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

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## Abstract

Improving captive conditions of pygmy slow lorises (Nekaris and Nijman have recently suggested that the pygmy slow loris should be called the pygmy loris and is distinctive enough to warrant a new genus, *Xanthonycticebu*) (*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, eight 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. We conclude that social housing of pygmy slow lorises improves their well-being by reducing stress levels, and that their group housing 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.

**KEYWORDS**

all-female groups, animal welfare, fecal glucocorticoids, social housing, stereotypic behaviors, *Xanthonycticebus*

# 1 | INTRODUCTION

Pygmy slow lorises (*Nycticebus pygmeaus*) are nocturnal primates endemic to Southeast Asia. It has recently been suggested that the pygmy slow loris should be called the pygmy loris and that it is distinctive enough to warrant a new genus, *Xanthonycticebus* (Nekaris & Nijman, 2022). Lorises are exudativorous, have a litter size of one to four, torpor during the winter months, and are the smallest and fastest of all the *Nycticebus* spp. (Cabana & Plowman, 2014; Gardiner et al., 2018; Madani & Nekaris, 2014; Ruf et al., 2015). Most of their threats are anthropogenic, including deforestation, hunting for the illegal pet trade, and their use in traditional medicine (Feng, 2012; Iseborn, 2011; Musing et al., 2016; Nahallage et al., 2008; Nekaris & Starr, 2015; Ratajszczak, 1998; Starr et al., 2010). 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 and 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 of the pet trade have resulted in an increase in the number of live wild animals seized and housed by government organizations (Fuller, 2022; 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 and prone to poor conditions and exploitation (Rivera et al., 2021; Zhou et al., 2016). In Japan, 400 individuals listed as *Nycticebus* spp. 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).

In the wild, earlier reports of lorises had rarely been recorded to form groups, but recent data suggest 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 (*Nycticebus pygmaeus*) females sleep with their young or other subadult females (Starr & Nekaris, 2020; Starr et al., 2011). Long-term field data in West Java of slow lorises (*Nycticebus javanicus*) show that they too often share sleeping sites with conspecifics (Nekaris, 2014), and home ranges overlap (Starr & 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).

For most primates in captivity, social housing is the default housing status, and it is an important requirement that should be met whenever possible (Coleman & Novak, 2017). When social housing and adequately complex living spaces are provided to primates, most animal welfare concerns can be alleviated (Hannibal et al., 2017; Lutz & Novak, 2005). Social housing has been well documented to decrease stereotypic behaviors and prevent chronic diseases related to stress in rhesus macaques (*Macaca mulatta*) (Bernstein, 1991; Leonardi et al., 2010), and promote general well-being in capuchin monkeys (*Cebus apella*) and squirrel monkeys (*Saimiri sciureus*) when paired together (Baker et al., 2012), including pygmy slow lorises (*N. pygmeaus*) (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 & Ziegler, 2002).

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 hypothalamic–pituitary–adrenal (HPA) axis activity, lowering fecal cortisol levels (Cohen & Willis, 1985). Similar relationships between stress and affiliative behaviors have been reported in various primate species. Strong social bonds between male Barbary macaques (*Macaca sylvanus*) help reduce environmental stress (Young et al., 2014), female yellow baboon grooming networks (*Papio cynocephalus*) help alleviate stress in the wild (Wittig et al., 2008), and affiliative interactions help reduce stress in chimpanzees (*Pan troglodytes*) from daily stressors (Wittig et al., 2016), while bonobos responding to other conspecifics distress has the same effect (Clay & 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 (*Nycticebus 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 (Daschbach et al., 1983).

When attempting social housing, a primate's social system and organization must be taken into account to maximize success, 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 the success and failures in housing lorises in captivity in European and American zoos (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.

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 may have lower fecal cortisol levels in compromised welfare conditions (Pawluski et al., 2017). Female squirrel monkeys (*S. sciureus*) 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 conditions, and behavior among adult female pygmy 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 Section 2), allowing us to assess the impact of housing conditions on hormones and stereotypies. Here, we 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.

## 2 | MATERIALS AND METHODS

### 2.1 | STUDY SITE AND SUBJECTS

The study took place at the Slow Loris Conservation Center (SLCC) in the Japan Monkey Center (JMC), where lorises confiscated at various airports in Japan between 2006 and 2007 were brought. The purpose of establishing the SLCC was to improve their welfare, conduct outreach education and awareness for visitors, and provide an opportunity for noninvasive research. The confiscated animals were moved in 2015 after the enclosures were refurbished for slow lorises. The enclosures were provided with adequate reverse-lighting cycles, where lamination levels between 0.00 and 0.18 lux (Yamanashi et al., 2021), where red lighting was only used during husbandry practices. There was 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 & Nekaris, 2015; Cabana & Plowman, 2014). Our eight study subjects were housed in small quarantine cages ($50 \times 50 \times 75$ cm; $0.3$ m$^3$) before being moved to the larger permanent enclosures (Table 1). The permanent enclosures ranged from two compartments of around $1.5$ m$^3$ ($0.91 \times 0.92 \times 1.78$ m) with two tunnels, to allow for slow introductions in the smallest one, a medium-sized enclosure consisting of two compartments with two tunnels ($128$ cm $\times 125$ cm $\times 207$ cm each compartment), and the largest enclosure with open space for social grouping ($239$ cm $\times 282$ cm $\times 249$ 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. The 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 four females in September 2017. The collection of fecal samples continued during the process until the summer of 2018, when male–female pairs were formed.

**TABLE 1** Data collected by subject before and after pairing.

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

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 (Alejandro et al., 2021; Yamanashi et al., 2021). This study conformed to "The Guide for Animal Research Ethics" of the JMC, the legal requirements of Japan, and the ASP Principle for Ethical Treatment of Nonhuman 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).



**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 four females; Roji, Tsubaki, Momiji, and Sakura in 2017–2018.
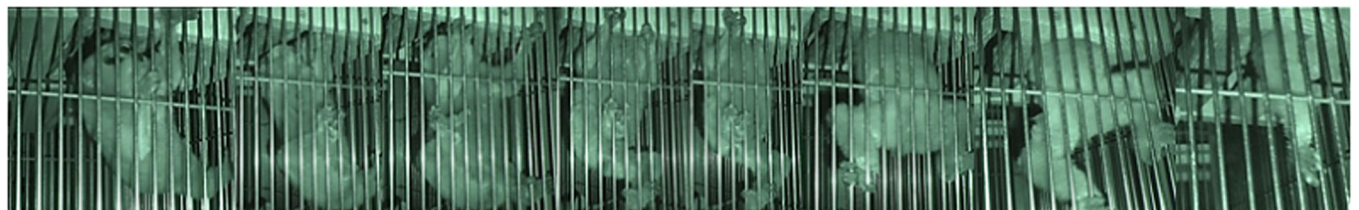
## 2.2 | BEHAVIORAL DATA COLLECTION

Behavioral data were collected in 10 min continuous focal sampling sessions following Martin and Bateson (1993), where the four main activities were recorded; move, rest, groom, feed, and all other behaviors recorded ad libitum, described in detail by Alejandro (2021) and others. Postural behaviors were classified following Glassman and Wells (1984). Daily 10 min focal recording sessions for each female were made with a hand-held night vision video camera, where the researcher was present and observing at 1-m distance from the enclosure, during the night cycle between 13:00 and 16:00 from June 2017 to March 2018, with an average of 3.14 (1.21 SD) observations per individual weekly. The same methods were used before and after group formation. Pair group observations before social housing commenced were conducted from June through September (3 months) and from September to February (5 months) postsocial-housing, while for the group of four, presocial-housing was performed from November through December (2 months) and December through February (3 months) in the social-housing condition. For behavioral data collection, we conducted instantaneous sampling at 1-min 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 box 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.

## 2.3 | PHYSIOLOGICAL STRESS

### 2.3.1 | Fecal sample preparation

Fecal samples were collected during light cycles by the husbandry staff (2016–2018) and J. A. (2017) during observation hours for



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

subsequent analysis by enzyme immunoassay (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 30 min at room temperature (24°C) at 5000 rpm. The supernatant was used for hormonal analyses by EIA.

### 2.3.2 | Cortisol EIA

The extracts of cortisol metabolites in the supernatant were analyzed using a modified EIA method previously used on Japanese macaques and other animals by Takeshita et al. (2018) and Kinoshita et al. (2011). Briefly, the fecal extracts were diluted 98- to 100-fold with EIA buffer (0.15-M NaCl, 0.04-M $Na_2HPO_4$, 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 immuno-globulin G (H&L) Affinity Pure (A16112: Invitrogen). To obtain the standard curves, 0.024–50 ng/mL of cortisol (H4001; Sigma-Aldrich Co.) diluted with EIA buffer was also dispensed into the wells in duplicates. Immediately after the addition of 50 μL of cortisol antiserum (×40000, FKA404E; Cosmo Bio Co., Ltd.) and an equal volume of horseradish peroxidase-conjugated cortisol (×80,000, FKA403; Cosmo Bio Co., Ltd.), 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 μL of 6 N $H_2SO_4$ 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.

### 2.3.3 | 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 the 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. Interassay variabilities were 18.9% (high) and 17.2% (low) and intra-assay variabilities were 13.2% (high) and 11.8% (low). Data with a coefficient of variation values that were higher than 10% were reassayed for the analysis.

## 2.4 | STATISTICAL ANALYSIS

All statistical analyses were conducted with RStudio version 4.02.0.0 (R Core Team, 2021). We used repeated measures of analysis of variance (ANOVA) to test for differences in hormonal profiles between (1) pre- and postsocial 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 & 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 multipair comparisons where ANOVA was conducted. Where main effects were significant, we used the post hoc Tukey's honestly significant difference test for pair-wise comparisons. All datasets were checked for normality with the Shapiro–Wilk (SW) 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, we 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 h). To compare fecal cortisol levels of socially housed females' versus subsequent male–female pairs, We used a nonparametric Wilcoxon-sign rank test. Statistical significance was set at $p < 0.05$ and two-tailed results were reported.

## 3 | RESULTS

Female pygmy slow lorises exhibited statistically significant lower levels of fecal cortisol once socially housed in an enriched enclosure ($F_{(1, 127)} = 3.24$, $p = 0.03$; 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 1 h 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 1 h 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 four 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 four had statistically significantly higher levels of fecal cortisol than two 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 ($n = 12$)
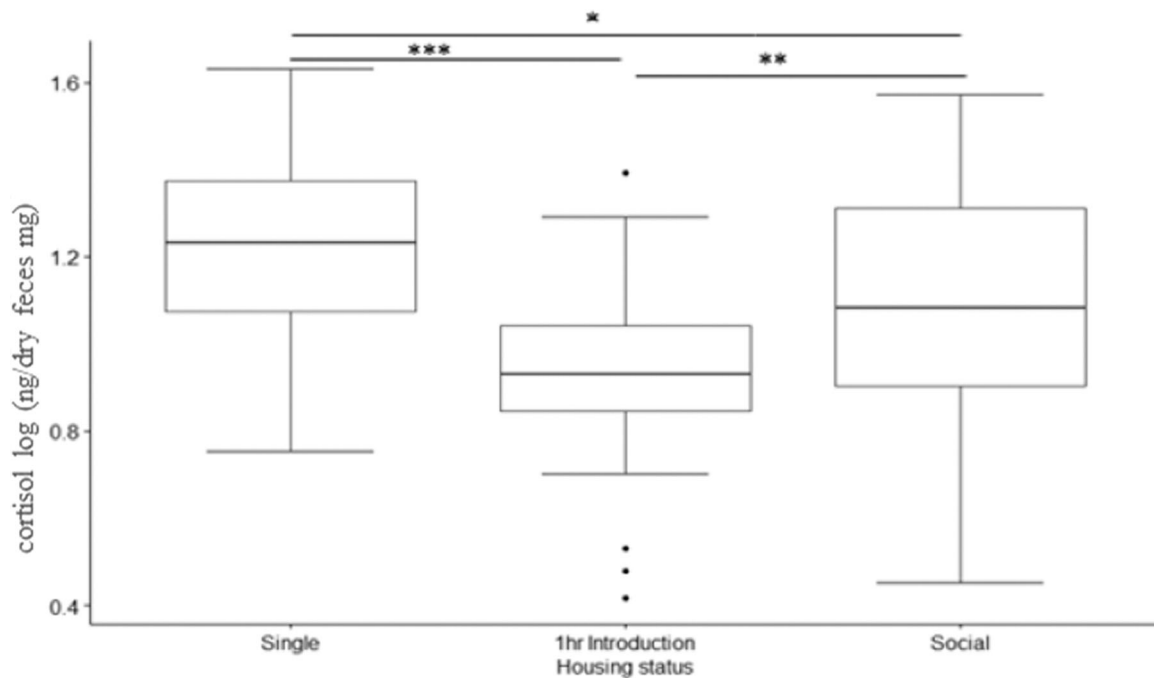
**FIGURE 3** Boxplot represents fecal cortisol log (ng/mL) in the three types of pairing process of the second pair, Mizuki and Nanten, where the boxes represent the lower and upper quartiles, whiskers represent the minimum and maximum, and the horizontal line the median, with significant codes set as *0.05, **0.01, ***0.001.
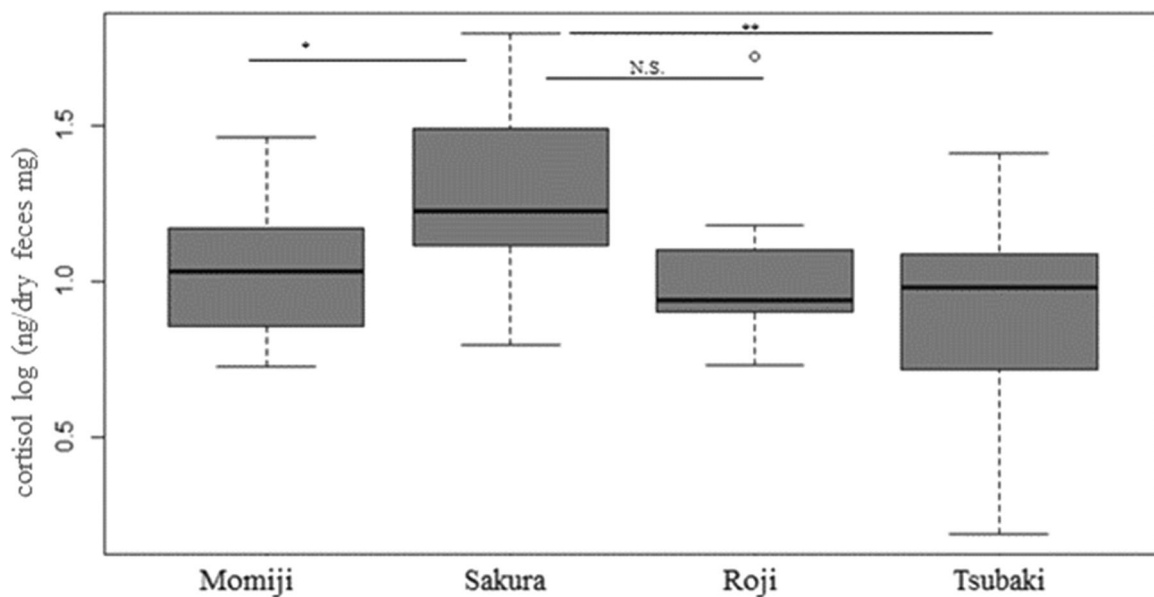


**FIGURE 4** Boxplot represents fecal cortisol log (ng/dry feces mg) comparing fecal cortisol of all four females in the singly housed condition compared with Sakura, which engaged in stereotypic behaviors. Boxes represent the lower and upper quartiles, whiskers represent the minimum and maximum, and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

$z = 2.58$, $p = 0.01$), 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

**TABLE 2** Results from linear mixed effects model (lme4 using drop-1 function) on associations between fecal cortisol on activity and postural behaviors.

|  | Estimate | SE | df | T value | p Value |
|---|---|---|---|---|---|
| **Cort~Behav** | | | | | |
| 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** | | | | | |
| 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 |

*Note*: 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 "****".

Abbreviation: REML, restricted maximum likelihood.

and male–female ($n = 6$) housing ($W = 12$, $z = 0.65$, $p = 0.39$) were observed.

## 4 | DISCUSSION

Social housing is critical for primates living in captivity because they are highly social animals and need social interactions to thrive. Primates that lack social interaction with conspecifics may develop stress-related behaviors including self-harm and stereotypical behaviors. Social housing also provides opportunities for primates to learn and develop skills necessary for survival in the wild. In addition, social housing helps to reduce aggression and other negative behaviors that can arise from social isolation while meeting ethical and regulatory standards. Nonetheless, primates have evolved to defend themselves from others both in captivity and in the wild, and many factors can affect the success of social housing.

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.

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 et al. (2008), that a minimum cage size of 2.5 m × 2.5 m × 2.5 m with enough furnishing should safely house one to three 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, we were 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-h introduction, and fully socially housed. From the weeks spent on each housing status, 1-h pairing showed the lowest levels of fecal cortisol. Intermittent pairing, daily separation, and repairing (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.

Regarding Prediction 3 that behaviors expressed when solitarily housed would be reflected in our physiological findings were 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-Negrín et al., 2009). Emphasizing the importance of resting, Ebinghaus et al. (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 on 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 & Suomi, 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 et al. (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 and others (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; Nycticebus 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.

Regarding Prediction 5, when we 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 & St. Claire, 1998; Crockett et al., 1994), but success rates varies between species (Jorgensen et al., 2015; Truelove et al., 2017), and within species depending on the institution (DiVincenti & 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 (Crockett et al., 1994), seem 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. We 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.

Unfortunately, we 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. It is possible that some animals were related or confiscated from the same area, or they might have all been from different countries, which could have affected our findings. Likewise, limited space did not allow for visual and olfactory separation between the male and female lorises, and inverted lighting

cycles could have affected their natural seasonal hormonal changes might have also affected our results. However, rhesus macaques (M. mulatta) are also seasonal breeders and are often paired indefinitely with conspecifics of the same sex with visual and olfactory access to the opposite sex, yet have equal pairing success (MacAllister et al., 2020). Additionally, since the animals were confiscated in 2006, most being in their early to mid-teens at the time of pairing, seasonal breeding changes might not have played such a big role. Our 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 unpairable and unreleasable 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 unreleasable 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 to 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, 3 died, 4 lost their radio collars, and 1 had to be recaught. Only two were in good condition after 2 months living in the wild (Kenyon et al., 2014; Osterberg & Nekaris, 2015; Pointdexter et al., 2017; Streicher et al., 2003). 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.

## 5 | 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. We 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.

## AUTHOR CONTRIBUTIONS

Conceptualization and collection of data was a collaboration between Josue Alejandro, Kei Nemoto, and Yumi Yamanashi. Hormonal analysis and sample processing were conducted by Keiko Mouri, Josue Alejandro, and Ching-wen Huang. Methodology, analysis design, and write-up were done by Josue Alejandro, Michael A. Huffman, Fred B. Bercovitch, and Yumi Yamanashi. All authors have read and agreed to the published version of the manuscript.

## CONFLICTS OF INTEREST STATEMENT

The authors declare no conflict of interest

## DATA AVAILABILITY STATEMENT

The data presented in this study are openly available in FigShare at 10.6084/m9.figshare.21827349.

## ETHICS STATEMENT

The study was conducted according to the guidelines and approved by the Institutional Review Board of the Primate Research Institute Kyoto University (protocol code 2018-159) and the Research Ethics Committee of the Japan Monkey Centre (protocol code 2015001; protocol code 2016001; protocol code 2017003).

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