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Environmental Drivers of Stress in Red Pandas (Ailurus fulgens) : Behavior and Space Use Changes in Response to Redesigned Naturalistic Enclosures

Alexis Michelle Lawson

Abstract

Animal welfare encompasses the physical and mental state of an animal, as well as how that animal is cared for. Recently, zoological settings have shifted from strictly housing animals to improving the welfare of the animal such that the goal of captivity is for residents to thrive. This is especially crucial as global wildlife numbers and biodiversity continues to decrease due to anthropogenic impacts such as poaching, mining, habitat destruction, fragmentation. Further confounding this issue is climate change, air pollution, invasive species, ocean acidification, and more. In particular, the red panda (*Ailurus fulgens*) is an endangered species whose declined 50% within last three generations (20 years). Thus, maintaining captive populations, and ensuring their subsequent reproductive success, is vital for the longevity and diversity of this species.

To examine the welfare of zoo-housed red pandas, behavioral assessments were made in response to different environmental variables, including visitor number, weather, temperature, environmental change, and the presence or absence of a conspecific. From February to December of 2022, observations were made for three-hour sessions one to two times per week at Essex County Turtle Back Zoo, located in West Orange, New Jersey.

It was found that zoo visitor numbers and time with a conspecific were most important factors in determining stereotypies in subjects. Behaviors indicative of stress were significantly correlated with higher temperatures and visitor numbers, while more time with a conspecific reduced stress-linked behaviors. It was also discovered that pacing duration was significantly predicted by environmental factors, with again, zoo visitor numbers being the most influential. Overall, this study identified significant correlations between environmental factors with space use and behavior, establishing a valuable starting point for the development of zoo management strategies with the goal of reducing stress and improving captive red panda welfare.

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Environmental Drivers of Stress in Red Pandas (*Ailurus fulgens*): Behavior and Space Use Changes in Response to Redesigned Naturalistic Enclosures

by

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Abbreviations

AZA- The Association of Zoos and Aquariums

SAFE- Saving Animals from Extinction

dB- Decibel

IUCN- International Union for Conservation of Nature

CITES- Convention on International Trade in Endangered Species

TBZ- Turtle Back Zoo

SSP- Species Survival Plan

PCA- Principal Component Analysis

ANOVA- Analysis of Variance

p- Probability

Introduction

1. Introduction

1.1. Animals in Captivity

As early as 2500 BCE, zoos, or more specifically, menageries of private collections owned by aristocrats, have been used to house animals under human care (Houlihan, 1996; Kisling, 2000). The ethical evolution of zoos, however, did not occur until the eighteenth century (Baratay & Hardouin-Fugier, 2002). In recent times, zoos, aquariums, and other captive settings have the obligation of providing top notch care for their residents, promoting positive welfare in the form of naturalistic environments, appropriate diets, mentally stimulating enrichment, and medical treatment (Fernandez et al., 2009). The Association of Zoos and Aquariums (AZA), established in 1924, has created standards that exemplify the importance of animal welfare, research, education, and conservation efforts.

In 2019 alone, the AZA allocated $230 million to field research in 127 countries (Falk, 2007). Facilities also work to directly reintroduce captive endangered animals to the wild (Condor Recovery Program; Che-Castaldo et al., 2018) and repair the ecosystems essential to their survival (2018's Malasia Saving Animals from Extinction (SAFE) orangutan program; Boshak, 2021). Zoos and aquariums also aid in animal rehabilitation, and in accordance with the U.S. Fish and Wildlife Service, help rescue and care for wild animals, such as sea turtles and otters, until they can be released back into their native habitats (Thompson, 2015). Furthermore, most zoological settings are used as educational tools for the public and research institutions; As of 2019, AZA facilities incorporated over

2,500 education programs on topics such as human-wildlife conflict, and even invested $26 million to research efforts spanning more than 550 species (Falk, 2007).

However, even with advancements in understanding and care practices for animals, there are still sources of stress induced by captive settings (Dawkins, 1990). Several abiotic environmental stressors have been shown to increase stereotypies across an array of species. First, sounds in captivity often include varying frequencies of unnatural noises, contrasting that of their natural environments, and sounds above 85 dB have been shown to induce stress in captive animals (Anthony et al., 1959). Further conditions, including thermal (Stoinski et al., 2002), weather (Ramsay, 1995), lighting (Bellhorn, 1980; Hediger, 2013), and enclosure design (Clubb & Mason, 2007) can all affect animal stress and result in behavioral stereotypies (Mason, 1991), which is expressed as highly repetitive, invariant, abnormal coping acts which are without apparent adaptive function (Mason, 1991).

Overexposure to stress in captive animals can cause major physiological issues, including weight loss, reduced immune system function, and decreased reproductive abilities (Fischer & Romero, 2019). It is therefore crucial to understand the signs of stress and different stereotypies presented by varying captive species to negate the potential negative effects of ‘zoochosis’ (Ringelestein, 2021). These include pacing, and repetitive or excessive head-bobbing, swaying, bar-biting, licking, and grooming (Fischer & Romero, 2019). When endangered species, including the red panda (*Ailurus fulgens*, Cuvier 1825) exhibit stereotypies such as intense pacing, irritation and/or aggression with conspecifics, and over-grooming (of the tail specifically) in captivity (Olson, 2001), it can, however, reduce not only the success of captive care, but also zoos' educational and conservation goals.

1.2. The Red Panda

The red panda (*Ailurus fulgens,* Cuvier 1825) has often been the center of taxonomic dispute. Because of their morphological qualities, including dentition, head shape, and tail pattern, the red panda was initially classified into the raccoon family (Procyonidae, Cuvier 1825), and then later considered to be part of the bear family (Ursidae; Lindzey, 1990; Leone & Wiens, 1956). Its colloquial name stems from the Nepali word, "ponya," which translates to "bamboo eater". While many believe that the red panda and giant panda are closely related, the two only share the quality of a pseudo-thumb, which aids in grasping bamboo when eating (Endo et al., 1999; Antón et al., 2006; Davis, 1964; Gould, 1982). Modern genetic testing has placed this species in its own taxonomic family, Ailuridae (Choudhury, 2001), and further phylogenetic analyses classifies the red panda in the order *Carnivora*, with the closest relatives being raccoons, weasels, and skunks (Eizirik et al., 2010).

Fully-grown red pandas weigh approximately 2.7 to 6.4 kilograms and have a body length of about 58 centimeters with a tail length of around 40 centimeters (Roberts & Gittleman, 1984). The red panda is characterized by their dense undercoat overlain with guard hair and bushy tails which provide both balance and protection against cold, harsh winds. Recognizable by their redbrown coat coloration and black underside, which camouflages the animal in their preferred fir trees, the red panda also has a white face and pointy ears with tear marks extending from the mouth to the eyes, which reflect sunlight from their vision (AZA Small Carnivore TAG, 2012). Red pandas rely on olfactory communication, scent marking with glands located on their footpads and anal region (Yonzon & Hunter, 1991), both of which release a colorless, odorless secretion which is undetectable to humans (Roberts & Gittleman, 1984). Unique to red pandas, an oral gland (located under the tongue) allows individuals to test these odors in a form of communication (Emura et al., 2009).

Red pandas are an arboreal species that inhabit temperate forests in the Himalayas and other high-altitude environments (CEPF, 2005; Sharma, et al. 2014; Yonzon & Hunter, 1991). In addition to their pseudo-thumbs, red pandas' fibula and tibia attachment allows for the rotation of the fibula around its axis, facilitating adept climbing including moving head-first down trees (Roberts & Gittleman, 1984; Fisher et al., 2008; Fisher, 2011). Their geographic range spans across India, Bhutan, China, Nepal, Tibet, and Myanmar (Dorji et al. 2012; Glatston, 1994). Optimal temperature ranges for red pandas in this region span 10 to 25 °C (50 to 77 °F; Roberts & Gittleman, 1984; Loeffler, 2011). When exposed to frigid conditions, the red panda can curl into a ball, lower its metabolic rate, and remain dormant in a state of torpor, allowing them to expend as little energy as a sloth (Watts & Cuyler, 1988; Fei, 2016), while in hot temperatures they can pant and splay out to maintain thermostasis (Princée & Glatston, 2016).

The red panda's metabolism and energy budget are very much limited by their obligatory bamboo-eater status (Yonzon & Hunter, 1991). While more than 90% of the red panda's diet is bamboo leaf tips and shoots (Fei et al., 2017; Panthi et al., 2015; Sharma et al., 2014), they will also feed on fruits, insects, and on occasion, birds, and small mammals (Yonzon & Hunter, 1991). The red panda is crepuscular (most active at dawn and dusk), in addition to being more active in the cold and mating season (Johnson et al., 1988; Roberts & Gittleman, 1984).

Red pandas are relatively solitary animals, whose home range only spans about one square mile (Thapa & Basnet, 2015). Individuals only encounter conspecifics during mating season (Pradhan et al., 2001; Yonzon & Hunter, 1991), which begins after the winter solstice in January and lasts until March. After fertilization, females can delay implantation ranging ninety-three to one hundred fifty-six days to ensure that the abundant seasons can support the high-energy gestation costs when the females' metabolic rates are slow (Curry et al., 2017; Roberts & Kessler, 1979). Gestation then lasts for approximately one hundred thirty-five days (Macdonald et al., 2005). Females build nests in tree holes and stumps, giving birth to two cubs between May and July (Yonzon & Hunter, 1991). Offspring remain with the mother until one year old, when both sexes emigrate to new territories and reach sexual maturity at around eighteen months (Roberts & Kessler, 1979). While females are no longer able to reproduce after the age of approximately twelve, males have the potential to reproduce the entirety of their lives (Northrop & Czekala, 2011). While in the wild this lifespan is, on average, thirteen years, in captivity, red pandas have reached twenty-three years of age (Heath & Platnick, 2008). By understanding species lifespan and reproductive potential, we can begin to address the larger picture: biodiversity and its resulting role in ecosystem sustainability.

1.3. Biodiversity & Conservation

Biodiversity includes all the variability of life on Earth, including genes, species, and biotas (Redford & Richter, 1999; Harper & Hawksworth, 1994; Swingland, 2001). Ecosystems rely on biodiversity (Rapport, 1998; Kumar, 2012); however, global biodiversity has been decreasing as anthropogenic impacts continue to increase, exponentially increasing species extinction rates (Grayson, 2001; Ceballos et al., 2015). Biodiversity and conservation are heavily influenced by global socio-political conditions (Wyborn et al., 2016). These conditions include the exploitation and destruction of natural resources and clearing or fragmentation of animal habitats for human activities such as agriculture and livestock farming (Thapa et al. 2018; Wei et al. 1999). These factors have a direct impact on red pandas.

Red pandas compete with livestock for bamboo, and the clearing of land continues to reduce food availability for the red panda (Wei et al., 1999). In addition, domesticated dogs have also been reported to hunt and transmit diseases to the red panda, and fragmentation has led to inbreeding within wild groups that are isolated (Glatston et al. 2015; Thapa et al. 2018).

Poverty and tourism also put pressure on protected areas (Nepal, 2000). Furthermore, plow-income countries often depend on forests for cash income or a direct source of food (Gray & Moseley, 2005), which leads to deforestation and habitat fragmentation when such activities are unsustainable, hindering biodiversity and conservation efforts (Appiah et al., 2009; Geist & Lambin, 2002). Hunting and trade have also continued to reduce this species' biodiversity and population size (Dorji et al., 2012).

Furthermore, climate change continues to negatively impact biodiversity, and as a result, wildlife populations must adapt to changing, as well as shrinking, environments or face extinction (Chevin et al., 2010; Hoffmann & Sgrò, 2011). Further complicating this problem is that laws and regulations are often not enforced, and overall investment in research and conservation is low. Because of this, zoological and other captive settings maintain red panda populations and promote reproductive success to preserve the species in the face, and to raise awareness, of such threats. To bring attention to both biodiversity and habitat preservation, particularly in low-income countries, a flagship species is often utilized.

A flagship species can be defined as a species that represents, or acts as an ambassador for, a particular habitat to support conservation in specific regions, and are often indicators of stable biodiversity (Dietz et al., 1994). The giant panda is the flagship species for the conservation of the Asian temperate forests it inhabits (Wei et al., 2018). The red panda, too, is a charismatic species that could be used to gain public traction and attention. Overall, the endangerment of the red panda, with its potential to be a flagship species, could be used to bring awareness and funding to larger conservation efforts in their native territory.

Currently, the International Union for Conservation of Nature (IUCN) Red List classifies the red panda as Endangered (Glatston et al., 2015) due to poaching, habitat degradation, and habitat loss (Hu et al. 2020; Thapa et al. 2018). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) also lists the red panda under Appendix I, preventing its trade (Choudhury, 2001). Despite laws and regulations to protect this species, hunting, logging, and livestock grazing in red panda habitat has reduced the species' populations by forty percent during the prior twenty years alone (Yin & Liu, 1993). As human populations continue to grow and expand throughout Asia, red panda habitat ranges will decrease.

1.4. Statement of Problem

To conserve and protect a species, it is best to first understand the proximate and ultimate causes of their loss, and the behaviors used to cope with such stressors. By understanding the environmental drivers or animal stress and the behaviors which indicate coping strategies, we can better understand when anthropogenic disturbances affect wild populations, and when populations have fully recovered from such stresses. Red panda conservation efforts in the wild are further complicated by their distinctive environments and the unobservable nature of their high-altitude residency (Kandel et al., 2015; Panthi et al. 2019). And as humans continue to encroach on these habitats, wild red panda populations will become increasingly more difficult to find. This demonstrates the importance of studying captive red pandas and promoting their reproductive success.

Several studies have examined red panda behavior in captivity (Spiezio et al., 2022; Khan et al., 2022). For example, one study analyzed the effects and importance of enrichment products and enclosure design on red panda welfare, providing a positive relationship between the number of logs offered and red panda activity levels (Khan et al., 2022). Another study examined the diet of captive red pandas, finding that high fiber diets improved both oral and gastrointestinal health in subjects more than gain-based foods supplemented with animal protein or fruits, for example (Nijboer & Dierenfeld, 2011). While efforts are beginning to be made to understand this endangered species, no study has considered all environmental factors that affect red panda behavior and welfare, or lack thereof. There are still gaps in our knowledge on how best to care for this species, and without this knowledge, we cannot successfully promote reproductive success and introduce new genes to the gene pool in captivity, nor can we use this information to better protect wild red pandas. The future of red pandas in captivity depends on our ability to distinguish stress-linked behaviors, environmental factors inducing these stereotypies, as well as how enclosure redesign and changes affects this species.

1.5. Research Objectives

Here, I investigate the effects of zoological practices, environmental factors, social stress, and human disturbances on the captive red panda. Specific objectives included:

1. Determine stress-inducing factors through defining stereotypic behaviors, such as repetitive pacing, as well as the intensity of said stereotypies.

2. Determine how space usage and behavior are impacted by fixed and random effects environmental factors including social stress, noise, visitor impact, weather, temperature, and presence and length of time with of a conspecific.
3. Determine how enclosure design and naturalistic elements affect red panda behavior and space use to promote welfare and replicate that of the natural environment.

1.6. Research Questions

1. Because there is limited literature on the topic, how do we characterize stereotypies in the red panda?
    a. What is the intensity or prevalence of these behaviors?
2. What fixed and random effects factors most affect the red panda, both positively and negatively?
    a. Will the presence or introduction of a conspecific alter observed behaviors and space use, especially over time?
    b. To what extent does the "visitor effect" affect red panda behavior?
    c. Does weather conditions and temperature affect the duration of behaviors performed, such as eating, grooming, and pacing?
3. What enrichment and environmental elements are most crucial to include within an enclosure, and how does enclosure change affect subjects?
    a. Will a change in enclosure elicit a stress response?

1.7. Research Hypotheses

1. Hypothesis 1

a. H_o: There is no statistically significant relationship between stress and perceived stereotypic behaviors including pacing, eating, and grooming behaviors.

b. H_a: There is a statistically significant relationship between stress levels and the presentation/intensity of stereotypic behaviors, most prominent being pacing.

2. Hypothesis 2

a. H_o: Environmental factors including the "visitor effect," temperature, weather, the presence or absence of a conspecific, and enrichment provided will not significantly alter red panda behavior.

b. H_a: There is a significant relationship between all tested environmental factors and red panda behavior.

3. Hypothesis 3

a. H_o: There is no significant relationship between appropriate and redesigned naturalistic enclosure elements, as well as complete enclosure moves, with red panda behavior.

b. H_a: Enclosure redesign/changes, or complete move in exhibits, significantly affects observed red panda behavior.

2. Materials and Methods

2.1. Participants

The sample was comprised of three red pandas (*Ailurus fulgens*) housed at Essex County Turtle Back Zoo (TBZ) in West Orange, New Jersey. The newest resident, a one-year-old female at the beginning of the study, was transferred from Kansas City Zoo to West Orange in June of 2022 as part of the Species Survival Plan (SSP). The second subject was a two-year-old male who is ranked highly based on AZA standards for his unique gene set. The third subject was a sixteen-year-old female.

2.2. Enclosure Setup

The first exhibit housing subjects measured 6.10 meters in length, 5.20 meters in width, and 4.00 meters in height (Figure 1). The enclosure was split in half, with the left (from the observer's view) housing the young pair together and the other side for the geriatric subject. Both halves included climbing branches spanning across the exhibit at varying heights; a litter box; a bed; enrichment usually in the form of plastic dog toys hiding fruits; logs; planters; and daily replenished bamboo and leaf-eater biscuits. The visitor viewing areas spanned the front half of the side/width of the pairs' side of the exhibit, as well as the entire length of the front of both halves. A fence surrounded the exhibit, keeping the public 0.60 meters from the enclosure mesh itself.

Seven months into this project, individuals were relocated to a new enclosure following the opening of an Asia exhibit (Figure 2). There was no disruption in observational data collection during this transition. The young duo occupied the new exhibit measuring 7.62 meters in length, 3.00 meters in width, and 6.100 meters in height, while the older subject was housed off-exhibit. Higher, and more abundant climbing branches were provided and at more varying heights, but no bed or litter pan. Instead, a ledge at the top of the exhibit was made accessible to this arboreal species to climb up to.

2.3. Behavioral Observation

A pilot study with no manipulations was carried out for a total of twelve hours to note baseline observations and develop an appropriate ethogram in February of 2022 (Table 1). Observations were conducted outside the enclosure, ranging from 0.61 to 3.00 meters back from the front side of the enclosure to not disrupt subjects' natural behaviors. Data were collected using Lincoln Park Zoo's monitoring software, ZooMonitor (Ross et al., 2016). Focal animal sampling was used for all subjects one to two times a week for three-hour sessions ending in December of 2022. A total of 176 hours of observation were collected at random times and on random days of the week spanning ten months. Only one person collected the data used for analysis, increasing the reliability across sessions.

Activity budgets for all three red pandas were determined with five-minute interval time point samples, creating a total of 2,119 intervals. Activities included lying; cooling; out of sight; active; repetitive; purposeless locomotion like walking; and climbing. Location, to measure proximity to each other and space use, was mapped in Zoomonitor on a computer-rendered image of the aerial view of the enclosure. This was encoded as X, Y coordinates ranging from 0-600 pixels, in which locations were mapped along with all interval and all-occurrence behaviors observed. An all-occurrence sampling method was used to document brief and sudden behaviors to determine the prevalence in "normal" versus "stereotypic" behaviors exhibited, as well as behaviors related to aggression or territorial disputes. For example, this included behaviors ranging from the focal individual scratching itself, to scent marking and sniffing exhibit elements.

State behavior data were also collected in Zoomonitor using the continuous behaviors feature to examine the prevalence of maintenance-related behaviors. This included playing; grooming; hanging from the enclosure; digging; body contact with conspecific; zookeeper presence in the exhibit; eating; and pacing.

2.4. Environmental Factors

In addition to examining how both the presence of a new conspecific and a change in enclosure affects red panda behavior, other environmental factors that could potentially affect behavior were considered. Prior to beginning a three-hour session of data collection in ZooMonitor, the observer listed the current weather condition from a list of options, manually typed the temperature (°F), listed if subjects had access to off exhibit holding, and manually typed in the number of visitors actively standing at the exhibit at the beginning of data collection. Furthermore, a box was provided to add any pertinent notes, like loud construction on premises that could potentially affect behaviors observed. Last, all daily ticket sales for 2022 were collected from the zoo to determine the total number of zoo visitors on days of observation sessions to accurately quantify visitor numbers for analysis.

2.5. Behavioral Statistical Analysis

2.5.1. Stress-inducing factors and related stereotypic behaviors.

A factor analysis was performed to quantify stereotypic behaviors exhibited by subjects and predict which behaviors are most linked to stress. By running this analysis, patterns in behaviors can be distinguished, and the underlying factors causing correlations in the different behaviors can be identified. These patterns can then be used to group related behaviors, such as over-grooming, eating, and pacing durations, which are all hypothesized to be indicators of stress in this captive species. Furthermore, principal component analyses (PCA) were performed to decrease the number of independent environmental variables in the data to define only key behaviors that most strongly contribute to stress in red pandas. Last, multiple regression analysis was performed to examine the nature of the relationship between individual environmental variables and behavioral responses that suggest the presence of stress as determined by the previous statistical analyses.

2.5.2. Effects of environmental factors on behavior and space use.

To measure the relationship between environmental factors, including temperature; conspecific presence; visitor number; time; and weather with observed continuous behaviors, the Spearman correlation coefficients were calculated. This was due to the small sample size and non-linear relationship between variables. In addition, activity budgets were calculated to measure the effects of these different environmental variables. To calculate this, the proportion of time spent in different behavioral interval status states was compared to the total amount of time subjects were visible on exhibit for observation. Finally, space use data based on X, Y coordinates were analyzed to quantify which areas of the exhibit subjects prefer to spend their time, as well as how their use of these areas change with differing environmental factors.

2.5.3. Naturalistic enclosure redesign and effects on well-being.

The proportion of time spent exhibiting key behaviors determined to be related to stress were used again to perform independent t-tests and the analysis of variance (ANOVA) statistical analyses, as well as a Chi-square analysis on nominal behavioral categories. This was to examine if there were any significant differences in activity between both exhibits inhabited. In addition, multiple regression analyses were performed to determine the nature of the relationship between behavior and enclosure, all while controlling for visitor numbers; temperature; conspecific presence and time together; and weather.

3. Results

3.1. Stress-inducing factors and related stereotypic behaviors.

The factor analysis and PCA suggest that three factors held predictive power when determining stereotypies, including zoo visitor numbers, temperature, and the length of time with a conspecific. This was also supported by the finding that the factors accounted for 71.42% of the total variance in the data (Table 2). Social interaction, which accounted for 37.82% of variance, was most influenced by the environmental stressor of zoo visitor numbers (Eigenvalue = 2.27, Factor Loading = 0.86; Table 3). As visitor counts increased, the more time conspecifics spent both in closer proximity and pacing. Factor two, internal stressors, indicated that as temperature increased, as did pacing durations, showing the resulting engagement in the enclosure (Eigenvalue = 1.01, Variation in Data = 16.91%, Factor Loading = 0.64; Figure 3). Other potential predicted factors were not included, meaning there were most likely other exhibit factors not studied affecting pacing. Overall, regardless of the exhibit, these findings in reduced stress-related behaviors over time might support the idea that subjects are able to adapt and cope better to their captive environment.

Last, multiple linear regression analyses demonstrated a positive correlation between zoo visitor numbers, and the length and number of stereotypies expressed by subjects. A least squares test showed that only pacing duration was significantly predicted by weather, temperature, time with a conspecific, enrichment, and zoo visitor numbers (F (14, 290) = 4.45, p <0.0001), with visitor numbers being the most important driver (Estimate = -0.21, SE = -0.074, T (290) = -2.80, p = 0.0054). Surprisingly, as time with a conspecific and zoo visitor numbers both increased together (Estimate = -0.011, SE = 0.0048, T (290) = -2.32, p = 0.021) pacing decreased, while the increase of conspecific presence with temperature (Estimate = 3.33, SE = 0.65, T (290) = 5.10, p <0.001) significantly increased pacing durations. Overall, multiple regression analysis was able to explain 17.67% of the variability in pacing duration.

3.2. Effects of environmental factors on behavior and space use.

Several environmental factors were associated with red panda space use and behavior. First, as temperature increased, grooming durations decreased (Kendall T= -0.134, p =0.040), with less grooming at higher temperatures reflecting increased lethargy or reduced activity levels in subjects at higher temperatures. Next, as visitor numbers increased, subjects utilized more of the length of the exhibit (Kendall T = 0.12, $p < .0001$). This might be indicative of their common pacing pattern along the length of the enclosure. Furthermore, as visitor numbers increased, subjects utilized less of the width of the exhibit (Kendall T= 0.023, p =0.044) suggests that subjects stay at higher elevations when crowds are larger. Overall, regarding increased visitor numbers, subjects paced the length of the enclosure more (larger x-coordinate range) while also staying further back and higher up (smaller y-coordinate range; Kendall T= -0.063, $p < 0.0001$). This indicates that parts of the enclosure were avoided by subjects, as these areas might be less accessible or attractive to subjects, leading to potential stress.

Determining the proportion of time spent performing stress-related behaviors provides further insight into red panda behavior in captivity. When examining the prevalence/frequency of continuous behaviors, pacing was the most frequent among red pandas, accounting for 9.55% of the total time observed. This is followed by eating (7.34%) and grooming (2.32%). Each subject, on average, spent 515.70 seconds (8.60 minutes) per three-hour sessions pacing; 396.36 seconds (6.61 minutes) eating; and 125.28 (2.10 minutes) seconds grooming. When examining the percentage of time these behaviors were performed and compared to one another, 49.70% was pacing; 38.23% was eating; and 12.07% was grooming. For activity budgets, behavioral intervals reported during observation were 38.41% in an active state; 45.02% in a lying state; and

16.56% out of sight (Figure 4). For interval alertness, 40.04% of intervals were reported as alert, while 59.96% were non-alert.

For important all-occurrence behaviors, including exploratory, territorial, social, and stress-linked behaviors, scent marking was the most frequent, accounting for 43.41% of all all-occurrence behaviors logged. This was followed by vigilance, or hyperawareness of noise or movement outside the exhibit, at 27.08%; exploratory behaviors with enrichment (3.72%); eye contact with a conspecific (8.65%); licking (2.67%); scratching (4.69%); chasing (0.56%); and sniffing (9.22%) (Figure 5). Overall, chasing was observed once every three sessions of data collection; exploratory behaviors twice per session; eye contact with conspecific five times per session; licking twice per session; scent marking twenty-six times per session; scratching three times per session; sniffing five times per session; and vigilance sixteen times per session. These findings show that several environmental factors affect behavior and space use in captive red pandas and can also be used as indicators of stress.

3.3. Naturalistic enclosure redesign and effects on well-being.

Overall, the increase in both pacing and eating durations following the enclosure move suggests that enclosure moves were stress-inducing for subjects. One-way analysis showed that grooming duration was unaffected by exhibit changes ($F(1,106) = 0.99$, $p = 0.32$). However, the ANOVA for eating ($F(1,314) = 3.09$, $p = 0.040$) indicated that subjects ate more following the move. Move status also affected pacing duration ($F(1, 348) = 17.49$, $p < 0.0001$). Prior to enclosure changes, the male and female subject paced for a total duration of 8,826 and 26,030 seconds, with means of 80.97 and 195.71 seconds, respectively; The number of pacing bouts before were N=109 and 133. Following the move, the male and female subject paced for a total duration of 21,915 and 3,726 seconds, respectively; The number of pacing bouts were N= 85 and 23. This showed that the female was more affected by the move to an entirely new zoo than the enclosure changes three months later, while the resident male experienced more stress during the move.

Chi-square analyses of move status on nominal behaviors were also performed. For both the parameter estimates and the effect likelihood ratio tests indicated that the model was positively significant and that move status did affect all-occurrence behaviors observed (χ^2 (21, N= 2) = 166.25, p <0.0001). This included territorial, exploratory, social, and stress-linked behaviors. Furthermore, analysis of move status on interval alertness showed positive significance for both the whole model tests and effect likelihood ratio tests (χ^2 (2, N= 2) = 27.34, p <0.0001). Findings of the chi-square indicated that move status affected observed interval statuses (χ^2 (3, N= 2) = 325.34, p < 0.0001), showing that enclosure moves do influence activity levels at different intervals.

Mixed model regression analysis using the fit least squares test showed that following the move status, as visitor numbers increased, grooming decreased (Estimate = -0.17, SE = 0.075, T (91) = -2.19, p = 0.031), in which less grooming was performed after the enclosure move (Figure 6). However, pacing durations were significantly affected by rainy weather conditions (Estimate = -245.34, SE = 60.17, T (349) = -4.08, p < 0.0001) more than enclosure move; When rainy, subjects paced more. Last, enclosure move status did not significantly affect eating durations, but were more affected by the interaction of temperature and weather ($p = 0.019$).

4. Discussion

It was found that both zoo visitor number and length of time spent with a conspecific were most important factors when predicting red panda stereotypic behaviors, with pacing duration being the most reliable indicator of stress. Previous research has identified stereotypic behaviors in captive red pandas, finding that the most common stress-linked behavior was pacing, which is consistent with the current findings (Meagher & Mason, 2012). It was also found that head-bobbing was the next most common stereotypy, which was not observed in the current study, indicating that red pandas could exhibit different stress responses (Meagher & Mason, 2012); future research should examine a larger range of behaviors when defining and quantifying stereotypies. It was also found that social companionship and diverse environmental enrichment reduces the presence of these behaviors (Young, 2013), supporting the idea that natural habitats with environmental stimulation can reduce stress. More specifically, climbing structures and hiding places reduces stereotypies (Swaisgood & Shepherdson, 2005), however, social interaction did not prove to be a predictor of stress in red pandas here. This could be due to subjects being introduced during the project's duration and not having an established relationship like most previous studies.

These results highlight the necessity for captive environments that resemble natural habitats with the opportunity for social and environmental stimulation to reduce stress-related behaviors. In addition, this also shows the importance of minimizing human disturbances while promoting natural social interactions and behaviors. Therefore, we can improve the welfare of red pandas in captivity by creating a more stimulating, enriching enclosure that promotes natural behaviors while mitigating stress-linked ones. Overall, the present provides insight into how we define stereotypic patterns in captive species, as well as the factors contributing to them, which can lead to the development of more effective zoological management practices.

Factors including temperature, conspecific presence, and visitor numbers proved to be influential on grooming, eating, and pacing durations as well, affecting what areas of the exhibit were utilized. In addition, territorial and social behaviors were expressed heavily under these conditions, such as scent marking, vigilance, and eye contact with a conspecific. Previous research supports the idea that increased visitor numbers affects behavior, like increased inactivity but decreased grooming and foraging, but did not find significance in its correlation to stereotypic behaviors (Sherwen et al. 2015). Another study found that when zoo visitor numbers were low, subjects used the entirety of their exhibit (Stoinski et al., 2012), supporting the behavior performed by current subjects and that human presence affects space use.

Finally, in a previous experiment, temperature was the most influential factor on stereotypic behaviors, supporting these results, but did not find significance in conspecific presence (Veasey et al., 1996), which again could be due to the transfer of a new female subject halfway through the duration of this study. Our findings indicate that certain environmental factors not only affect behavioral repertoires, but also induce stress, in which further research would be needed to examine these relationships to begin establishing interventions with the aim of improving red panda welfare. Again, the findings can help not only with addressing management practices, but the overall design and management of zoological enclosures.

Enclosure redesign and complete enclosure moves were determined to be stress-inducing for subjects. For the male, while the number of pacing bouts did decrease following the move, the mean duration of these bouts and total time pacing were over three times greater; It is important to note that the before period spanned nine months while the after spanned three months. This, however, was not the same for the female, who expressed more stress through pacing upon her arrival to Turtle Back Zoo and meeting a new conspecific than the enclosure moves three months later.

Similar results were observed in captive felid, primate, and carnivore research, finding that enclosure redesign increases stereotypies across many species, supporting the claim that changes should be minimized to reduce potential stressors (Carlstead & Shepherdson, 1994). Enclosure size has also been analyzed, finding that larger, more complex enclosures decrease pacing while increasing exploratory and foraging behaviors (Garner et al., 2003). Overall, the present findings suggest that enclosure redesign requires careful consideration and caution when it comes to captive species' welfare, as it may induce stereotypies and signs of stress. Zoological settings and animal welfare organizations should push to minimize the need for enclosure relocations and implement protocols to reduce moving stressors when necessary.

In summary, this study highlights the importance of a deeper understanding of the complexities between space use, behavior, and environmental variables. Findings do suggest that management strategies could be implemented to address these factors and promote red panda welfare. This includes providing more environmental enrichment, managing visitor numbers and their movement patterns, and increasing time spent with conspecifics, while also monitoring temperature fluctuations. While this experiment provides valuable insight into the well-being of captive red pandas, it is important to note the small sample size in observation and that this might not be generalizable to all captive red pandas. Further research can be conducted to confirm these findings and determine the underlying impacts of environmental factors on red panda behavior and overall welfare. Furthermore, research into behavioral observation with physiological measures, such as fecal cortisol assays, can help determine the nature of the pacing behavior increases and its potential welfare implications. Nonetheless, this present study serves as a starting point for the future development and effectiveness of management protocols to promote the health of red pandas in captivity, with the overarching goal of protecting this endangered species for decades to come.

Appendix

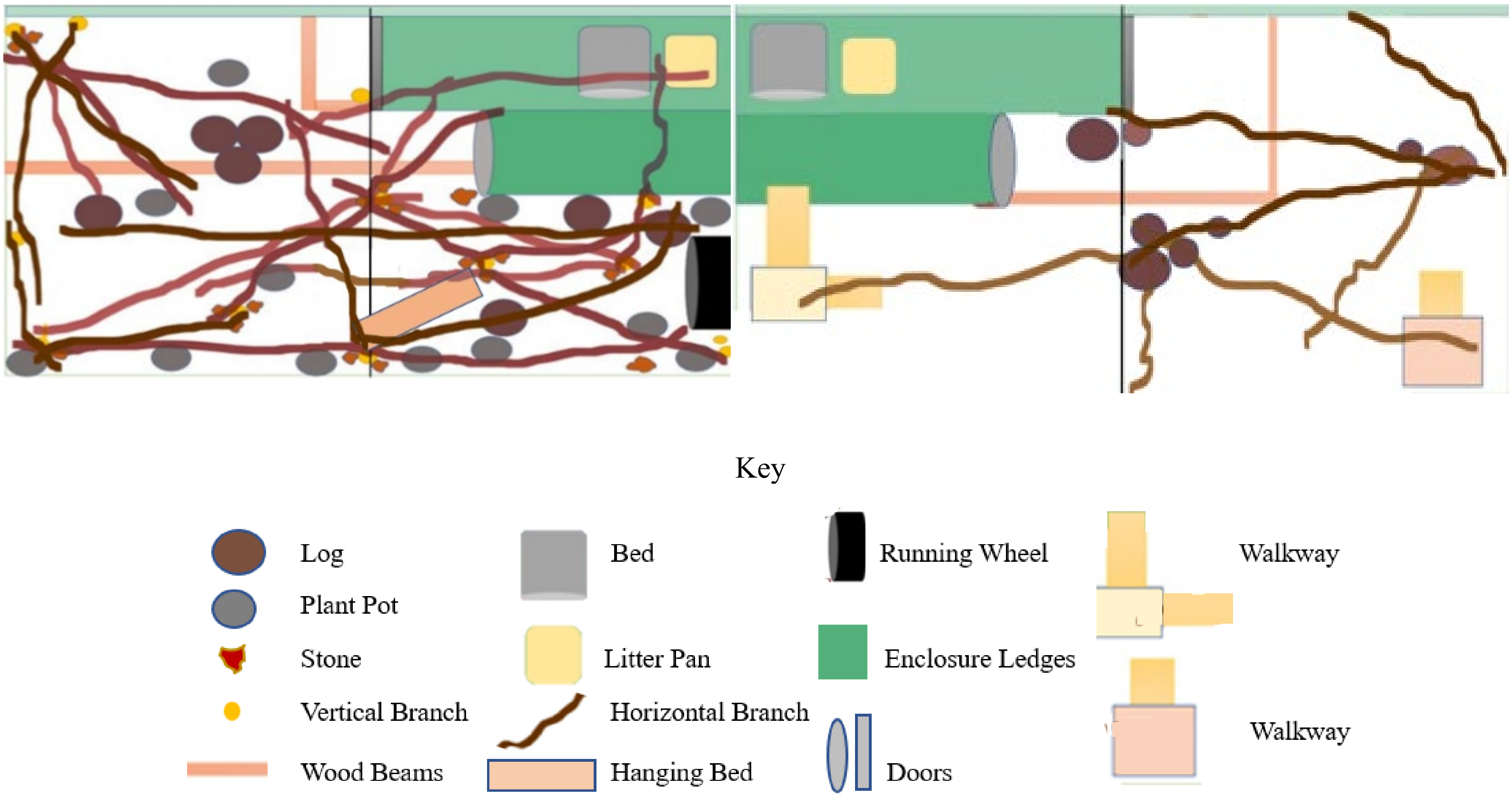

Figure 1. Computer-rendered aerial view of red panda (*Ailurus fulgens*) exhibit split into halves at the start of March 2022 at Essex County Turtle Back Zoo in West Orange, New Jersey.

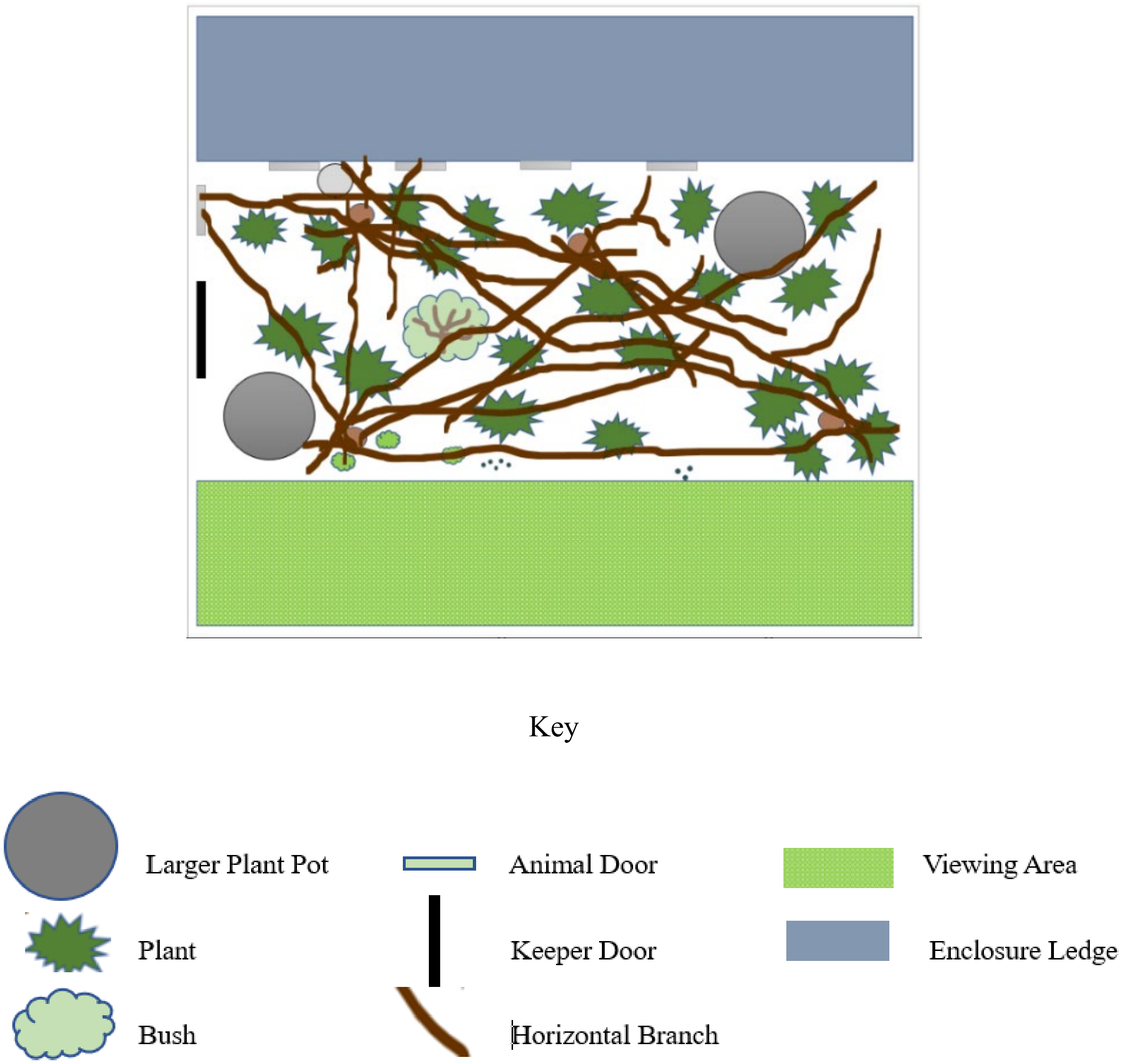

Figure 2. Computer-rendered aerial view of new red panda (*Ailurus fulgens*) exhibit at the start of September 2022 at Essex County Turtle Back Zoo in West Orange, New Jersey.

Table 1. Red panda (*Ailurus fulgens*) ethogram including time-point interval, continuous, and all-occurrence behaviors.

Interval Behaviors

Code	Behavior	Modifier	Definition
LYI	Lying	Alert, Non-alert	Lying inactive (either curled in a ball or lying flat out
OOS	Out of Sight		Continuous stretch of time out of sight (believed to be inactive)
ACT	Active	On Ground, On Ledges, On Branches	Standing upright, moving around, or sitting moving head around

Continuous Behaviors

Code	Behavior	Modifier	Definition
GRO	Grooming	Given, Received, Body Part Groomed	The practice of brushing and cleaning the coat of an animal
PAC	Pacing	Straight Line, Circle, Random Path	Repetitive route in enclosure- predictable pattern, limited response/ awareness to outside stimuli
EAT	Eating	Browse, Vegetables, Fruits, Pellets, Bamboo	Standing upright, moving around, or sitting moving head around

All-Occurrence Behaviors

Code	Behavior	Modifier	Definition
SCR	Scratch	Self, Other, Ground, Object, Body Location	Using claws to scratch a part of one's own body, a conspecific, or keeper
VIG	Vigilance	Inside our Outside Exhibit, Object Involved	Observation within or outside enclosure (of a conspecific or object)
EXP	Exploratory	Object Involved	Exploratory/territorial investigation of enclosure, interaction with furnishings within enclosure
SCE	Scent Marking	Object Involved	Rubbing of genital regions either sideways or front to back
SNF	Sniffing	Body Location, Object Involved	Olfactory investigation of an object or a non-animal
LCK	Licking	Object Licked	Oral/taste investigation of an object or a non-animal
EYE	Eye Contact	Visitor, Researcher, Keeper, Conspecific, Non-conspecific	Two conspecifics, or red panda and non-conspecific, making eye contact (stare)
FIG	Fight	Initiate, Receive	Initiate or experience acts of physical aggression

Table 2. The red panda (*Ailurus fulgens*) behavioral eigenvalues, percentages of variance explained, and cumulative percentages for factor analysis.

Number	Eigenvalue	Percent (%)	Cumulative Percent (%)
1	2.269	37.816	37.816
2	1.0147	16.912	54.728
3	1.0016	16.693	71.421
4	0.98	16.334	87.755
5	0.4947	8.246	96.001
6	0.24	3.999	100

Table 3. The red panda (*Ailurus fulgens*) rotated factor loadings for factor analysis on environmental factors.

	Factor 1	Factor 2	Factor 3
Zoo Visitor Number	0.864014	-0.090967	-0.064811
Temperature	0.622371	0.071477	-0.04605
Time With A Conspecific	-0.863139	0.112433	-0.034121
Pace Duration	-0.070847	0.643524	-0.00102
Groom Duration	-0.021293	-0.001019	0.637904
Eat Duration	0.045305	0.00235	0.000811

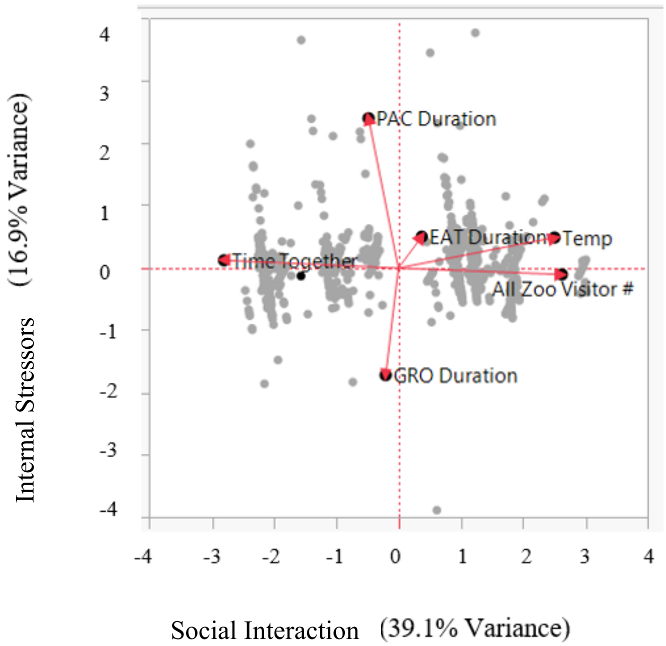

Figure 3. Biplot of the relationship between environmental factors and red panda (*Ailurus fulgens*) observed behaviors.

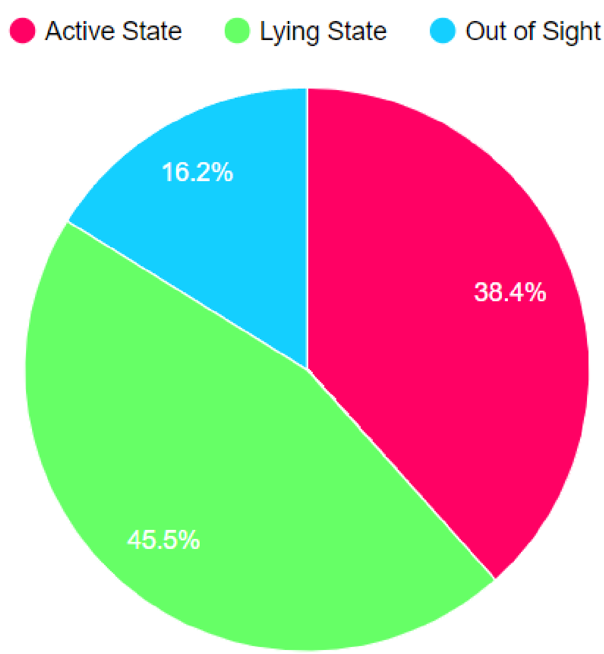

Figure 4. Activity budgets of interval statuses for the red panda (*Ailurus fulgens*) subjects.

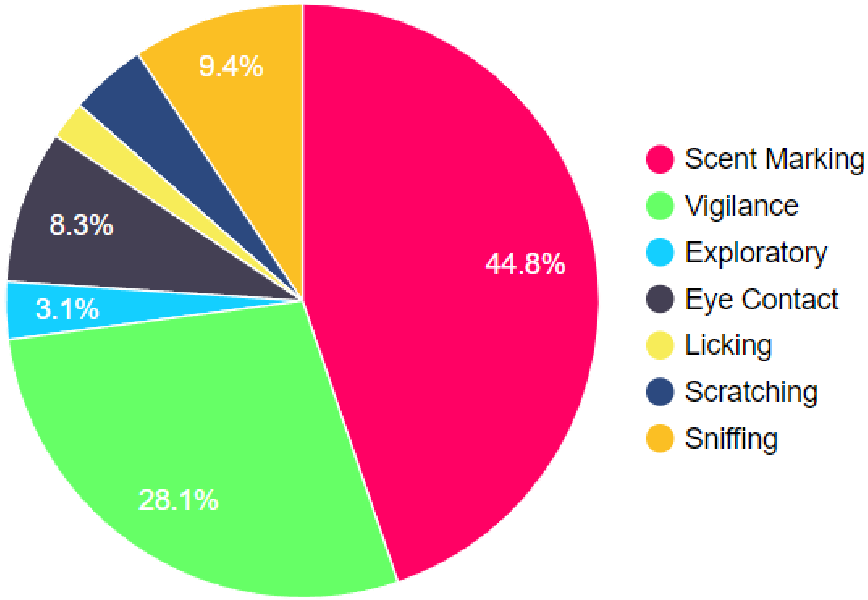

Figure 5. Prevalence/frequency of all-occurrence behaviors for red panda (*Ailurus fulgens*) subjects.

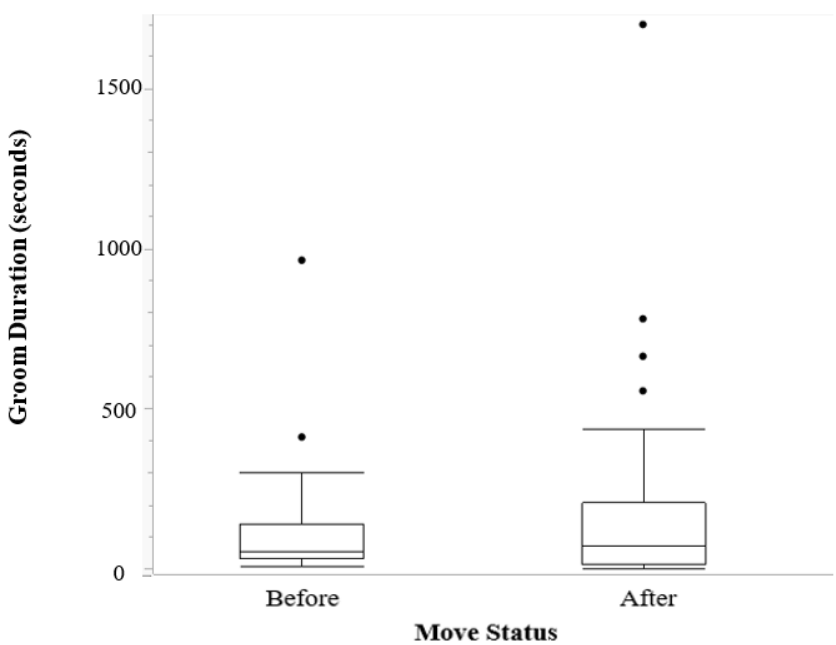

Figure 6. Box plot of grooming duration of red pandas (*Ailurus fulgens)* before and after enclosure moves.

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