were good practice, but gave no reason. Five people thought that it might improve welfare, mentioning the 3 R’s principles (Russel and Burch 1959) and the additional R (remembrance) suggested by Iliff (2002). They mention comments such as it was “a reminder of the sacrifice of the animals' lives and perhaps an incentive for reducing the number used”, that animal memorials could “enhance the way people connect with animals” and had the potential “to improve current captive settings for the animals”.

While 70 (74%) participants thought that the animal memorial ceremonies were positive for various reasons, 25 (26%) had the opposite opinion. The two most common reasons for the negative attitude were that they are too human-focused (n=6), commenting that “people just want to feel less guilty”, the memorials only serve a selfish “self-satisfaction”, or they “can be harmful if it [sic] anthropomorphizes animals”. Nearly an equal number (n=5) thought that such ceremonies were not good for their job as they believed it did not help improve their job, working environment, and that those resources should be implemented in the animals that are still living and the people taking care of them. Three people (2% of our sample) thought that animal ceremonies were hypocritical, three believed that they were too religious or spiritual in nature (n=3), and three

suggested that they can be too formal (n=3). Additional reasons given by individuals were “not necessary for all staff/students/animals - just certain animals that are well known/loved”. And one added that “the death of a healthy animal may help to prevent more animals from suffering if they are in human care under limited space or resources.”.

Participants were asked if there are better ways in which they think their institutions can improve the well-being of the staff and the animals instead of having memorial services and a wide range of responses was obtained. The most frequent recommendation was to invest more resources and time into living animals, such as building larger cages and providing access to the outside for captive animals. A complete list of respondent ideas for improving the well-being of animals is presented in Table 3.

In response to the question of what have they done to make themselves feel better, and how have they coped with the loss of an animal, participants responses varied greatly. Among them was to find support from friends, distracting themselves with other activities, focusing on the animals that are still alive and to think of the contributions the animal may have played, and to think of the good interactions the human had while the animal was alive. And lastly, when participants were asked if they had any additional thoughts that they wished to share about their spiritual beliefs that might help to think about memorials for animals, about 34% (n=38) of the participants answered (32% male, 68% female). Most responded that they had nothing more to add, while 32% (n=12) claimed that they were not spiritual, religious and/or atheists. 15% (n=6) of individuals suggested that due to their religious beliefs or in that animals have souls, reincarnation, or believe in animal heaven, animal memorials are needed; while 13% (n=5) of people suggested that although they are not spiritual, they do believe in the benefit of the



ceremonies. Both positive and negative opinions on animal memorial ceremonies were represented by most continents.

## **5.5. Discussion**

### *5.5.1. General findings*

My aims for conducting the survey were met as I gathered responses on animal memorial ceremonies of people from various countries and with different types of human-animal interactions that work with or around animals. Although not all of my predictions were supported as I did not find significant differences on attitudes towards animal memorials based on gender, region/country, or by self-reported strength of bonds respondents had with their animals; nonetheless, I found people who worked directly with animals such as husbandry caretakers to view these ceremonies significantly more positive than those that do not. Moreover, respondents who reported to feel more negative emotions related to compassion fatigue were more likely to agree that these ceremonies are needed than those who reported on having fewer negative responses. It is these differences that I found that may give us a better understanding as to why some institutions conduct these ceremonies and why they may be perceived differently depending on how people interact with animals.

Perhaps our most unexpected finding was that whether individuals had a positive or negative viewpoint about animal memorials was not dependent upon whether they engaged in non-invasive or invasive research. It is possible that many participants who do engage in invasive research at an institution do not carry out the invasive research themselves or their job can be multifaceted, where the invasive part is only a fraction of their work. Another possibility is that they are aware of the animal's contributions to science and animal memorials may not be needed

or they may spend less time interacting with each animal than caregivers. Our sample only included 7% (n=8) of respondents that engaged in invasive research, of which only 3/8 reported having animal memorial services at their workplace.

#### *5.5.2. Attitudes towards ceremonies and the type of human-animal bond*

As predicted, I found that people who had reported to have experienced compassion fatigue, compared to those who had not, were more agreeable towards the statement that having animal memorial ceremonies are beneficial or needed. Moreover, our model showed that the only significant predictor of agreeing that animal memorial ceremonies are beneficial or necessary was the number of grief-related feelings (compassion fatigue) people have once the animals they work with and have created bonds with die. A recent study conducted by Thurston and others (2021) on the prevalence of compassion fatigue on laboratory animal personnel (n=200) during the COVID-19 pandemic found that 86% of participants had experienced compassion fatigue at some point in their careers, with 41% experiencing an event (new or worsening symptoms of compassion fatigue) during the pandemic, and 90% of participants who reported an event also reported subsequent effects on their personal or professional lives. As 29% of all respondents found that the main stressor at work was animal euthanasia, while only 36.8% of institutions offered access to compassion fatigue information (lectures, handouts, webinars); access counseling or mental health services for employees. When they were asked what their institution could have done better to avoid or treat their compassion fatigue (n=72), although the top three suggestions were better communication with leadership, more personal appreciation, and compensation, 13% reported they did not know or were unsure. Although the study did not explore animal memorials as institutional support, our results indicate that it could be a way to alleviate and give awareness to this emerging problem.

Buckmaster (2015) found that naming animals in the laboratory has become more popular and suggested that such a practice creates a stronger bond between an animal and a caregiver. Therefore, I had expected that workers who named animals would think differently of animal memorial ceremonies than those who had not, but our findings did not support our prediction. Borkfelt (2011) argues that when we name animals, we are exercising power over the animals that we name, such that it can affect our behavior towards them and we tend to see them as less than us (humans). Yet the Borkfelt also acknowledges that not naming an animal can mean distancing ourselves from animals and disregarding their likeness to ourselves, which makes it easier to justify harmful treatment, or to have disenfranchised grief when it dies (Spain et al., 2019). Cohen (2012) suggested that in human societies animals are not granted the same moral importance as people, making humans superior to animals, so the act of naming an animal is thought of as human superiority. The suggestion that by naming animals, human beings are somehow exhibiting a superior attitude about the human species conflicts with the idea that by naming animals we consider them as companions that are similar to human beings. People name their dogs and many treat their dogs as family members (Walsh, 2009). Anecdotally, we do not know of anybody that gives their companion animal a number to try to avoid acting as a higher being by naming it. I suggest that the reasons I did not find differences in attitudes toward animal memorial ceremonies between humans that name their work animals and those that do not are complex, with many variables at play other than human-animal interactions. In the survey, one possibility as to why my prediction was not met was that many animals had names before other humans worked with them. Respondents stated that some of their animals already had names before they started working with them and it may be one of the limitations, I had in my study that inhibits me to explore this variable any further. Future work should try to answer this question by researching newly introduced

animals to the workplace, giving employees or people who work with animals the option to name or not-name their animals, and asking them how they feel about animal memorials afterward.

I had predicted that having animals housed in the workplace would increase opportunities for more interactions, but many people reported working at places where animals are housed where they do not have contact with animals at all. Our results revealed no differences in opinions about animal ceremonies between people that have animals housed at their workplace and those that do not. Human-animal interactions may be more frequent depending on the type of role or job with animals a person may have, rather than if the work place houses animals or not.

### *5.5.3. Attitudes towards ceremonies based on cultural differences*

European respondents seemed more neutral than those from other continents, which might be related to cultural differences in human-animal interactions. In the UK and US, where animals can be buried with the owner or in the same plot, have increased in recent years (Schuurnam and Redmalm, 2019). In Sweden, pet cemeteries are similar to that of humans with religious decorations, giving them equal treatment to that of humans, while in neighboring Norway, these practices are seen less and are more modest in appearance (Gustavsson, 2013). One respondent from South America wrote that it is “bad luck to bury animals, as they bring a bad spiritual omen”. At some point in the early history of Japan, animal memorials were erected, but with the introduction of Buddhism in the 6th century, the killing of animals was considered sinful. Since the re-opening of Japan to the outside world in 1860, the culture of animal memorials reappeared (Kast, 1994), with Japan emerging in the 1950s, along with Thailand (Iliff, 2002), as a leader in conducting animal memorials for research animals. Louis-Caruana (2020) suggests that all religions converge in a fundamental principle of our responsibility towards animals, where animal

concern becomes both a moral and a religious obligation, but our findings revealed that religious belief had no impact on attitudes towards animal ceremonies. Cultural traditions, personal beliefs, and institutional practices all play a role if animal memorial ceremonies are held or not, but I did find that they occur in all continents, with a variety of attitudes between and within countries.

#### *5.5.4. Attitudes towards ceremonies and the cost of caring*

As the complexity and variety of human-animal interactions evolve, animal welfare advocates in pursuit of the ethical treatment of animals, especially in captivity, keeps growing (Bekoff and Hettinger, 1994). In research settings, positive interactions between animals and caregivers not only ensure the welfare of the animal, but also the welfare and safety of the caregiver. Respondents who were more emotional about animal deaths and who gave responses similar to those described by people experiencing symptoms of compassion fatigue (anger, sadness, frustration, confusion, helplessness, feeling conflicted, loss of appetite, and guilt, among others) reported in works done with humans coping with stress associated with compassion fatigue (Cocker and Joss, 2016), agreed with the statement that animal memorial ceremonies are beneficial and that they are 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. Our 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 our 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. Although the trend nowadays is to engage more and more with electronic gadgetry (Rolston 2017), the natural resources of the planet are disappearing, and animals are threatened more often with extinction. 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 our dead non-human relatives while potentially alleviating the emotional fatigue for the humans that take care of them.

## **5.6. Conclusion**

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 we found that animal caretakers were significantly more agreeable to having animal memorial ceremonies than other participants engaging in other human-animal-related jobs. Our 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 our regression model, we 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. Although the trend nowadays is to engage more and more with electronic gadgetry (Rolston, 2017), the natural resources of the planet are disappearing, and animals are threatened more often with extinction. We suggest that the animal-human bond is gaining strength and we suspect that it will result in more institutions adopting animal memorial ceremonies to honor our dead non-human relatives while potentially alleviating the emotional fatigue for the humans that take care of them.

## 5.7. Figures and Tables

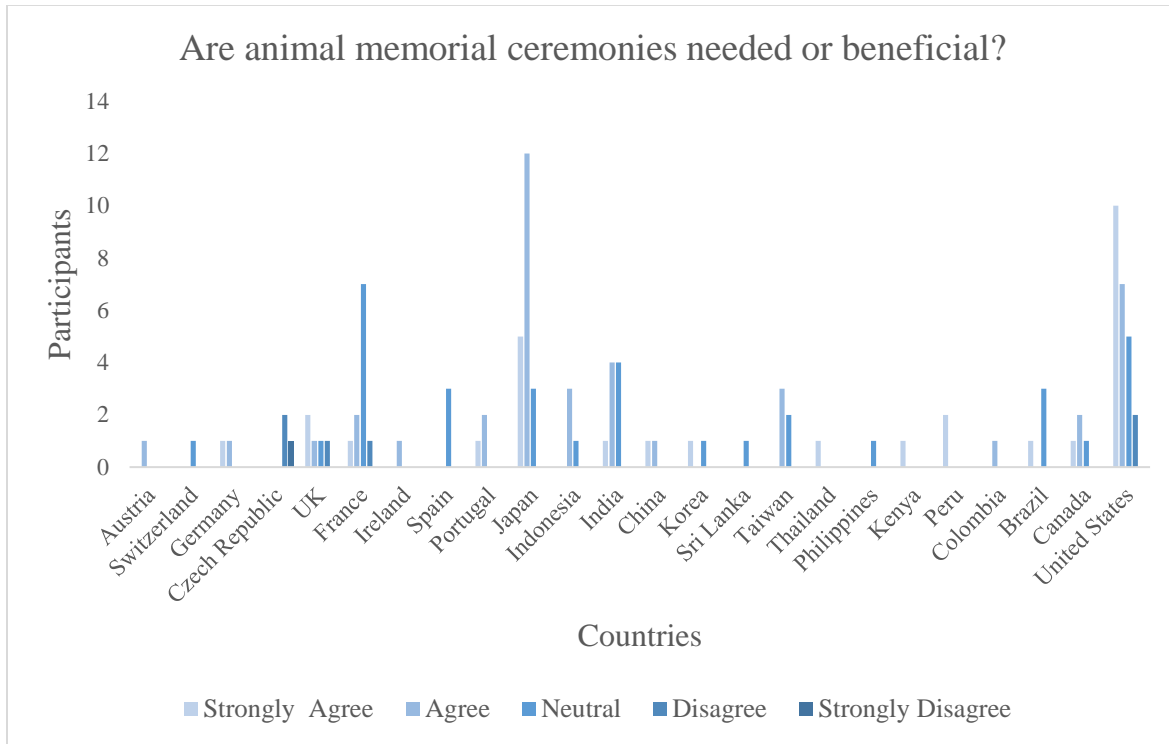


Figure 1. Number of responses to the 5-pt Likert question to what degree are animal memorial ceremonies beneficial or needed.



Figure 2. (Right) Monkey Memorial Pond at the ONPRC, (center) staff of a primate center created figures for their departed monkeys, and (left) Monkey Memorial Service at PRI.

1. Gender
2. Select Age Group: <18; 18 – 24; 25 – 34; 35 – 44; 45 – 54; 55 – 64; 65-74; >75-year-old
3. Country of birth
4. Highest level of education
5. Are you associated with a place where animals are housed?
6. Please select the region where you work (study, research, observe, manage, etc.) with animals.
7. What type of institution or place are you affiliated with? (Zoo, Research Facility, Sanctuary Farming Industry, Rescue Center, Semi-Wild Protected Area, Wildlife, or Other)
8. What is your role at the institution or place you are affiliated with? (Administrative, Husbandry Staff/Animal Caregiver, Invasive Research, Curator, Veterinary/Health Services, Student, Volunteer, Wildlife Observer, Non-invasive Captive Research, Non-invasive Free-ranging or Semi, Free-Ranging Animals, or Other)
9. If you currently work or have ever worked directly with animals, how often do/did you work directly with animals? (Daily, Weekly, Monthly, Yearly, Other)
10. Have you ever heard of memorial services for animals? (Y/N)
11. Does your institution or workplace have a memorial service for your departed animals or equivalent practice? Examples can be but are not limited to: pictures of animals, statues, paintings, ceremonies, rest areas, moments of silence, etc. (Y/N)
12. If your institution or workplace does have a memorial service or space to honor dead animals, please describe briefly what it entails. (Descriptive)
13. If your work is related to animals and your institution or workplace does not have a memorial service, do you think it is needed? (Y/N/Other)
14. If there is a memorial service for animals, is it only a tribute to the dead animals or is there a space or discussion for staff who worked with them? (Ceremony Only for Animals, For Animals and Human Workers, For Animals, Human Workers, and Other (Spiritual), Other)
15. To what extent do you agree that animal memorials are beneficial or needed. (1 Strongly Agree- 5 Strongly Disagree)
16. Are there better ways your institution can improve the welfare of staff and animals rather than memorials, if so, give a short description? (Essay)
17. Have you made a special bond with one particular animal?
18. Have you ever named an animal you worked with?
19. Have you ever felt extreme grief after losing an animal?
20. If you have felt extreme grief after losing an animal, what were the negative effects? (Anger, Sadness, Frustration, Confusion, Helplessness, Conflicted, Loss of appetite, Guilt, Other)
21. If you have lost an animal, what made you feel better afterward/how did you cope? (Essay)
22. What is your overall feeling towards animal memorials? Please describe in briefly in a few words.
23. Is there anything else you'd like to share about the spiritual beliefs that might make you think about animal memorials in a certain way? (Essay)

Table 1. Survey questions.



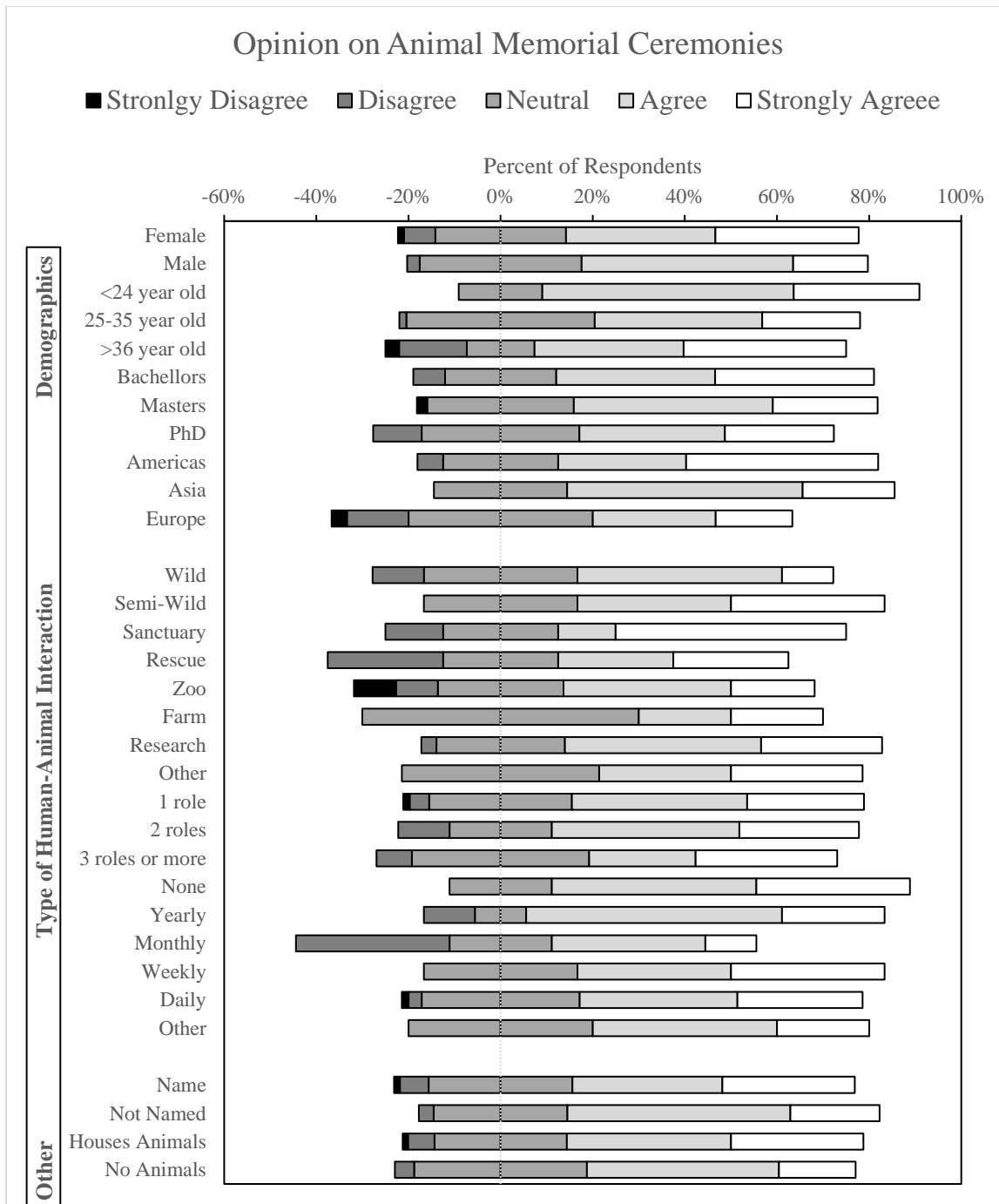


Figure 3. The proportion of responses to Likert questions on Animal Memorial Ceremonies is based on participants' demographics and type of human-animal relations.

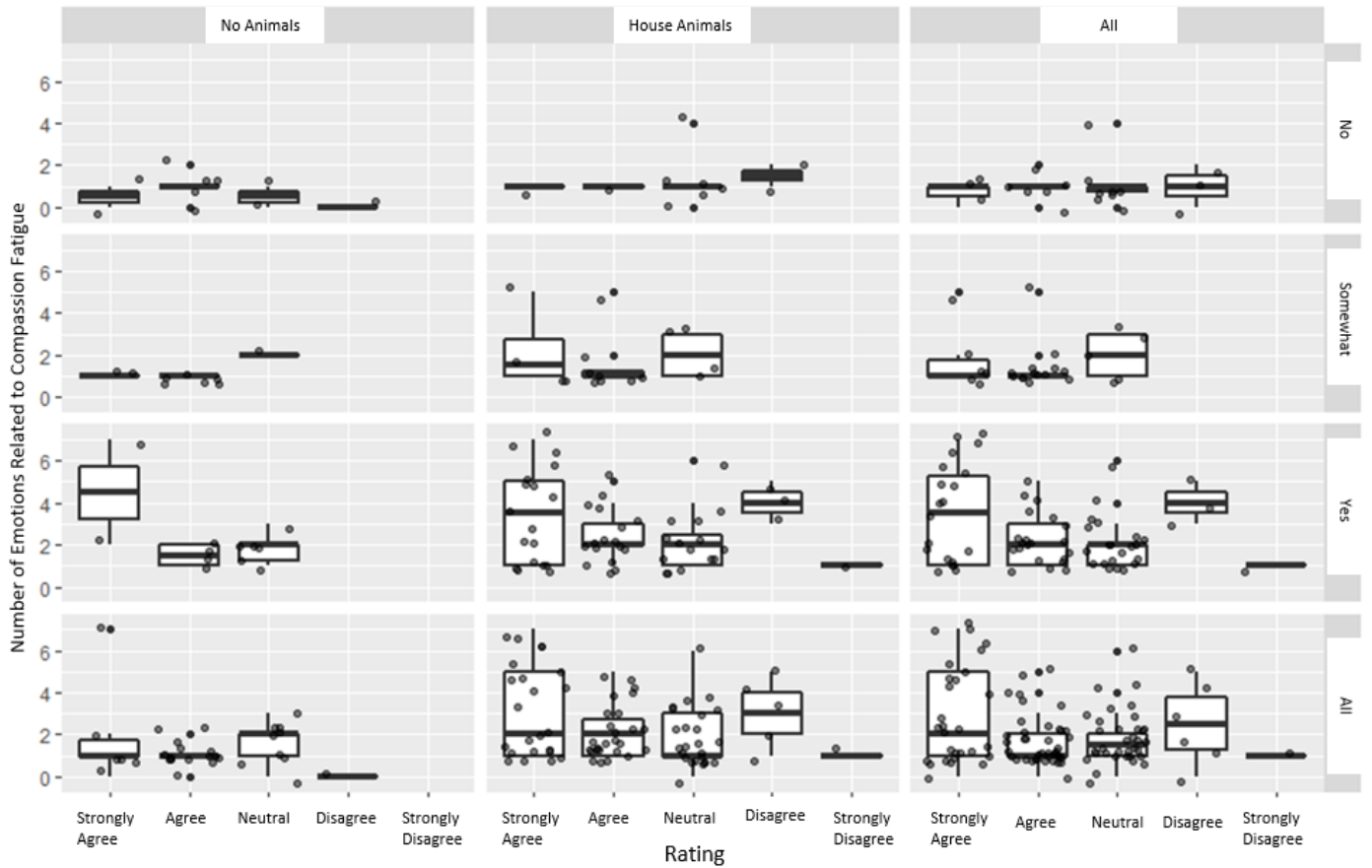


Figure 4. Boxplot representing the number of emotions related to Compassion Fatigue to every level of Rating of Agreeability on a 1- 5 scale, from Strongly Agree to Strongly Disagree, for values of marginal relationships for those respondents that Have Animals where they work or not, and if they have Experienced Grief after losing an animal.

<b>Intercepts</b>	<b>Value</b>	<b>Std. Error</b>	<b>t-Value</b>	<b>p-Value</b>
Sex	0.287	0.048	0.594	0.552
Age	0.020	.044	-0.45	0.649
Education	0.326	0.263	1.23	0.215
Region	0.833	2.03	0.409	0.681
Wild	1.19	2.61	0.458	0.646
Semi-Wild	0.223	2.78	0.079	0.936
Sanctuary	-1.13	2.70	-0.41	0.674
Rescue	2.29	2.89	0.795	0.426
Zoo	0.309	2.67	0.115	0.908
Farm	0.830	2.76	0.300	0.764
Research	0.282	2.56	0.110	0.912
Number of Roles	-0.11	0.403	-0.28	0.774
Have Animals	0.609	0.729	0.834	0.403
Frequency Interactions	0.062	0.190	0.326	0.743
Name Animals	0.233	0.666	0.349	0.726
Extreme Grief Yes	-0.23	0.672	-0.35	0.722
Grief Somewhat	-1.02	0.744	-1.38	0.166
Compassion Fatigue	0.456	0.183	-2.49	0.012**
Residual Deviance	214	NA	NA	NA
AIC	306	NA	NA	NA

Table 2. Results of the ordinal logistic regression model, with respondents' ratings on animal memorial ceremonies (5 level: Strongly Disagree- Strongly Agree) set as the dependent variable, and the respondent's demographics, type of human-animal relationships, and experiences as the predictor variables. Statistical significance level codes: \*\* 0.01.

For Animals
1) <b>Improve living conditions (n=20):</b> larger caging enclosures; enrichment; naturalistic enclosures; constant stimulation (cognitive); general welfare improvement; more space between visitors and animals; decrease stress in transportation, captures, medical procedures, collaring.
2) <b>Construct retirement spaces (n=5):</b> enclosures for animals retired from research; a place to retire from exhibit; remove from the group if beneficial to the individual; prohibit visitors in wild areas for vulnerable individuals and groups; send to sanctuaries.
3) <b>Avoid immediate removal (n=2):</b> allow conspecifics living in the same enclosure time to examine their dead.
For People
1) <b>Hold regular meetings (n=10)</b> or seminars to discuss: emotional impact; an event that leads to death (prevention); staff expectations; potential research outcomes on living animals; improvements for welfare and conservation for living animals; the importance of research and conservation efforts; type and quality of interactions between animals and staff.
2) <b>Improve education (n=10)</b> on: animal welfare; staff-animal habituation and proper husbandry training, positive reinforcement (stress reduction); the research, conservation, and outreach aims (highlight importance of staff roles).
3) <b>Increase benefits (n=6)</b> to staff: Health insurance/mental health; accommodation at the workplace for grief; proper salaries to hire and retain qualified staff; to improve work areas.
4) <b>Celebrate the living (n=4):</b> appreciation days; birthdays; enrichment for animals and staff day; updates of animals in the field, or captive facilities.
5) <b>Other (n=4):</b> Audio-visuals (pictures and videos) for staff to keep; individualized memorials.
For institutions:
1) <b>Revisit the 3 Rs (n=9):</b> reduce the number of individuals in invasive experiments; reduce the number of deaths; review ethic guidelines; incorporate husbandry staff to IACUC guidelines; more rigorous IACUC focus on prevention; more pre-research planning for staff to prepare
2) <b>Implement new guidelines (n=6):</b> guidelines for when a groupmate or cage mate dies to optimize welfare; incorporate a farewell day before euthanasia; equitable attention across animal species (i.e. equally between monkeys and apes); stop memorials until all other prevention measures are improved (welfare, conservation, disease prevention); memorials for animals not involved in research but housed in research facilities; virtual or physical memorial board to share stories of dead animals.
3) <b>Perform euthanasia (n=4):</b> explore different ways to practice euthanasia; increase euthanasia if conditions are not proper; decrease euthanasia in general; memorial services send confusing messages about husbandry practices that include euthanasia and should be stopped.

Table 3. Suggestions from respondents for potential alternatives to holding animal memorial ceremonies.

**Chapter 6.**  
*General Discussion*

### 6.1. *Summary of findings*

The principal objective of this study was to examine how different behavioral management plans influence the health and well-being of captive primates. The findings demonstrated that vegetated enclosures enabled male Japanese macaques (*Macaca fuscata*) to engage in more naturalistic behaviors than those living in a non-vegetated enclosure. When given the opportunity, female pygmy slow lorises (*Nycticebus pygmeaus*) huddled together in nest boxes and had lower glucocorticoid concentrations than when housed alone. Finally, memorial ceremonies that pay tribute to dead animals can have positive consequences for human health and welfare. The intimate bond that can form between an animal's caretaker and the animal has been viewed as a potential danger to scientific neutrality, but is now promoted by many facilities. Caretakers that devote themselves to the utmost care of their captive animals may suffer emotionally when the animals that they know die. The research presented in the preceding chapters has implications not only for captive nonhuman primate welfare, but for the welfare of animal caretakers.

### 6.2. *Benefits of vegetated-naturalistic enclosures for primates*

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 non-vegetated, large, outdoor enclosures. Captive, especially those housed in very limited space in laboratories or zoos, as well as some companion and sport animals, are often highly restricted in their activities and exhibit a variety of behavioral issues when their species-specific needs are not met (Kyley-Worthigton, 1989). Many animal species live in complex social and ecological environments in the wild. Non-human primate species are among the few species that are studied

extensively in research facilities, but in the wild, they frequently reside in social and ecological environments that are complex and, more importantly, challenging to simulate (Schapiro et al., 2014). To preserve and improve the welfare of primates employed in research, efforts are often concentrated on novel methods, setups, and tools in the form of enrichment or presence of conspecifics that functionally mimic natural situations and give the animals the chance to act in ways that are consistent with their species and time-budgets (Jaman and Huffman, 2008). The improvement of animal welfare in captive, non-natural situations is a difficult process yet crucial for many primates due to their various social and ecological requirements (Coleman, 2011).

A number of studies of nonhuman primates have reported that those living in more naturalistic environments appear to behave more like their conspecifics living in the wild. The finding that male Japanese macaques living in a vegetated enclosure have an activity budget resembling that of wild Japanese macaques more than those living in a non-vegetated enclosure mirrors recent findings (Inoue and Shimada, 2020) on the activity budgets of chimpanzees (*Pan troglodytes*) living in a zoo (Tama Zoo) compared to their wild counterparts (Mahale). Adequate zoo housing provides enrichment and suitable housing conditions, allowing captive individuals to behave in ways that mimic the behavior of those living in the wild. In Sulawesi, crested black macaques (*Macaca nigra*) those housed in large and complex naturalistic enclosures had similar activity budgets compared to their wild counterparts (Melfi and Feistner, 2002). Using activity budgets to compare and evaluate improvements to enclosures such as placement of natural enrichment has also been shown to affect activity budgets by promoting the natural foraging behavior of cotton-top tamarin (*Saguinus oedipus*) and the golden lion tamarin (*Leontopithecus r. rosalia*) (Steen, 1995), as well as in ring-tailed lemurs (*Lemur catta*), with activities resembling those that are seen in the wild once transferred to natural captive habitats (Keith-Lucas et al., 1999).

Changes in activity budgets for assessing welfare are not only limited to primates, but they have also been successfully used to monitor welfare states in dolphins (*Tursiops truncatus*) as faster swimming behaviors can be used as an early and easy-to-measure indicator to assess potential stressful conditions such as environmental stressors (Serres et al., 2020).

Practices that promote feeding and foraging behaviors when vegetation cannot be self-sustaining in an enclosure have been discussed in research regarding captive chimpanzees (Bloomsith et al., 1988). Unfortunately, the benefits found in Japanese macaques living in naturalistic enclosures depend on the presence of vegetation, but after over a decade of living in the same enclosure, the vegetation remains, but many of the trees have been lost. The new enclosures at Onobora are three times larger than the vegetated enclosures in the main campus of PRI reported by Jaman and Huffman (2008) and have kept much of the vegetation, but may degrade over time. Although the composition of groups formed and the size of the enclosure is closer to those reported for wild populations of Japanese macaques, 50 individuals in a 3.7km<sup>2</sup> (Izumiyama et al., 2003) in Niigata, groups size in captivity increase every year without an increase in vegetation, and the space is still much smaller, with only 3,900m<sup>3</sup> for 40-60 individuals. Management should address either an increase in provisioned food to minimize dependency on vegetation and/or remove animals more frequently as reproductive females will have offspring almost every year. As mother-infant bonds are also encouraged, leaving offspring in the group until they are needed for research use is common, and a group can triple in size in a few years. Future design of vegetated enclosures should take into account not only the socio-ecological factors of the primates they house but also the limitations in maintaining stable group numbers to maintain vegetative sustainability. Similar findings have been found in rhesus macaques at Cayo Santiago, where access to vegetation was given. Close to half of their diet was based on the



provisioned food, and the other half on natural vegetation (Marriott et al., 1989). If animals are housed in enclosures that are designed to duplicate as much as possible the natural habitat, then some primate species that we are accustomed to seeing in zoos around the world may be only seen in the countries they are endemic.

### 6.3. *Benefits of sociality for primates*

Unfortunately, there are no activity budgets reported for pygmy slow lorises living in the wild to compare with those living in captivity. However, the transfer to social from solitary housing led to a high level of gregariousness, sharing nest boxes, and spending about 10% of their time engaged in social grooming. They also showed a decrease in locomotion and a disappearance of stereotypic behaviors, as well as a decline in glucocorticoid levels. The findings add to the growing information that female pygmy slow lorises are not solitary animals (Fitch-Snyder and Ehrlic, 2003, Yamanashi et al., 2021). Improvements in their social environment in captivity should enhance their welfare as much as providing animals with more complex living areas and with more foraging opportunities.

Stress-relieving behaviors like playing are associated with ideal living conditions, appear to be more common in highly enriched enclosures and are considered very important for animal welfare (Novak and Suomi, 1988; Claxton, 2011). Immature Japanese macaques living in the vegetated enclosure spent significantly more time playing with others than immature macaques living in non-vegetated enclosures did. These findings concur with research done on other primate species that reveal how naturalistic environments promote more social play behaviors in primates than lower-quality environments. For example, Li and Rogers (2004) found that juvenile white-headed langurs (*Trachypithecus leucocephalus*) living in high-quality habitats engaged in

significantly more time engaged in social play than the group in a low-quality habitat, while a study comparing the behaviors of captive Hatinh and Delacour's langurs (*Trachypithecus hatinhensis* and *T. delacouri*) living in caged vs semi-free ranging naturalistic enclosures found that immatures living in vegetated enclosures had a greater amount of time spent socializing and playing than their caged counterparts (Phan and Stevens, 2012). The negative relationship between stressful environments and the absence or decrease of social play has also been used to monitor animal welfare. A large number of visitors and their proximity to orangutans (*Pongo pygmaeus* and *P. abelii*) in their exhibit was also associated with a decrease in play behavior (Choo et al., 2011). In children, play stimulates the reward system in the brain which can help with focus and activity and helps developing social skills, interests, and talents that they can use later in life (Wang and Aamodt, 2012).

The female pygmy lorises studied were not observed playing, but they were adults and they did show a high degree of affiliative behaviors, such as nesting together mostly every night and increasing their time spent social grooming. Self-grooming also significantly increased when moved to social housing, which presents a puzzle because self-grooming has often been used to describe and monitor an increase of stress, anxiety, or pre-conflict behaviors in primates (e.g., female crested black macaques (*Macaca nigra*)(Aureli and Yates, 2010); chimpanzees (*P. troglodytes*) (Fraser et al., 2008)). However, self-grooming can also be an indicator of positive animal welfare. One study looking at the link between emotional states and social and self-grooming in Barbary macaques (*Macaca sylvanus*) found that, unlike previous studies, an increase in self-grooming was not due to a higher risk of receiving aggression, environmental stressors, or preceding an initiation conflict, but arise from an emotional response to changes in activity (e.g. from grooming to traveling) and/or frustration at the termination of social grooming for both the

animal in the receiving end and the groomee (Molesti and Majolo, 2013). Crucial to recognizing the benefits of social housing was the finding that female pygmy slow lorises decreased their fecal cortisol levels, formed affiliative interactions quickly, and nested together. Although there might be initial stress moving into a new environment and being housed with unrelated individuals, an increase in both self and social grooming might outweigh any of the stress associated with changing into a new environment and may be an overall positive benefit of social housing this species, historically labeled as being semi-solitary.

#### 6.4. *Stereotypies and stress in primates*

Stereotypies are defined as repetitive behaviors with no apparent function (Mason, 1991). They may develop spontaneously in animal populations, and they can serve as valuable models for behavioral disorders in humans and animals as they provide additional tools for investigating the physiological, developmental, and environmental impact on behaviors (Lutz, 2014). They have been extensively used to measure improved states of welfare with a decrease or disappearance of them in various primate species. Relationships between these behaviors and physiological parameters are quite notable in macaques (Novak et al., 2012), although they have also been studied in farm animals since they can be interpreted to indicate inadequate living spaces such as environmental design or welfare (Dantzer, 1986). Stereotypic behaviors occurred in both Japanese macaques and pygmy slow lorises, but they exclusively occurred in the less favorable housing condition (non-vegetated enclosure in macaques and singly housed in the lorises). No stereotypical behavior was observed among animals living in enriched enclosures.

In macaques, I observed what I termed “bar-hanging”, which subjectively could be described as a type of game or resting behavior rather than a stress indicator, but other repetitive

behaviors with no apparent function such as twirling, head bobbing, self-biting without injury were noted. These types of activity have been linked to increasing HPA-axis activity and increased heart rate in rhesus macaques (*Macaca mulatta*) (Marinus et al., 1999; Lutz et al., 2003), possible indicators of individuals unable to or attempting to naturally reach homeostasis. The presence of these behaviors in the non-vegetated enclosure are similar to those reported in captive tufted capuchins (*Cebus apella*), where these stereotypic behaviors were associated with higher levels of fecal cortisol compared to those that did not engage in stereotypic behaviors (Pomerantz et al., 2012).

Acute stress in healthy individuals activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in a significant rise in corticosteroids which is followed by a return to baseline when the stressful event has passed. Effective coping mechanisms enable the corticosteroid level to quickly revert to baseline when the source of stress persists. Without any coping mechanisms, corticosteroid levels might stay high for a long time and have negative impacts on an animal's health and well-being. Historically, researchers have grouped various stereotypies because they are thought to represent a homogeneous group of behaviors (Waitt and Buchannan-Smith, 2001), but recent studies (Poirier and Bateson, 2017) have shown that different stereotypies can be attributed to a variety of reasons and emotional states within the same species; therefore, cross-species comparisons regarding the effects of a certain stereotypy in a particular species on a different species is difficult. However, the comparison between macaques living in vegetated vs non-vegetated enclosures regarding stereotypical behaviors resembles previous reports because these behaviors were not present in the enriched enclosure. Earlier studies attempting to decrease stereotypy (and stress) in macaques found that they declined when given ample space (Draper and Bernstein, 1963) The amount of time spent in stereotypic behaviors was cut in half after being

moved to better enclosures (Kaufman et al., 2004). Moving lion-tailed macaques from indoor caging to group-living in socially enriched outdoor enclosures had a positive influence on their behavioral repertoire and welfare by decreasing levels of stereotypic behaviors (Mallapur et al., 2007).

The pygmy slow loris, Sakura, that engaged in stereotypic behavior when alone stopped self-squeezing through a nest box when socially housed, although the same type of nest box was present. Studies on the prevalence of stereotypic behaviors of prosimians housed in captivity have shown that in 10 genera close to 13% have been observed exhibiting stress-related behaviors which have been attributed to the type of housing provided (Tarou et al., 2005). A relationship between stress and stereotypic behaviors has been widely reported in macaques (Lutz et al., 2003; Pomerantz et al., 2012), but have been underreported in strepsirrhines, and these findings can have positive implications for the management of pygmy slow lorises in captivity. Increased enclosure space, combined with housing with conspecifics seems to improve the welfare of female pygmy slow lorises.

#### *6.5. Animal memorial ceremonies, human-animal interactions, and animal welfare*

The idea of evaluating our human-animal relationships with the animals we keep has been well discussed since the beginning of the last century, especially with farm animals, such as broiler chickens housed in small enclosures, and the conflicts that arise from keeping horses in good conditions only to race and push them to their limits (Harrison, 1964). However, keeping animals for research purposes rather than farming is relatively a new phenomenon in our evolution of human-animal interactions. Moreover, keeping primates for research purposes is also quite recent. Prior to the turn of the 19<sup>th</sup> century, the scientific community wanted to identify the key neuronal

pathways in the brain that are responsible for the cortical control of movement and vision through non-human primate models. This led to the establishment in 1912 of the Primate Station in Tenerife in the Canary Islands by Max Rothmann, neurophysiologist at the University of Berlin and sponsored by the Royal Prussian Academy of Sciences (Teuber, 1994). The Station became famous by Teuber's observations on chimpanzee's "affective calls" and "gestural language", and more notably, Wolfgang Kohler's empirical studies of chimpanzee problem solving that led to his first publication, "Intelligenzprüfungen an Anthropoiden I" by the Royal Prussian Academy of Sciences in Berlin and later his book, *The Mentality of Apes* (1925) (Ulric, 2002). Soon many other countries formed primate centers of their own and one practice that started along with animals we research are animal memorial ceremonies. A survey of 120 research centers in Japan (Nishikawa and Morishita, 2012) found that 95.1% of the 69% of total respondents replied that they have animal memorial ceremonies. The results revealed that 45.7% of the ceremonies were religious, while 44.5% were non-religious and 9.6% reported it as another type of ceremony. Close to half of the respondents that reported it to be a religious ceremony noted that it was Buddhist while the other half said it was Shinto based.

**Chapter 5** presents an expansion of these earlier reports by considering places outside of Japan where animals are housed, where animal ceremonies are taking place, and how people working with animals perceive these ceremonies. The only predictor variable that agreed with the statement that animal memorial ceremonies are needed, or considered to be a good activity, was the number of negative emotional responses related to compassion fatigue when respondents lost an animal they had worked with. Compassion fatigue, a state of exhaustion and dysfunction biologically, psychologically, and socially as a result of prolonged exposure to compassion stress, has been well documented in affecting healthcare workers (Cocker and Joss, 2016), as well as

healthcare workers for animals (Van Hooser et al., 2021). These studies have found that the stresses people can experience while working with animals may be further complicated by each individual's self-understanding and emotional investment in the human-animal bond. Findings such as these have created institutional management changes that include emotional support and are becoming more proactive with end-points for animals in research. Workers who are affected the most by departed animals are also most likely to favor animal memorial ceremonies. People who have experienced compassion fatigue may need to be given some precautions before starting to work at a research facility. Moreover, institutions may consider the growing interest in animal care and welfare to include human animals that work with nonhuman animals and possibly provide workers that may have a more salient response to an animal's death a space to grieve. Animal memorial ceremonies have the potential to improve animal welfare, as they promote closer human-animal bonds, draw attention to a crucial similarity between humans and animals that the law does not recognize (Narver et al., 2017).

#### *6.6. Conclusions, limitations, and future perspectives*

Improvements to how we keep primates in captivity in the form of better enclosures and behavioral management strategies are having positive implications for the welfare states of Japanese macaques and pygmy slow lorises. Providing vegetated naturalistic enclosures for male Japanese macaques bred for research purposes gave them more opportunities to engage in species-specific behaviors, while also decreased the amount of stereotypic and aggressive behaviors. Forming female pygmy slow loris groups in captivity seems to be an appropriate management strategy as they opt to engage in affiliative behaviors and spend their time close to each other, while also reducing stress and stereotypic behaviors. Finally, human grief can emerge from working with captive animals who die or are sacrificed, resulting in a positive viewpoint about

animal memorial ceremonies. Some people grieve when their pets die, so one might have expected that the death of a captive animal that one has formed a bond with would produce a similar emotional state.

Because female reproductive state is artificially controlled among the Japanese macaques at PRI, my study was limited to males. The removal of immature individuals as a management strategy could have influenced the behavior of male Japanese macaques which could have affected my results. Additionally, for macaques to live in self-sustaining vegetated enclosures, a yearly rotation to adjacent enclosures was needed, which might have also affected activity going from a highly foraged area to an untouched area. The enclosure also had irrigation systems, electrical fencing, tree maintenance, and perches, which all required a much larger space than the non-vegetated enclosure I compared it with. Vegetation also functions as visual barriers that reduce the number of aggressive interactions. Vertical spaces in the form of trees and high perches could also benefit monkeys by avoiding others when aggressive interactions start, but these spatial indices were not measured. Finally, differences in family lineages (Minou for the vegetated and Takayama for the non-vegetated) might have affected how animals behaved and interacted with each other. Stereotypic behaviors and hair loss may be more or less prevalent in females compared to males, but my study subjects were only male Japanese macaques. Although providing animals a vegetated enclosure is good for their well-being, it is not a cure-all solution for all challenges that come from macaques living in captivity. Vegetated enclosures require a large initial financial investment, more space, and still require population manipulation in order to maintain the quality of the enclosure.

The research on pygmy slow lorises suffered from a small sample size. Eight females were studied, but only 6 provided both behavioral and physiological data. Moreover, no details about



their husbandry, health, or life histories before their confiscation in 2006 exist. A few of the lorises might have been captive all of their lives while others had just been captured from the wild. Their precise ages were unknown but it had been over a decade since all were confiscated. Therefore, some if not most of the females were reaching geriatric life stages, as they were approaching the 19.3-year life expectancy reported in captive individuals (Zehr et al., 2014). A recent discussion with loris experts informed me that female-female pairings work in situ until they reach breeding season, and once the season starts, they seem to be incompatible as pairs. Life histories, age, and whether or not they enter a breeding season in ex-situ indoors may all affect successful social bonding among captive female pygmy slow lorises.

The animal memorial ceremonies survey had a total of 23 questions, but I missed a very important question that occurred to me after the survey was completed. The type of animal or species they worked with could have had an impact on their perspective about animal ceremonies if emotional responses to the death of an animal depends on the species. Perhaps animals genetically closer to us results in more salient affective states, or species that are considered “cuter” have more of an impact on the worker. Finally, my conclusions are biased toward people who work or are around animals, and responses could have been different if I had recruited more participants that do not work with animals.

Captive management strategies can and should improve the lives of captive primates without requiring many technological advances. They can be easily reproduced at in-situ institutions. Although technological advancements have improved captive management in the form of sophisticated enrichment and management, some basic strategies can readily be replicated in primate host countries where resources may not be easily available also (S.E. Asia, S. America, Africa). On the other hand, ensuring the health and well-being of primates might mean that some

species should not be kept in places where they are now resident in a captive situation. My hope is that adopting good animal welfare practices will have a positive impact on how we treat our nonhuman primate relatives no matter where they are housed. As noted in the introduction, animal welfare and animal ethics are merging together as they both strive to maximize the quality of life for captive individuals.

## 7.0. References

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