The behaviour and welfare of zoo-housed Japanese macaques (*Macaca fuscata*)

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Abstract

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There have been an increasing number of research projects on zoo animal welfare in the past decades. However, these studies all tend to use the same species (mainly great apes) as subjects. In this thesis, we analyzed the effect of visitor presence and enclosure design on the activity budget and space use of the Japanese macaques at the Zoo de Granby, Granby, QC. In addition, we quantified the shift in the female dominance hierarchy that occurred when members of the two original groups of macaques, observed in the old enclosure in 2014, were merged to form one group in 2015, to later be introduced to a new enclosure. The results suggest that visitor presence may have had a small impact on the activity budget and space use, but its effect on welfare not apparent and trends were not consistent between or within groups. The macaques' behaviour and space use patterns suggested habituation to the new enclosure, but did not clearly indicate an increase in welfare compared to the old enclosure. It is likely that the changes in hierarchy confounded the effects of the new habitat. Individuals' ranks changed significantly between groups, and again midway through the 2015 field season. There was evidence that age was an important factor in determining the new rank of an individual, but the order in which the individual was added to the group was not. It is clear from this thesis that both visitor effect and enclosure changes are multifaceted topics which require continued research.

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Contribution of Authors

As the first author of this work, I was responsible for the majority of the design, set up, data collection and analysis, and writing of the manuscripts related to this thesis. Both chapters were co-authored by Dr. Robert Weladji and Patrick Paré, who served as mentors and supervised the work. The general concept of Chapter 1 was devised by Dr. Robert Weladji and Patrick Paré and both provided advice and insight with the experimental design. Dr. Weladji assisted with statistical analysis and edited the manuscript for both chapters. Patrick Paré assisted in the coordination of the field work, reviewed early versions of the manuscript and provided background information on the study animals at the Zoo de Granby.

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General Introduction

Zoos have existed in some form since as far back as ancient Egypt (Bostock, 1993; Graetz, 1995). Once mainly in existence for religious purposes or as a show of power and wealth, zoos slowly evolved into places for public viewing of exotic animals by the 16th century (Bostock, 1993). It wasn't until the 1900s that zoos began to move away from sterile, barren habitats and adopt a more natural environment, along with larger, more naturalistic enclosures (Graetz, 1995). In the past century, the zoo community has continued to progress, with the growing prevalence of organizations such as the American Association of Zoos and Aquariums (AZA), which promote scientific research and higher welfare standards (Stoinski et al., 1998). Though zoo-like institutions have existed for centuries, many people still question if keeping animals in zoos is humane. The issue of whether zoo animal experience good or bad welfare from being in captivity is still debated (Bostock, 1993).

One of the major problems in welfare research is that welfare, as it refers to animals, does not yet have a widely accepted definition; it often varies depending on whether it is being applied to agricultural animals, laboratory animals or to companion animals. Traditionally, many definitions implied that good welfare was derived from the absence of negative states such as hunger, thirst and sickness (Maple and Perdue, 2013). By this definition, zoo animals would be experiencing very good welfare, as they are generally well provided for in terms of food, water and veterinary care. More recent definitions have shifted more towards focusing on what an animal "wants", "perceives" or "feels" (Maple and Perdue 2013). An animal suffers a decrease in welfare when it is trapped in a situation where they are willing to pay a high energetic price to escape, or is deprived of something they would be willing to pay a high energetic price to get (Dawkins, 1990). Since animals in zoos have little control over many aspects of their environment and may not be able to act in the way they "want" or "feel", it is more likely, under this newer interpretation, that zoo animals can suffer from poor welfare.

In 2005, the Animal Welfare Committee established by the AZA produced a working definition of zoo animal welfare. They describe animal welfare in the zoo context as being "the degree to which an animal can cope with challenges in its environment as determined by a combination of measures of health (including pre-clinical physiological responses) and measures of psychological well-being" (Barber and Mellen 2008).The measures commonly used by

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researchers to study these changes in welfare include physiology, using metrics such as stress hormone levels and physical indicators (e.g. presence of injuries or reproductive success). Psychological well-being is generally assessed by ethological measures, through the comparison of an animals' behaviour to what is deemed to be normal or desirable behaviour (Dawkins, 1980; Hill and Broom, 2009; Hosey et al., 2013). It is under this definition of welfare that we will precede in this study.

There are numerous records from early zoo history indicating poor welfare in zoo animals, such as high mortality rates. In the 1950s, the species-specific needs of zoo animals, compared to other domesticated animals, were brought to the attention of both the public and the scientific community (Melfi, 2009). In modern zoos, animals often exceed the life expectancy of their wild counterparts due to better nutrition, veterinary care and lack of predation, suggesting the state of welfare for zoo animals has significantly improved. Much attention is being focused on the various ways in which the zoo environment may pose a challenge to an animal's wellbeing (Morgan and Tromborg, 2007). The design of enclosures and habitat use has become a point of interest in zoos (Little and Sommer, 2002; Mallapur et al., 2002; Ross et al., 2011a; Ross and Lukas, 2006; Ross et al., 2010). Modern innovations allow institutions to design more complex habitats that mirror an animal's natural habitat and improve the visitor experience (Graetz, 1995). However this increased technology also gives zoos and researchers more to consider when evaluating the welfare implications of an enclosure.

A large body of research has focused on the effect of visitor presence on zoo animal welfare, also referred to as "the visitor effect" (Davey, 2007). Human presence is an unusual stimulus for most wild animals. Early research by Chamove et al., (1988), suggested that the presence of large numbers of people is a source of stress for captive animals as well. Zoo animals not only have to cope with the human presence, but also the presence of other animals, particularly conspecifics (Morgan and Tromborg, 2007). Fortunately, most zoos have realized that housing social animals singly is very detrimental to their welfare as it often leads to impaired social behaviour and other behavioural abnormalities (Harlow and Suomi, 1971), and the practice is now widely avoided. However, this has resulted in the development of other questionable habits in the social housing of animals, such as overly-large groups, or the

formation of male bachelor groups in a species where this is not observed in nature (Morgan and Tromborg, 2007; Sha et al., 2013).

The continued pursuit of knowledge on the subject of zoo animal welfare and general zoo biology has resulted in an increase in studies on this subject (Stoinski et al., 1998). In primates, welfare and behaviour are both popular research topics (Melfi, 2005) Studies of these types are common as the data collection is generally non-invasive, the experimental design does not need to be overly complex, and the results are very helpful to the collaborating zoological institution (Melfi, 2005). However, research in zoos tends to be biased towards the most charismatic animals, such as the large felids and great apes, regardless of their numbers in the zoo population (Melfi, 2005; Melfi, 2009). An extreme example of this is the bonobo (*Pan paniscus)*, which was numbered at less than fifty individuals in captivity in 2009 spread between fewer than five institutions in the AZA, and yet there were approximately ninety research projects performed in zoos on this species alone (Melfi, 2009). It is clear that zoo researchers need to diversify their study species in order to gain a more thorough understanding of the aforementioned subjects.

The aim of this thesis was to increase our knowledge of the effects of the zoo environment on the behaviour and welfare of the Japanese macaque (*Macaca fuscata*). Japanese macaques are a species of old world monkey (family: Cercopithecidae) endemic to the islands of Japan (Appendix A). They are the most northern-living nonhuman primate, inhabiting the subtropical to subarctic habitats that occur between 30°N to 41°N (Hamada et al., 1996). Due to the variability of their habitat, Japanese macaques tend to exhibit a wide range of adaptations in their morphology, including variations in colour, body size and fur density (Hamada et al., 1996). As a species, Japanese macaques are listed as least concern on the IUCN Red List (Watanabe and Tokita, 2008). They are one of the most extensively researched primate species, with a large body of literature available on their ecology and behaviour. However, this literature is confined almost exclusively to the field and the laboratory.

In 2014, the Zoo de Granby housed fourteen adult Japanese macaques (two groups of seven), the majority of whom were suffering from some degree of hair loss. Hair loss in primates has been linked to a number of variables, including aging, hormones, nutritional deficiencies and stress (Novak and Meyer, 2009). It is commonly observed in laboratory primates as a result of sterile and unstimulating housing conditions (Kroeker et al., 2014; Steinmetz et al., 2006).

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Though less frequent and often less severe, alopecia (hair loss) in wild and zoo-housed populations is not unheard-of (Florence and Peel, 1977; Zhang, 2011). In the wild, Japanese macaques are more likely to develop alopecia if they are provisioned by humans (Zhang, 2011). In the 1970s, when the Japanese macaques at the Calgary Zoo were suffering from a severe bout of hair loss, research suggested that it was due to excess stress as no other pathologies were observed (Florence and Peel, 1977). Skin biopsies and other veterinary tests performed on the Zoo de Granby Japanese macaques did not reveal any medical causes of alopecia and changes in diet did not improve coat condition. Therefore, an exogenous stressor may have been the cause of their observed hair loss. The first chapter of this thesis will use behaviour, specifically activity budget and space use, as a means of welfare assessment to investigate two prominent zoo-specific stressors in the Zoo de Granby Japanese macaques: visitor presence and enclosure design. The second chapter of this thesis is a short analysis and commentary on the changes in the dominance hierarchy of the Zoo de Granby Japanese macaques throughout the observation period and its potential effects on the welfare of these animals.

Chapter 1:

The effect of visitor presence and enclosure design on the behaviour of zoo-housed Japanese macaques

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Abstract

Two important stressors in the zoo environment are the presence of visitors and the artificial habitat. In 2015, two groups of Japanese macaques at the Granby Zoo, Granby, QC, were merged and transferred to a newly built enclosure. We studied their behaviour before and after the transition to investigate the effect of enclosure design on the troop's welfare. As zoo attendance varies significantly throughout the season, we also tested the hypothesis that the Japanese macaques' welfare would be affected (negatively or positively) by the presence of visitors. We performed generalized linear mixed models to analyze the effect of phase (high, low and no visitor), enclosure, and week of observation in the new enclosure, on the activity budget in the Japanese macaques, and to determine the effect of three visitor variables (sound level, attendance and crowd size) on the rate of occurrence of seven behaviours used as indicators of welfare. The effect of phase and week of observation (in the new enclosure) on space use was determined using generalized linear models. We found that both activity and space use varied between phase and all three visitor variables correlated with one or more behaviour categories in at least one of the groups. However, there was no strong support for any visitor effect. Results from the new enclosure suggested habituation did occur. Though behaviour in the new enclosure differed from in the old, no welfare benefits were apparent. Further research is needed to further clarify the effect of these stressors on Japanese macaques.

Introduction

The use of behaviour as a welfare indicator is widespread in the scientific community (Dawkins, 1980; Dawkins, 1990). Poor welfare is a negative state that generally results in undesirable or maladaptive behaviour (Dawkins ,1990) Deviations from "normal" behaviour are believed to arise when animals is intensely and chronically stressed by perceived threats, or frustrated, meaning they feel a lack of control and predictability in their environment and an inability to fulfill their biological needs (Dawkins, 1990; Broom, 1991). Though behaviour varies between species and between individuals, there are several behavioural indicators of low welfare that are commonly accepted. Stereotypic behaviours, that is, highly repetitive behaviours that appear to serve no proximate or ultimate purpose, tend to increase in stressful situations (Mason, 1991). This type of behaviour is not well understood, but it has been suggested that it could act as do-it-yourself enrichment for animals lacking stimulation, or as a calming coping mechanism (i.e. the "mantra" effect) when animals are in a stressful environment (Mason and Latham, 2004). In addition to abnormal behaviours, any normal behaviour, such as self-grooming or resting, that is performed significantly more or less than in a wild individual, may indicate reduced welfare in captive animals (Broom, 1991). In particular, excessive aggression, directed both at other individuals and at oneself, is considered to stem from frustration; this has been found during delayed feeding of captive stump-tailed macaques (*Macaca arctoides*) (Waitt and Buchanan-Smith, 2001).

Behaviour is not only considered to be indicative of poor welfare; there has been a recent push to include indicators of positive affect states (i.e. good well-being) in zoo welfare studies as well (Whitham and Wielebnowski, 2013). These are behaviours such as play and other non-aggressive social interactions (Mitchell and Hosey, 2005; Whitham and Wielebnowski, 2013). Even vigilance could be a sign of an animal's non-threatened interest towards humans, especially if accompanied by greeting behaviours (Mitchell and Hosey, 2005). This study will use behaviour, specifically activity budget and space use, as a means of welfare assessment to investigate two prominent zoo-specific stimuli, visitor presence and enclosure, specifically the change of enclosure, in Japanese macaques (*Macaca fuscata*), an understudied zoo animal.

The near constant presence of numerous humans is one of the main factors that set the zoo environment apart from the field and the laboratory (Hosey and Druck, 1987). Aggression,

directed towards both animals and humans, tends to increase as visitor density increases, suggesting that human presence is a source of stress (Cooke and Schillaci, 2007; Soriano et al., 2013). In the majority of primates, aggression towards humans appears to be competitive, as it is characterized by excessive dominant displays (Cooke and Schillaci, 2007). High visitor densities often correlate with stereotypic behaviours as well, such as the repetitive pacing commonly observed in large felines (Sellinger and Ha, 2005). Animals may even avoid being in sight when the number of guests is too great (Soriano et al., 2013). It is now believed that visitor effect is not explained by a single variable (i.e. visitor number) but by a composite of multiple variables such as visitor age, sex and activity level, which may contribute more to stress than visitor numbers alone (Birke, 2002; Cooke and Schillaci, 2007; Owen et al., 2004). Alternately, some argue that human presence can be enriching, particularly for social animals such as primates, and that positive interactions with humans can increase welfare (Baker, 2004). Therefore we cannot assume that all species, even closely-related species, will react similarly to the same stimuli.

Enclosure design plays perhaps a more important role in zoo biology than visitor effect as a well-designed enclosure can buffer other stressors (Clark et al., 2012; Stoinski et al., 2001). Captive animals in complex and spacious environments often express a wide range of behaviours and an activity budget similar to that of their wild counterparts (Melfi and Feistner, 2002). Past research has identified certain habitat characteristics to be directly related to behaviour; for example the presence of vegetation often results in increased foraging and decreased grooming in macaques (Beisner and Isbell, 2008; Jaman and Huffman, 2008). Enclosure size has also been reported as an important characteristic, as increased proximity to conspecifics in a small enclosure leads to increased confrontations (Hogan et al., 1988; Little and Sommer, 2002). Though space is important, how animals use the space they are provided with does not necessarily relate to habitat size; hence, bigger is not always better (Hediger, 1970; Stoinski et al., 2001). Animals require areas in their habitat that correspond to ecologically significant fixed points in their natural territories (e.g. pools for swimming, dens for sleeping). Enclosures must therefore be designed with an animal's biology in mind. Behavioural studies can help in developing enclosures that best accommodate the animal's needs (Forthman and Bakeman, 1992; Hebert and Bard, 2000; Stoinski et al., 2001).

The aim of this study was to assess the activity budget and space use of the Japanese macaques at the Zoo de Granby, Granby, QC, Canada, and investigate how visitor presence and enclosure design affect their welfare. We expected that the behaviour of the Japanese macaques at the Zoo de Granby would differ in activity budget from macaques troops not housed in zoos due to the different stressors and environmental pressures they experience. Due to the variation in visitor density throughout the year at the Zoo de Granby, it was possible to investigate the effect of visitor density and activity, as indicated by ambient noise levels, on Japanese macaques' behaviour. Though there is evidence of ambivalence and even positive interest of zoo animals towards visitors, the majority of studies in both primates and other mammals suggest a negative or stressful influence of visitors (Davey, 2007). We chose to test two opposing hypotheses: (1) visitors are a source of stress and/or frustration for zoo-house Japanese macaques, and thus decrease their welfare, and (2) visitors are a source of enrichment for zoo housed Japanese macaques and their presences increases welfare. If a decrease in welfare occurred due to visitor presence, we predicted that behaviours associated with a negative emotional state such as aggression, self-directed behaviours and abnormal behaviours would increase with increasing visitor number and activity (indicated by sound level) and behaviours associated with a positive emotional state, such as non-aggressive social contact, would decrease (Mitchell and Hosey, 2005). The opposite trends would be expected if an increase in welfare occurred. Changes in space use relative to visitor presence and activity were predicted as well, as increased use of housing, high structures and the back of the enclosure when there are higher numbers of visitors may indicate avoidance, whereas increased use of areas close to visitors could indicate interest.

Observations of the same individuals in two distinct enclosures are rare, though some literature on the subject does exist. Studies of this type have been performed with langur monkeys, *Presbytis entellus* (Little and Sommer, 2002), and with great apes, *Pan troglodytes* and *Gorilla gorilla gorilla* (Ross et al., 2011b) A study involving the observation of these Japanese macaques throughout their transition to a new enclosure was, therefore, a unique opportunity. To take advantage of this, we tested the hypothesis that larger, more structurally complex enclosures that are designed specifically for the animal of interest promote better welfare in zoo animal. If this is true, we would expect to see a change in the behaviour of the Japanese macaques between the old enclosure and the new enclosure, and that these changes would indicate better welfare. Possible behavioural changes that would indicate this are a decrease in aggression and abnormal

behaviours due to lower levels of stress and frustration, an increase in species typical behaviour such as moving and foraging, and an increase in behaviours indicating positive affect states like play and object manipulation (Whitham and Wielebnowski, 2013). Aggression may also decrease in a larger enclosure; there would be greater potential for decreased proximity to undesirable individual, and more escape opportunities when compared to the old, smaller enclosure. We expect to observe decreased inactivity and increased locomotion and foraging behaviour should be observed as the new enclosure is more structurally complex and contains a greater variety of vegetation.

Furthermore, this research examined the idea that primates make selective use of their enclosure (Hebert and Bard, 2000; Ross et al., 2011a). We predicted that the macaques would spend a significantly larger proportion of their time in specific areas of their enclosures compared to others. A habituation period, in which we would observe a permanent lessening of a response to a stimulus (the new enclosure) as a result of active learning during repeated exposure, was also anticipated (Thorp, 1956); this is common with these types of enclosure transitions in zoo animals (see Ogden et al., 1990). An observation that space use becomes less equal in the new enclosure as exploration diminishes and preferred areas are decided upon would support also this. We would also expect to see more exploratory behaviours such as object manipulation, vigilance and locomotion at the beginning of the data collection period in the new enclosure compared to the end of the data collection period.

Methods

Subjects, Study area and Husbandry

This study was performed at the Zoo de Granby in Granby, Quebec. The Zoo de Granby was founded in 1953. It is currently accredited by the Canadian Association of Zoos and Aquariums (CAZA) and the Association of Zoos and Aquariums (AZA), and is a member of the World Association of Zoos and Aquariums (WAZA).

During the 2014 field season, we observed the fourteen Japanese macaques at the Zoo de Granby, who were housed as two groups of seven (groups one and two; Appendix B). They had been housed in groups resembling those at the time of study for the past seven years; therefore the social structure of the groups was relatively stable at the beginning of this study. All individuals were sexually mature adults, though the group was non-reproducing as males had all been castrated or received vasectomies. The founders of the troops came from the Bioparco di Roma, Rome, Italy, in 2002 (Majolo et al., 2005). These individuals are likely the ancestors of a group of 27 Japanese macaques (9 males and 18 females) captured near mount Takasaky, Oita prefecture, in Southern Japan, May 1977 (Majolo et al., 2005). All individuals observed in this study were born in captivity.

The 2014 on-display enclosure in which the macaques were observed (Appendix C) was decades old and originally housed a polar bear before being co-opted for the Japanese macaques. The two troops were rotated between the display enclosure and an off-display area on a weekly basis. In 2015, a third group of ten individuals (five from each of the original groups) were transferred to a new enclosure completed May 2015 (group three; Appendix B). The new enclosure was designed specifically for the Japanese macaques under the guidance of the conservation and research department of the Zoo de Granby (Appendix C).

Regular enrichment and feeding schedules were followed during the study period. This consisted of one enrichment item (e.g. scattered grains, branches, hay, etc.) between 9:00 and 10:00, and one enrichment item along with cut fruits and vegetables between 17:00 and 18:00 when the zoo was open or between 16:00 and 17:00 when the zoo is closed. In order to place the food and enrichment in the enclosure, the monkeys were temporarily transferred to their indoor holding areas. In the 2014 enclosure, a snack was also provide between 13:00 and 14:00, during

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which formulated monkey chow was passed through the fencing while the monkeys were still in the enclosure.

Behavioural Observations

Data for this analysis were collected July to October 2014, April/May 2015, when the macaques were introduced to their new enclosure, and July to September, 2015. Data collection began as early as 9:30, when the macaques were let out into the display enclosure. Sampling continued for 7.5 hours total, excluding three fifteen minute breaks and a pause for the afternoon feeding and enrichment, and usually ended between 17:15 and 18:00. The exception to this was in May 2015, when the macaques were let out later (10:00 - 11:00) and brought in early (16:30 - 17:30); the average day during this period was approximately 6.5 hours, including breaks. The focal individual sampling technique was used, whereby a single individual was observed for a period of ten minutes (Martin and Bateson, 2007). Individuals were assigned a number and sampled in numeric order, starting with a different individual each day, ensuring that each individual was observed at every time slot in order to reduce error due to temporal variations in behaviour. Behaviours were recorded instantaneously every 15 seconds for a total of 40 sampling points per period. The ethogram employed (Table 1.1) was based on that used by Maruhashi (1981). Social behaviour was divided into affiliative, submissive and dominant behaviour, each of which was then further divided into subcategories. In addition, two types of exploration, object/environment manipulation and vigilance, were added to better measure interactions with the environment and habituation in the new habitat.

Spatial Use Data

Space use of the macaques, scan sampling was performed (Martin and Bateson, 2007). During a scan, the vertical and horizontal positions of the visible individuals were instantaneously recorded. Vertical space was divided into four levels in the enclosure: (1) ground level to approximately 1.5m off the ground; (2) approximately 1.5m to 3m; (3) approximately 3m to 4.5m; and (4) approximately 4.5m and above. Horizontal quadrants were designated by letters (illustrated in Figures 1.1 and 1.2). The division of the horizontal spaces was made based mainly on the functional significance of the different areas. Moving individuals were recorded as being in the vertical level and horizontal area in which they were first observed during the

scan. Scans were performed at the start of each day, after every ten minute focal session and after the three scheduled rest periods.

Visitor Density and Intensity Data

Three visitor phases were determined based on the zoos opening schedule for the season and the local schools holiday schedule. For the 2014 field season, data collected from July 15th to August 23rd were classified as "High" season, when the zoo is open for full or extended hours in all areas, and all schools are on holiday. Data from August 25th to September 1st and additional weekends in September and October, were classified as "Low" season, characterized by the zoo being open for reduced hours in some or all areas and some or all schools in session. Data collected on weekdays from September 2nd onwards were classified as "No" visitor season as the zoo was closed to visitors, with the exception of the occasional tour group. In 2015, data collected July 21st to August 19th were classified as "High", data from May 16th to 18th, May 23rd to the end of June, August 24th to 30th, and September 5th to 7th, 12th and 13th were classified as "Low" and data from April to May 15th, May 19th to 22[nd,] and weekdays from August 31st onwards were classified as "No" visitor season. Daily zoo attendance was provided by the Zoo de Granby.

Crowd size was also recorded instantaneously at each 15 second interval of the focal sampling session. Crowd was recorded as categories, similar to those used by Choo et al. (2011) (Table 1.2). In the 2014 enclosure, crowd size was determined by quickly scanning and counting the number of visitors on the path directly around the enclosure. However, due to the size of the 2015 enclosure and barriers to visibility, it was not possible to count the number of people around the entire perimeter. The enclosure was divided into left and right sides and only visitors in the viewing areas on one side were counted, depending on where the animal was positioned in the enclosure (Figure 1.3). We believe that this was still an acceptable measure of crowd size as the perimeter of the 2014 enclosure was approximately equal to the perimeter of half the new enclosure, making the number of people it could accommodate comparable. In addition, animals tend to take more notice of visitors that are in closer proximity to them, meaning that the number of visitors on the opposite side would be less relevant as it relates to behaviour (Choo et al., 2011).

Visitor intensity was represented by ambient noise levels. Ambient noise level was measured in decibels using a Reed Model ST-805 Sound Level Meter. The maximum sound level experienced in the viewing area around the enclosure was recorded directly before each ten minute focal session. The settings of the meter were at slow time weighting, which checks average levels of fluctuating noise and "A" weighting, for general sound, as opposed to "B" weighting, which is for low frequency noise. Level range was set to Low (30-100dB), as the meter will automatically switch to High (60-130dB) if the measurement exceeds the low range.

Statistical Analysis

Six Kendall's rank correlations were performed to compare the three Zoo de Granby macaque groups' activity budget to the activity budget of a wild population (Hanya, 2004) and a research population (Jaman and Huffman, 2008). A significant positive correlation would indicate the order of predominance of the behaviours was the same between the two groups being analyzed. Some of the categories of behaviours from zoo groups were combined in order to be comparable to the other studies. Object manipulation, vigilance, inactive, dominance, submission and other were combined to form the category "Resting", and abnormal behaviour and self-grooming were excluded, in order to compare the activity budget to the data from wild population. To compare the zoo macaques' activity budget and the research macaques' activity budget, the categories self-grooming and allogrooming were combined to form "Grooming", affiliative behaviour and object manipulation was combined to create "Object Manipulation and Play", and other and abnormal behaviours were excluded. The activity budget during the entire field season of each group was used for this analysis as both papers of interest provided activity budgets in this manner. Data from May 2015 were excluded from this analysis as the macaques were still adjusting to the new enclosure and changed social grouping at this time. The Kendall's rank correlations were performed at the 5% level of significance in R 3.2.3 (R Core Team, 2015).

Frequency of observations of a given behaviour during each focal were used for the following five models. Any focals missing more than one minute of observations were removed from the analysis (Jansen and Vogel, 2006; Lehner, 1996; Vyas, 2006). Due to low occurrences of some of the behaviours, the original ethogram was re-organized. Object manipulation and affiliative behaviour were combined to form the category of positive affect indicators, referred to as "Positive behaviours", submissive and dominant behaviours were combined to form

"Agonistic behaviours", and abnormal and other behaviours were combined to form "Other". Three generalized linear mixed model were used for each group to assess: 1) the variation in the occurrence of the different behaviours 2) the effect of phase on the variation in the occurrence of the difference behaviours and 3) the effect of the three visitor variables on a given behaviour, while controlling for time of day, using the data from July to September/October in 2014 and 2015. Two models were performed using different subsets of data to assess habituation in the new enclosure and the effect of enclosure on activity budget respectively, while controlling for both time of day and phase. Generalized linear mixed models with a negative binomial distribution and log link function were run using Proc Glimmix in SAS 9.4 (SAS Institute Inc. 2013). A 5% level of significance was adopted for all tests. We included individual ID in our models as a random term to account for pseudoreplication. The natural logarithm of the total number of "in sight" observations was used as an offset to account for differences in the number of observations in a focal (Agresti, 1996). Therefore, the results of the generalized linear mixed models pertain to the rate of observation of a given behaviour during a focal. Due high number of terms in each model and the large number of models which were performed, non-significant results are not reported in the results section.

The first model run for each group tested the effect of activity type, a categorical variable with nine levels, on the rate of occurrences recorded during a focal. The second tested the effect of activity type, phase and the interaction between activity type and phase, on the rate of occurrences recorded in a focal, for each group. In this model, we were mainly interested in the interaction term, as it would indicate whether the relationship between number of occurrences in a focal and activity type differs between phases, and if significant, would support the further analysis of the visitor variables. The third model was performed within each group for the seven behaviours of interest (allogrooming, autogrooming, agonistic behaviour, inactivity, other behaviours, positive affect indicators and vigilance). It tested the effect of attendance, crowd size, sound level and time of day on the rate of occurrence of the behaviour of interest. Backward selection was used for the third model, whereby all predictors are initially included and the one with the smallest and non-significant partial F-statistics is dropped (Quinn and Keough, 2002), using an α to drop of 0.1.

In order to compensate for a potential sensitization or desensitization to visitor effect (i.e. being less affected on the first day exposed to visitors compared to later days and vice versa), particularly for the 2014 enclosure, where the two groups were cycled on- and off-display each week, a three day running mean was calculated using the daily attendance for the day of the observations and the two previous days. If the group was off-display during those days, the daily attendance was considered to be zero. This value was used in the analyses in place of the true daily attendance and will henceforth be referred to as "attendance". The mode crowd size during each focal was determined and used as the categorical predictor "crowd size", with three levels (1, 2 and 3). Time of day was represented by the categorical predictor "period", where the level "AM" consisted of start times between 9:00 and 12:00, "PM1" consisted of start times between 12:00 and 15:00 and "PM2" consisted of start times later than 15:00. A number of focals were lacking data for sound level as the sound level meter could not be used when it was raining, and we experienced some technical issues during the 2015 that caused the readings to be unreliable, therefore these focals were excluded for this model. Pairwise comparisons of categorical variables were performed using a Tukey-Kramer correction.

For the habituation model, we used data from the first seven days that the macaques of group three were in their new enclosure with no access to the indoor enclosure (May 20th to 26th), which was labelled as the "early week", and the last seven day of observation (between September 10th and 17th), labelled as the "late week". The model tested the effect of activity type, week and the interaction between week and activity type, as well as phase, period and their interaction with activity, on the rate of occurrences recorded during a focal.

In the enclosure comparison model, we tested how the rate of occurrences recorded during a focal were affected by activity type, group and the interaction between group and activity type, as well as phase, period and their interaction with activity. To reduce the potential confounding effect of the visitor variables and to assure individuals had sufficiently habituated to the new enclosure, only data from late August to early October 2014 and late August to late September 2015, during the "No Visitor" and "Low Visitor" phases, was used. Group was used as a categorical predictor, with groups one and two representing the old enclosure and group three representing the new enclosure. Individuals in groups one and two who were not present in group three were excluded from the analysis. The interaction term would indicate whether the

effect of activity pattern on rate of occurrences was dependent on group and, by proxy, enclosure. Pairwise comparisons were performed using Tukey-Kramer corrections for the interaction terms of activity type and week for the first model, and activity type and group for the second.

Proportion of individuals observed in a given horizontal area was used as the response variable in the analysis of the space use. Proportions were calculated for each time period of each day by summing the number of observations of any individual in the area of interest during the scans in that period, then dividing this sum by the total number of observations of individuals in all areas during that period. The same method was used to calculate the proportion of individuals in a given vertical level. Generalized linear models, using a binomial distribution and logit link function, were run using Proc GenMod in SAS 9.4 (SAS Institute Inc. 2013). For each group, separate space use models were run for horizontal and vertical space use, analyzing the effect of area or level, respectively, on the proportion of observation in a period as well as effect of period and phase, the interaction between period and area/level and the interaction between phase and area/level. To assess the pairwise comparisons of the least squares means of a given area or level to all other areas or level, and to assess the difference in least squares means of the same area or level during different phases or time periods, a Tukey-Kramer correction was performed after each model for area or level and the two interaction terms.

Spatial habituation was also assessed in the same way as for behaviour. Using data from the "early week" and "late week", these two models analyzed the effect of area/level, week and the interaction between week and activity type, as well phase, period and their interaction with area/level, on the proportion of observations during a period. These were followed by a Tukey-Kramer post hoc test on the interaction between week and area or level. All tests were performed at the 5% level of significance.

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Results

Activity Budget

The rate of the various behaviours differed significantly in group one ($F_{(8,5217)}$=212.95, p<0.001), group two ($F_{(8,5362)}$=246.14, p<0.001) and group three ($F_{(8,8573)}$=515.76, p<0.001). The dominant behaviours were vigilance and allogrooming for group one, vigilance and autogrooming for group two, and allogrooming alone for group three (Figure 1.4). Conflict was the least frequent behaviour for all three groups. Activity budgets did not correlate with what was observed for the wild troop (all groups: τ=0, T=3, p=0.625; Figure 1.5) or the research troop (groups one and three: τ=0.333, T=14, p=0.191; group two: τ =0.238, T=13, p=0.281; Figure 1.6). This means that the order of predominance of the behaviours in the zoo groups was significantly different than that of the wild and research groups.

Visitor effect

The rate of the various behaviours differed significantly between phases in all three groups (group one: $F_{(16, 5044)}$ =2.83, p<0. 001; group two: $F_{(16, 5314)}$ =2.58, p<0.001; group three: $F_{(16, 8662)}$ =3.77, p<0.001; Figure 1.7). Period had a significant effect on rate of allogrooming in all groups (group one: $F_{(2, 500.1)}$ =8.42, p>0.001; group two: $F_{(2,541.1)}$ =6.10, p=0.002; group three: $F_{(2,818.2)}$ =7.39, p<0.001). In group one ($F_{(2,500.3)}$=3.14, p=0.044; Figure 1.8) and group two ($F_{(2,544.4)}$=3.44, p=0.033; Figure 1.9) the rate of allogrooming differed significantly between crowd sizes; for both , there was more allogrooming at crowd sizes of 20 or more people compared to crowd sizes of less than ten people. The rate of allogrooming also decreased with sound level (estimate ± SE=-0.030±0.015; $F_{(1,550.6)}$ =4.16, p=0.042; Figure 1.10) in group two. None of the three visitor variables significantly influenced rate of allogrooming in the new enclosure (all p>0.05). The rate of autogrooming varied with periods only for group three ($F_{(2,849.5)}$ =5.69, p=0.004) and was not affected by any of the other variables in each of the three groups (p>0.05).

In group one, the rate of agonistic behaviours decreased with sound level (-0.102±0.029; $F_{(1,467)}$ =12.75, p<0.001; Figure 1.11) and increased with attendance ($8.70 \times 10^{-5} \pm 4.40 \times 10^{-5}$; $F_{(1,467)}$ =4.01, p=0.046; Figure 1.12). The rate of inactivity differed significantly with period in the old enclosure (group one: $F_{(2,440)}$ =3.11, p=0.045; group two: $F_{(2,568.2)}$ =3.75, p=0.024) and in the

new enclosure ($F_{(2,808.9)} = 9.27$, p<0.001). In the new enclosure, rate of inactivity also increased significantly with attendance ($5.60 \times 10^{-5} \pm 2.60 \times 10^{-5}$; $F_{(1,801.1)} = 4.51$, p=0.034; Figure 1.13) and did not vary with crowd size after the adjustment. The rate of positive behaviours differed between time periods for group one ($F_{(2,363.5)} = 5.77$, p= 0.003) and group three ($F_{(2,857.6)} = 3.80$, p=0.023). The rate of positive behaviours differed significantly among crowd size levels for group one ($F_{(2,342)} = 5.17$, p=0.006; Figure 1.15), but not for groups two or three; it was lower at crowd sizes with 20 or more people compared to crowd sizes smaller than ten people.

Vigilance decreased with attendance in group two ($-4.00 \times 10^{-5} \pm 1.10 \times 10^{-5}$; $F_{(1,604)} = 12.42$, p<0.001; Figure 1.15) and group three ($-4.00 \times 10^{-5} \pm 1.40 \times 10^{-5}$; $F_{(1,843.4)} = 7.33$, p=0.007; Figure 1.16). Vigilance also varied with periods in groups two ($F_{(2,593.1)} = 3.86$, p=0.022) and three ($F_{(2,849)} = 8.11$, p<0.001), and with crowd size only in the old enclosure (group one: $F_{(2,506.1)} = 4.00$, p=0.019; group two: $F_{(2,600.5)} = 3.39$, p=0.034); there was significantly less vigilance at crowd sizes of 20 or more people compared to crowd sizes of less than ten people (Figure 1.17), while the opposite trend was observed in group two (Figure 1.18). There was no significant effect of the visitor variables on other behaviours (which includes abnormal behaviours) for all three groups (p>0.5). There was a significant effect of period on the rate of other behaviour in group one ($F_{(2, 90.04)} = 6.76$, p=0.002).

Behavioural Habituation

The rate of the behaviours varied between the early week and late week in the 2015 enclosure ($F_{(8, 2374)} = 8.88$, p<0.001). There was significantly less allogrooming, less inactivity and less positive behaviours in the early week than in the late week (Figure 1.19). Vigilance and locomotion were performed for often in the early week as well.

Enclosure

The comparison of the activity budget between groups revealed a significant interaction between activity type and group ($F_{(16, 7839)} = 8.60$, p<0.001). Specifically, groups one and three were significantly more inactive than group two, whereas group two autogroomed significantly more than both groups one and three. Group one showed more positive behaviours than groups two and three. Group three also appeared to be less vigilant and performed more allogrooming than group two (Figure 1.20).

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General Space Use

For vertical space use, the proportion of observations differed significantly between levels for group one ($\chi^2_{(3)}$ =3652.44, p <0.001), group two ($\chi^2_{(3)}$ =1907.61, p <0.001) and group three ($\chi^2_{(3)}$ =13368.2, p <0.001). Both group two and group three had the highest mean proportion of observations in level two, whereas group one had a higher proportion of observations in level one (Figure 1.21). There was a significant difference between the proportion of observations in the horizontal areas for all three groups (group one: $\chi^2_{(10)}$ =2425.73, p <0.001; group two: $\chi^2_{(10)}$ =4076.37, p <0.001; group three: $\chi^2_{(11)}$ =21069.4, p <0.001). The house features (area K) were the most used area by both groups in the old enclosure, with areas A (back) and J (back outer-left), and areas A, J and H (front outer-left) being used the least by group one and group two, respectively (group one: Figure 1.22). In the new enclosure, the mountain feature and houses were the most used (area L), followed by areas F (in front of the viewing window), G (the water feature) and I (the climbing structure). Areas J and H (both outer right) had the lowest proportion of observations (Figure 1.23).

The models for all three groups showed significant interactions between vertical level and phase (group one: $\chi^2_{(6)}$= 50.98, p<0.001; group two: $\chi^2_{(6)}$=54.02, p<0.001; group three: $\chi^2_{(6)}$=166.7, p<0.001; Figure 1.24), between horizontal area and phase (group one: $\chi^2_{(20)}$= 239.66, p<0.001, Figure 1.25; group two: $\chi^2_{(20)}$= 49.09, p<0.001, Figure 1.26; group three: $\chi^2_{(22)}$=128.18, p<0.001, Figure 1.27) and between horizontal area and period (group one: $\chi^2_{(20)}$= 115.38, p<0.001; group two: $\chi^2_{(20)}$= 168.79, p<0.001; group three: $\chi^2_{(22)}$=359.87, p<0.001). The interaction between vertical area and period was only significant for groups two ($\chi^2_{(6)}$ = 42.4, p<0.001) and three ($\chi^2_{(6)}$ = 260.06, p<0.001).

Space Use Habituation

In the new enclosure, there was a significant interaction between level and week for vertical space use ($\chi^2_{(3)}$ =99.68, p<0.001; Figure 1.28) and between area and week for horizontal space use ($\chi^2_{(11)}$ =369.33, p<0.001; Figure 1.29). This means that the use of at least one of the horizontal areas and at least one of the vertical levels differed between early and late week. In addition, the interaction between level and phase was significant for vertical space use ($\chi^2_{(6)}$ =25.46, p<0.001) and the interaction between area and period was significant for horizontal

space use ($\chi^2_{(22)}$ =70.73, p<0.001); meaning that the use of at least one area and at least one level differed between phases for both vertical space use and horizontal space use.

Discussion

Empirical studies are needed to better understand how housing and husbandry practices impact the welfare of animals in zoos (Melfi, 2009). In this study, we determined the activity budget and space use patterns for the Japanese macaques housed at Zoo de Granby, to explore how two zoo-specific stressors, visitor presence and enclosure, affect their behaviour and welfare. In general, the three groups observed in this research performed a high amount of vigilance, allogrooming and autogrooming. In comparison, there was relatively little agonistic behaviour, positive affect indicators or other behaviour.

The Zoo de Granby Japanese macaques did not display an excessive amount of aggressive behaviour. Over the field seasons for each of the groups, agonistic behaviour accounted for only 0.4% to 0.6% of the activity budget, which is similar to what was observed in a research troop (Jaman and Huffman, 2008). Most individuals did not perform any abnormal or stereotypic behaviour, which is generally attributed to stress (Broom, 1991). However, the activity budgets themselves of both groups were very different from the wild and research populations used for comparison. All three of the Zoo de Granby groups of macaques performed much more grooming, and vigilance and less feeding and inactivity than the wild and research troops. Hebert and Bard (2000) state that captive animals often feed less than wild counterparts, which was true for these zoo-housed macaques and this leaves a large amount of time that must be filled with other activities; in this case that time was filled with vigilance and grooming.

We speculated that the high level of vigilance observed in the zoo was due to visitor presence, as the majority of observation days were when the zoo was open to the public. Vigilance is a common anti-predatory behaviour. Though zoo animals may view humans as only a non-lethal disturbance and not a true predatory threat, the two are believed to be analogous as they incur similar trade-offs between avoiding the perceived risk and performing more beneficial activities such as feeding or mating (Frid and Dill, 2002). This perceived risk would likely not be present in the research troop, where there are no natural predators and other disturbances would be relatively minimal. High levels of vigilance towards humans may also indicate that a primate views them as a social threat, as displaying a vigilant stance is considered an indicator of social-anxiety in low-ranking baboons (Sapolsky and Share, 2004). On the contrary, some believe that vigilance towards humans could simply show and animal's interest in them and their behaviour;

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thus, it could show an enriching effect (Mitchell and Hosey, 2005). However, the potential presence of negative visitor effect was also suggested by the large disparity in grooming rates between the Zoo de Granby Japanese macaques and the wild and research macaques. Allogrooming is used in primates to ease social tension and reconcile after confrontations (Schino et al., 2005) and self-grooming, though playing an important role in hygiene and self-maintenance, is also considered displacement behaviour and linked to anxiety (Diezinger and Anderson, 1986).

We found that the Japanese macaques' activity budget varied between the high, low and no visitor period. This result, combined with the large discrepancies between the activity budget of the Zoo de Granby macaques and the wild and research groups, gave strong support for the further examination of this stimulus. We chose to measure three commonly-used variables to represent visitor effect: sound level, crowd size around the enclosure and daily attendance at the zoo. All three variables significantly affected at least one activity type in at least one of the groups; however effects were not always consistent between groups. As with the gibbons in the study by Cooke and Shillaci (2007), both macaque groups in the old enclosure allogroomed more at the largest crowd size compared to the smallest, which may be indicative of an attempt to relieve tension. However in one of the old enclosure groups, group two, allogrooming also decreased with increasing sound level. A similar pattern was observed with the rate of agonistic behaviour in group one, which both increased with attendance and decreased with sound.

It is important to note that when measuring ambient noise levels in urban zoos, noise levels is generally higher when visitors present but not always so (Quadros et al., 2014). Construction and surrounding traffic could also cause increases in sound levels. Animals that have lived their entire lives in zoos could also become habituated to noise and either not react to it or react differently than expected. While studying the effect of noise on jaguars (*Panthera onca*), Sellinger and Ha (2005) observed more stereotypic pacing at moderate noise levels compared to both low and high noise levels. We noticed that sudden noises, such as thunder or the dropping of equipment, often solicited a behavioural response (e.g. branch shaking behaviour), whereas consistent noises, like chainsaws, did not. Overall, the lack of clear association of allogrooming and visitor presence, and the lack of correlation of autogrooming with any of the three visitor variable would suggest that the hair loss currently observed in the

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Zoo de Granby Japanese macaques is not likely due to stress-induced over-grooming caused by the number or activity level of zoo guests.

Our observation of less positive behaviours such as affiliation in group one at the largest crowd size, is in accordance with previous research (Chamove et al 1988). At the Singapore Zoo, orangutans were found to both perform less play behaviour when visitors were standing closer to them, but only in one of the two habitats in which they were studied (Choo et al., 2011). We also found an increase in inactivity with zoo attendance in group three and a decrease in vigilance with zoo attendance for both group two and group three, which is opposite to what has been observed in other primates (Chamove et al 1988) as well as in brown bears (*Ursus arctos arctos*) (Soriano 2013). Even within our study, contradicting results were found. In the old enclosure group one had a lower rate of vigilance at large crowd sizes, whereas group two had higher rates of vigilance at large crowd sizes. It is clear that visitor presence does not necessarily act in a similar way on all groups, even those of the same species. This is further supported when we take into account the differences in space use of the Zoo de Granby macaques between the three visitor phases.

The Japanese macaques were observed in all horizontal areas of their enclosures, unlike chimpanzees and gorillas at the Lincoln Park Zoo, Chicago, Illinois, which were observed in only 56.5% and 28.5% of the available quadrants in their enclosures, respectively (Ross et al., 2011a). However, all macaque groups still showed very clear selectivity. This is in agreement with other studies on primate space use (Hebert and Bard, 2000; Hedeen, 1982; Stoinski et al., 2001) and on space use in other mammals, such as wild boar, *Sus scrofa* (Blasetti et al., 1988), and Nile hippo, *Hippopotamus amphibius* (Blowers et al., 2012). In the old enclosure, the two groups (group one and group two) were observed in the area of the den and house structures approximately twice as often as in any other area. In particular, it was anecdotally noted that the tops of the houses were a favored spot for grooming and resting. Previous studies have found that wild boar and Indian leopards (*Panthera pardus*) both display significant area by behaviour interactions, meaning that specific areas of the enclosure are used for specific behavioural purposes (Blasetti et al., 1988; Mallapur et al., 2002). In 2015, approximately half of all observations were of individuals in or on the caverns and house features. As Japanese macaques

prefer to rest in sitting and lying positions (Chatani, 2003), the flat roof of the houses and caverns would be more stable than branches when resting off the ground.

The water feature area was the second most used for both groups in 2014, and was one of the three areas tied for second most used in the 2015 enclosure, despite none of the groups showing a great interest in actually entering the water. Many macaque species have been recorded bathing in water in the wild (Robins and Waitt, 2011). In colder areas of their range, Japanese often enter hot spring, a behaviour which is believed to be thermoregulatory in nature (Zhang et al., 2007). Populations have also learned bathing behaviour by being coaxed into water with food (Kawai, 1965). Other zoos, such as the Central Park Zoo and the Detroit Zoo have had success with water enrichment (Goodwin, 1999; Robins and Waitt, 2011). Only three macaques in this study purposefully entered the water in either enclosure (Mago, Madjae and Shiwa); however, they did use the water for washing food and other objects, a species-typical macaque behaviour (Kawai, 1965; Robins and Waitt, 2011). The artificial stone surface around the water may have also been favoured due to the microclimate it created. Commonly-used enclosure construction materials such as concrete or gunite transfer heat differently than a vegetated substrate such as grass (Brown and Gillespie, 1995; Langman et al., 1996).

Looking at vertical space use, we found that the Japanese macaques at the Zoo de Granby spend the majority of their time either at ground level or between 1.5m to 3m off the ground. Primates exhibit possibly the most diverse movement and spatial behaviour than any other mammal, hence building adequate housing for captive individuals can be a challenge. In the wild, Japanese macaques use both terrestrial and arboreal substrates when feeding, resting and traveling (Chatani, 2003). They generally spend approximately 40% to 70% of their time on the ground (Chatani, 2003). This is similar to what we observed, with the ratio of terrestrial to arboreal use being from approximately 30/70 in group two to 50/50 in group one, the old enclosure groups, and 45/65 in the new enclosure. The preference to be off the ground in two of our three groups could be related to the classification of levels used in this study. For our purposes, levels higher than ground level included both tree structures and the tops of the houses and mountain features, whereas Chatani (2003) only classified trees and vines as arboreal.

Throughout the study as a whole, the Zoo de Granby Japanese macaques did not prefer or avoid neither areas close to visitors nor areas further from. However, in both groups in the 2014

enclosure, the proportion of individuals observed at ground level was higher when the zoo was closed to visitors. Higher areas are often perceived as being safer by an animal when visitors are present (Choo et al., 2011; Coe, 1985). The horizontal space use also varied between phases, but only group one showed differences consistent with the visitor effect hypothesis. In this group, area A, at the back of the enclosure, was used significantly more during the high visitor phase compared to the no visitor phase, and areas H and F were used significantly less. In group three, differences in space use between the no, low and high visitor season did not clearly indicate any visitor effect

A number of reasons for variation in visitor effect have been suggested in previous literature including enclosure design, size of social group of the animals, size and weight of individual animals (Chamove et al., 1988; Choo et al., 2011; Cooke and Schillaci, 2007; Soriano et al., 2013). As group three was in the new, larger, more naturalistic enclosure than group one and two, and consisted of ten individuals as opposed to seven, it is not surprising that their behaviour appeared to be less affected by visitor presence than the other two groups. Nevertheless, we did anecdotally note a greater number of threat displays towards guests at the viewing window of the new enclosure, compared to the chain link viewing areas in both the new and the old enclosure. This may have been due to lack of a perceived barrier between them and the visitors, or due to the improved visibility of movements and facial expressions. Primates have been found to act territorially towards humans (Cooke and Schillaci, 2007). It would be beneficial to investigate this further, as previous research has found a potentially negative effect of viewing windows in some species (Clark et al., 2012). In this study by Clark et al. (2012), gorillas were found to display less negative visitor vigilance when semi-opaque privacy screens are placed over viewing windows so that only visitors general outline could be seen. Between the two groups in the old enclosure, the difference in visitor effect may come down to individual differences. In their study on visitor generated sound, Quadros et al. (2014) did not observe any significant visitor effect on the behaviour of a group as a whole, but did find individual-level behavioural differences relating to noise in brown howler monkeys (*Alouatta guariba*), ocelots (*Leopardus pardalis*) and a number of other species. They hypothesized that this was due to how the individuals perceived the stimulus differently.

In summary, it appears that the presence of visitors did impact the behaviour of the Japanese macaques at the Zoo de Granby; group three had the least number of behaviours that correlated with the visitor variables, potentially because of the design of the new enclosure. However, the direction of the changes in behaviour relative to the visitor variables did not consistently support either of the two hypotheses in any of the three groups. Therefore, we did not perceive any evident decrease or increase in welfare based on the behavioural indicators measured. Some negative interactions (aggression) and positive interactions (affiliative behaviour) with visitors were observed, particularly in the new enclosure, and further research is recommended to better clarify this subject

While exploring our second zoo-specific stressor, enclosure design, we chose to also test the hypothesis that the macaques would undergo a habituation period when first introduced to the 2015 enclosure. Our behavioural results were consistent with this hypothesis. During the first week that the Zoo de Granby Japanese macaques were fully exposed to the 2015 outdoor enclosure, with access to the indoor pavilion restricted, the group moved more, was less inactive and more vigilant than in the last week in which data was recorded, approximately four months later. The only result that was not consistent with habituation was the lower rate of positive affect indicators (which included object manipulation) in the early week compared to the late week. As the positive affect indicators category was a combination of object manipulation and affiliative behaviour, it is possible that this result is due to the latter behaviour. Changing habitats can be a stressful experience for captive animals, as has been suggested by both physiological and behavioural evidences (Goymann et al., 1999; Ross et al., 2011b) and, therefore, could result in a temporary decline in the rate of positive affect behaviours. In addition, object manipulation was relatively uncommon during both the 2014 and 2015 field season, which may indicate that its presence or absence is not a good representation of exploration in these Japanese macaques, unlike in the great apes (Ogden et al., 1990).

Patterns of space use in the Zoo de Granby Japanese macaques were less in accordance with the habituation hypothesis. They did reveal some interesting trends; the first of which was the increased use of the highest vertical level, 4.5m off the ground and above, in the early week compared to the late week. Use of this level could be indicative of exploration, as being in an elevated position would increase the field of view and facilitate visual evaluations of a novel

area. Alternately, increased use of the highest level could be a neophobic response to a novel environment; arboreal primates tend to be less fearful when positioned higher in trees (Hirsch, 2002).

As expected within the context of the vertical space use results, the Zoo de Granby macaques were observed more often in the horizontal area containing the climbing structure in the early week. Though they made significantly more use of areas H, J and K, which were used very little in the late week, horizontal space patterns still indicated much more selectivity in the early week than was predicted for early in the habituation process, always favouring the dens and houses (area L). In their study on gorilla adaptations to a novel environment, Ogden et al. (1990) found that individuals stayed relative clumped together during the first three months in their new enclosure and did not start to disperse to explore more until after this period; even after a year, individuals had not entered 40% of their new habitat. It has been suggested that primates associate areas of their enclosure with specific characteristics, such as comfort and safety (Ross et al., 2011a; Ross et al., 2009). In both our study and that of Ogden et al. (1990), it is possible that individuals were able to quickly assess the novel environment for characteristics of interest based on their previous experiences, without necessitating an extended period of exploration in each area. This is supported by the observation that level and area preferences in the Zoo de Granby Japanese macaques were not only consistent within the 2015 season but also between the 2014 and 2015 season.

In order to test the hypothesis that the new enclosure would improve the welfare of the Zoo de Granby Japanese macaques, we compared the behaviour of the three groups during the last ten days of their field season, in order to give group three the maximum amount of time possible to habituate to their new environment. The activity budgets of the three groups differed significantly, but these group differences did not clearly suggest an effect of enclosure design. Group three had increased rates of movement and decreased rates of vigilance, both signalling a possible shift towards more "wild-like" behaviour, but there was also a decrease in feeding and foraging, and an increase in allogrooming compared to the old enclosure. In langurs and wild horse (*Equus ferus przewalskii*), all types of social interaction, including social grooming, were less frequent in a larger enclosure compared to a smaller enclosure (Hogan et al., 1988; Little and Sommer, 2002). This was likely due to the decreased proximity between individuals. The

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observed increase in allogrooming in the Zoo de Granby Japanese macaques may have been related to recent changes in the social dynamics of group three.

The decrease in foraging in the new enclosure was unexpected, as this enclosure offered a greater diversity of vegetation than the old enclosure. Previous studies on zoo and research have consistently found increased feeding and foraging in larger enclosures with more vegetation (Hogan et al., 1988; Beisner and Isbell, 2008; Jaman and Huffman, 2008). Discontinuing the mid-day snack that was provided to the macaques after they were moved to the new enclosure could have contributed to this. In the old enclosure, the snack appeared to stimulate an extended bout of foraging on both the monkey chow and on the vegetation in the enclosure. Group three also displayed rates of behaviour that were intermediate between group one and two, as for autogrooming, or rates of behaviour that were very similar to one of the previous groups, but different from the other, as for inactivity. It is important to note that the behavioural differences discussed above were not all statistically significant.

Previous studies have found a wide range of behavioural responses to novel environments. Primates may display large differences in behaviours such as eating, resting and allogrooming when moved to a new enclosure, or relatively conservative differences (Little and Sommer, 2002; Ross et al., 2011b). Though it is important to gain data on the same individuals in two different environments to control for individual-level differences in behaviour, many animals currently housed in zoos are no longer in the extremely barren and restrictive habitats that were common decades ago. A change from an enclosure that is relatively naturalistic, but a bit lacking in size or complexity (as was the 2014 enclosure of the Zoo de Granby Japanese macaques) to a bigger better-designed naturalistic enclosure might not elicit a large change in welfare in either the positive or the negative direction. This is what was found in the study on the gorillas and chimpanzees at the Lincoln Park Zoo (Ross et al., 2011b). In addition, it is evident from both this study and the studies by Ogden et al. (1990) and Ross et al. (2011b) that primates must habituate to a new environment. The length of this study may not have been sufficient for the animals to truly experience the long-term welfare benefits of their new environment. Finally, there were confounding variables in this study that may have masked the behavioural differences of individuals between the two enclosures, in particular, the change in membership and social structure between the three groups. Previous experience of the animals, time needed to habituate

and additional stressors that the animals may be experiencing are all factors that should be considered by both researchers and zoo management when evaluating the merits of a new habitat.

Though the results for visitor effect and enclosure design were ambiguous in this study, it serves to highlight the complex nature of research in zoos. It appears that the presence of visitors did impact the behaviour of the Japanese macaques at the Zoo de Granby, its impact on their welfare was not clear. It is evident that visitor presence does not necessarily act in a similar way on all groups, even those of the same species. As all three visitor-related variables contributed to predicting behaviour, this study supports the consideration and use of multiple measures when exploring this phenomenon. The study of transferring animals to a new environment proved to be equally complex due to confounding variables such as habituation time. Thus, we recommend more thorough research protocol that allows for sufficient recording of all relevant predictors and confounding variables, whether the topic of interest is visitor effect, enclosure design or other components of zoo biology.
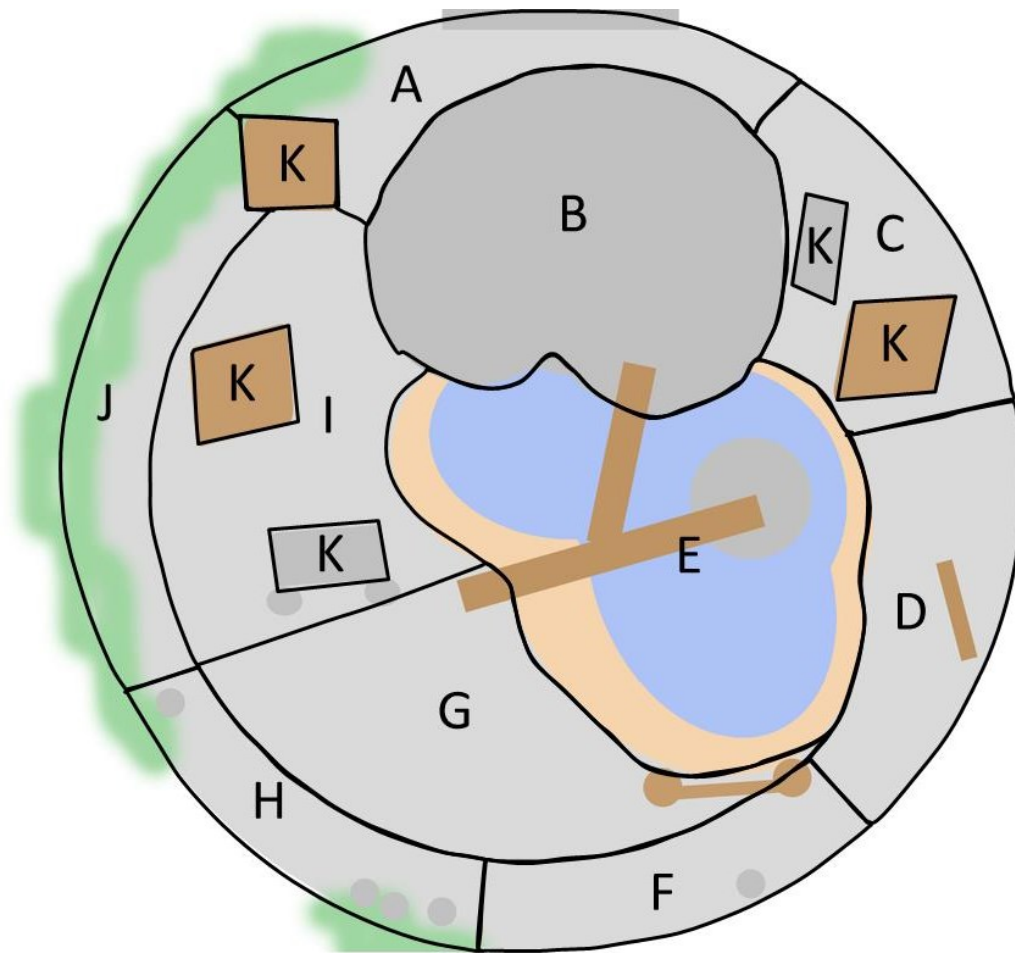
Tables and Figures

Table 1.1. The ethogram of behaviours recorded during the sampling period for the activity budget, adapted from Maruhashi (1981). For behaviours directed towards another individual, identity of both individuals involved in the behaviour will be recorded when possible.

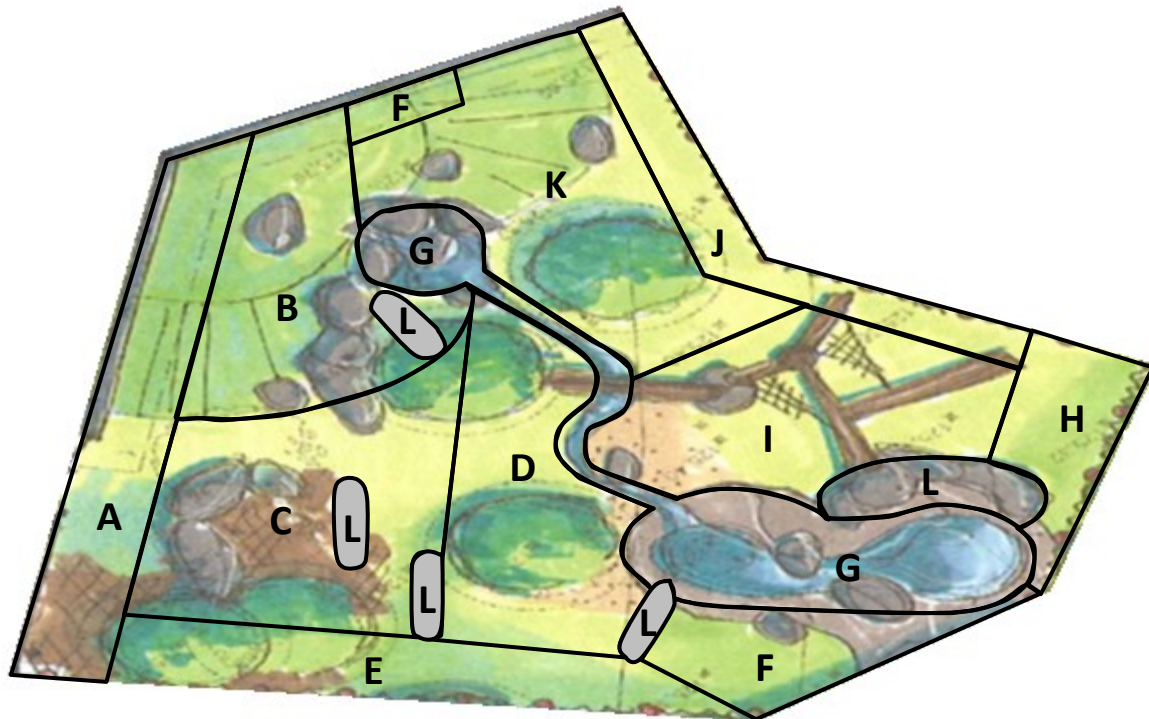| Activity	Definitions
Inactive	Animal is relaxed and not involved in any other activity
Moving	All types of locomotion (walking, running, climbing, etc.) not included in any other activity
Exploratory Behaviour	**Object Manipulation (OM):** Picking up objects or part of the enclosure and/or manipulating them with their hands or feet (Ogden et al., 1990) **Vigilance (V):** While stationary, animal is alert and is actively looking around the enclosure or at an animal or visitor (see "vigilance" in Soriano et al. (2013) and "scanning" in Martin and Réale (2008))
Allogroom	Animal is grooming another animal, or is being groomed by another animal.
Autogroom	Animal is grooming or scratching itself
Feeding/ Foraging	Intake of solid food or water, including the process of searching for food items on the ground or in vegetation, digging for and cleaning food
Affiliative behaviours	Positive interactions between individuals. Will be defined in notes as: **Affiliative Contact (AC):** non-violent physical contact between individual. Excludes allogrooming (Kapsalis and Berman, 1996; O'Keeffe et al., 1982/83) **Social Play :** non-aggressive competitive interactions (Aldis, 1975)
Submissive Behaviour	Agonistic behaviours indicating fear or submission. The behaviour observed was classified into the following subcategories (de Waal et al., 1976) **Flight (F)** - Fast withdrawing locomotion from another individual **Flight-intention (FI)**: Postures or movements expressing a tendency to flee such as crouching or shrinking. **Submission (S):** submissive facial expressions such as teeth-bearing
Dominant Behaviour	Agonistic behaviour of an aggressive or dominant nature. The behaviour observed was classified into the following subcategories (de Waal et al., 1976): **Threat (T) -** Facial expressions and postures that convey agonistic intentions. Branch shaking and leaping, were also be included in this category **Chase (C)** - Quick and brusque movements towards another individual **Physical Assault (PA)** – Varying degrees of violent physical contacts.
Abnormal Behaviour	Behaviour not considered to be a part of an animal's natural repertoire such as self-aggression, autoerotic stimulation and stereotypic behaviours (Ogura, 2012)
Other	Behaviours that are not included in any of the above categorised
Not In Sight	Animal is not visible to the observer

Table 1.2. Categories for recording instantaneous crowd size at the Japanese macaque enclosures.

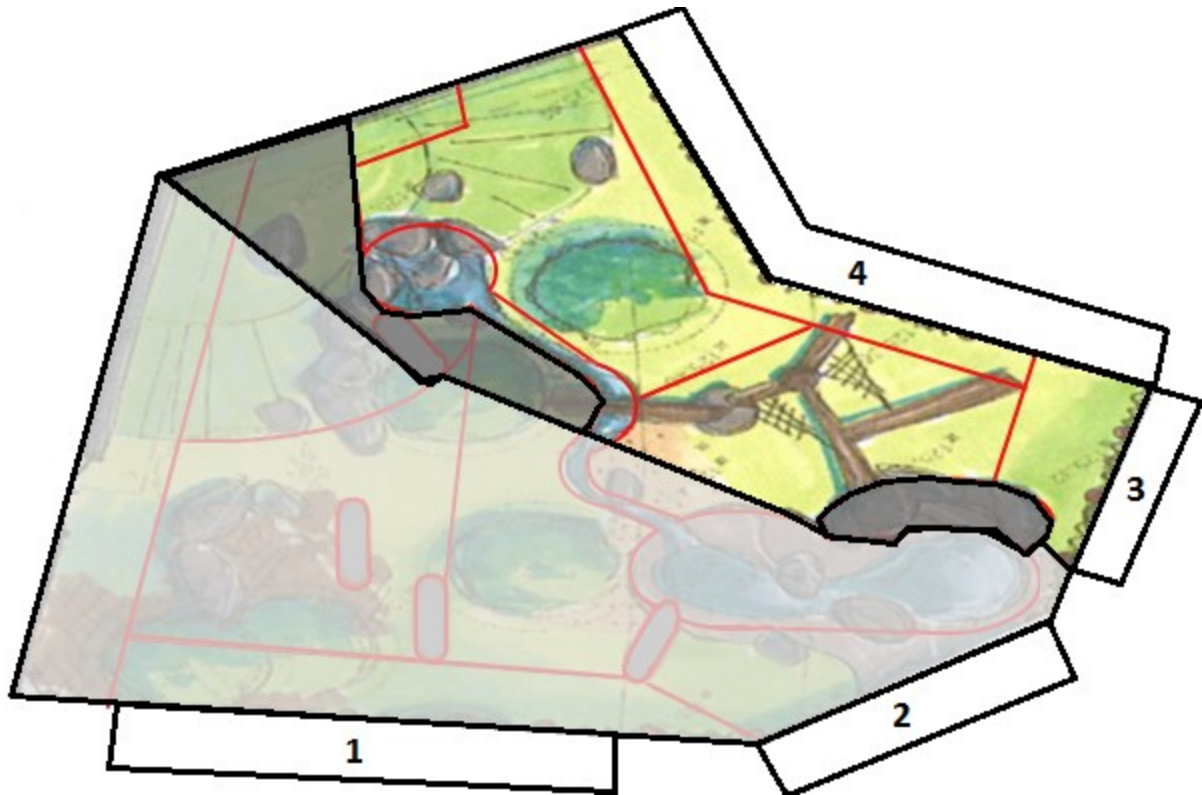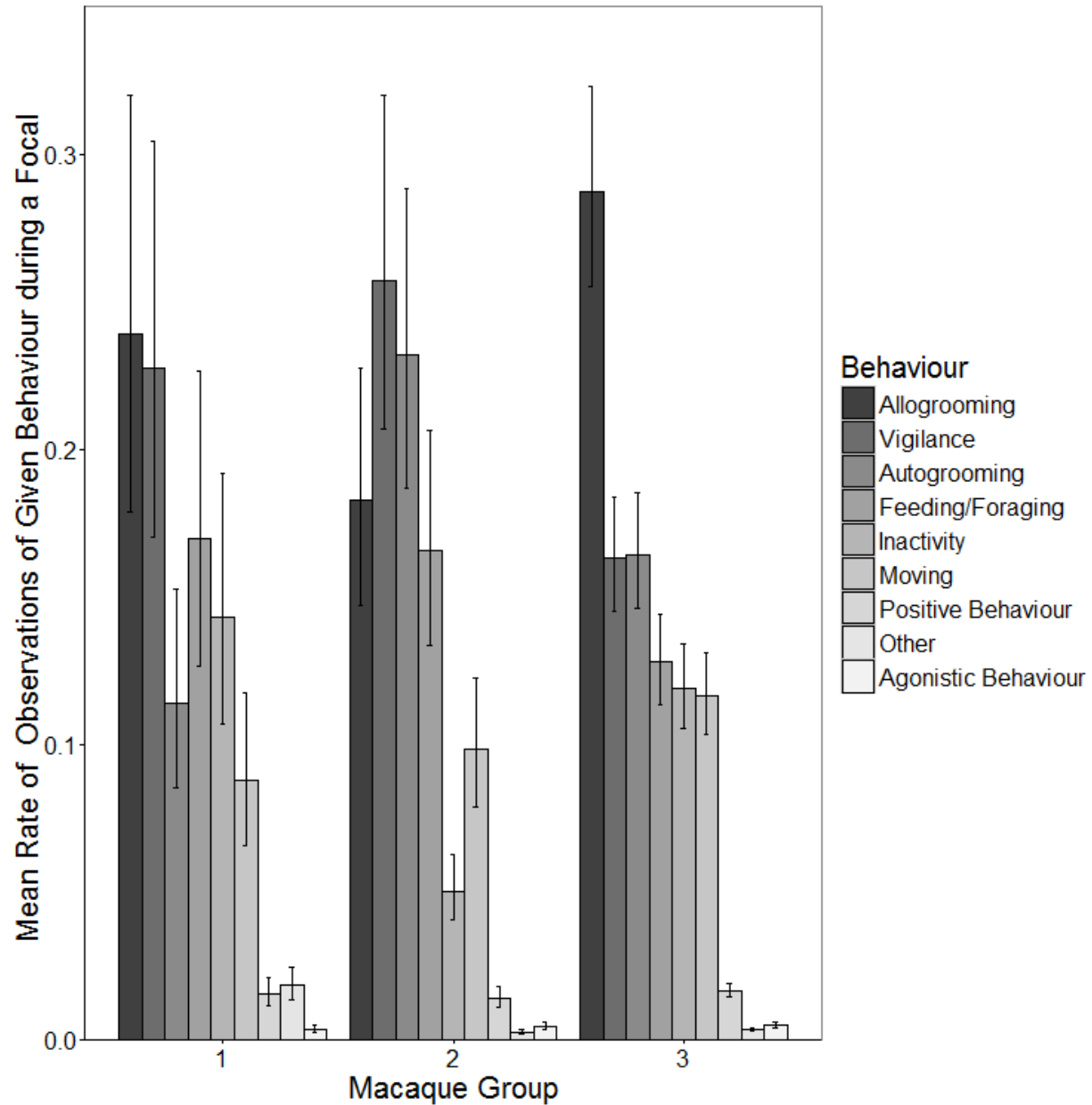| Category	Associated Rank	Number of visitors
Small	1	0 to 9 (excludes observer)
Medium	2	10 to 19
Large	3	20 +

Figure 1.1. A top-view, schematic image of the 2014 Japanese macaque enclosure showing how the horizontal space was divided. Section A is at the back of the enclosure, behind the rock feature, making it the most hidden from visitors. Section B is the rock feature. Sections C, D and F are the outer right and outer middle, which are all in full sight of visitors. Section E is the water feature and surrounding area, easily distinguished by its lack of vegetation. Section H and section J are the front outer-left and back outer-left respectively, both of which are slightly more protected areas as there ferns covering much of the fencing on that side. Section G and Section I are the inner middle and the inner left, respectively and section K is all the dens and houses combined.
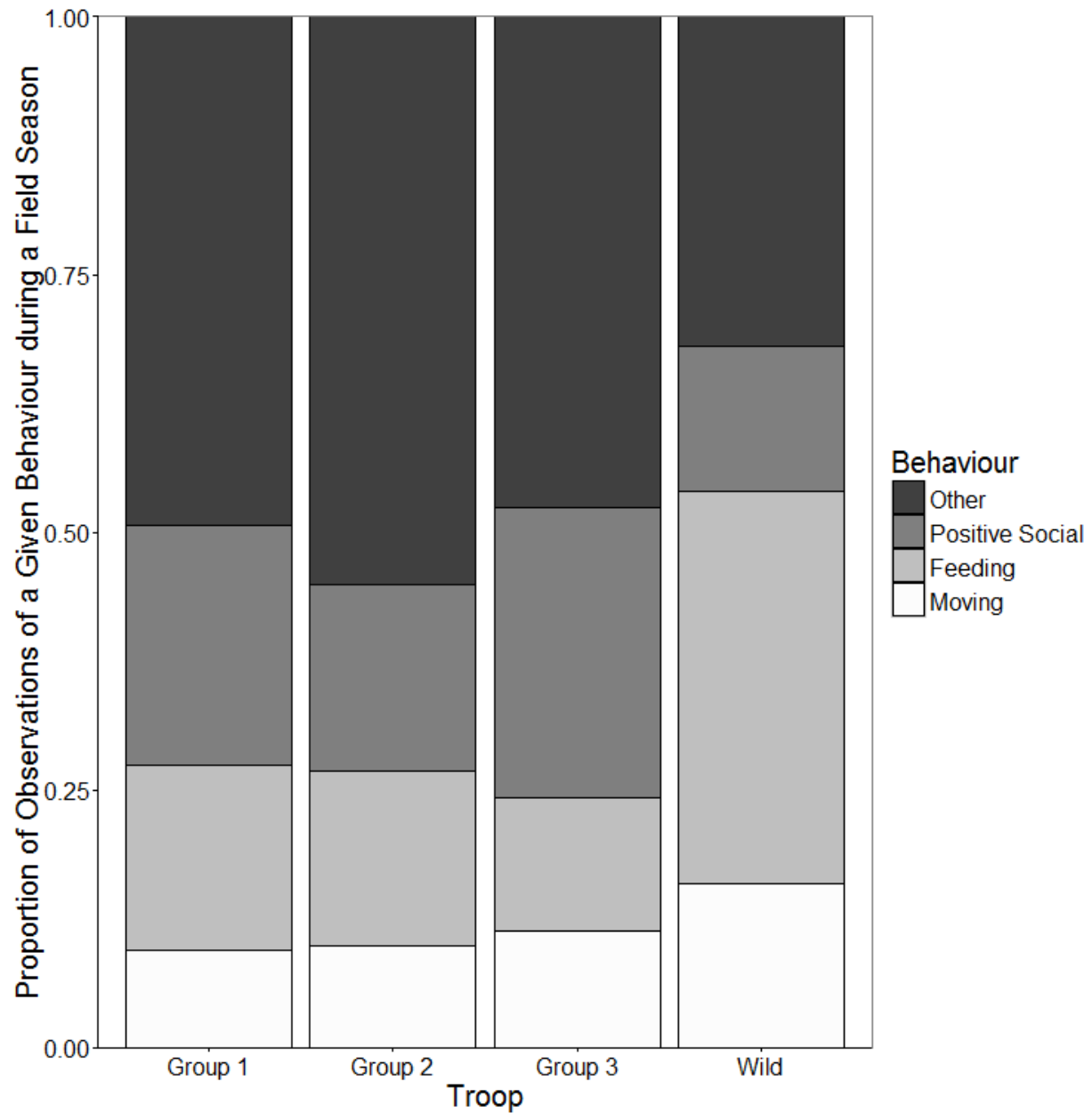
Figure 1.2. A top-view, schematic image of the 2015 Japanese macaque enclosure showing how the horizontal space was divided. Section A is at the back of the enclosure, one of the furthest from visitors. Section B and K are the left and right sides of the hill, respectively. Sections C and D are the inner left and E is the outer left. Section F is the area in front and a small area at the back, which are in front of viewing windows and in full sight of visitors. Section G is the water feature and surrounding 0.25m of rock. Section H and section J are the outer right. Section I is the wood structure and the area underneath and section L is all the dens, houses and the large stone cavern feature combined.
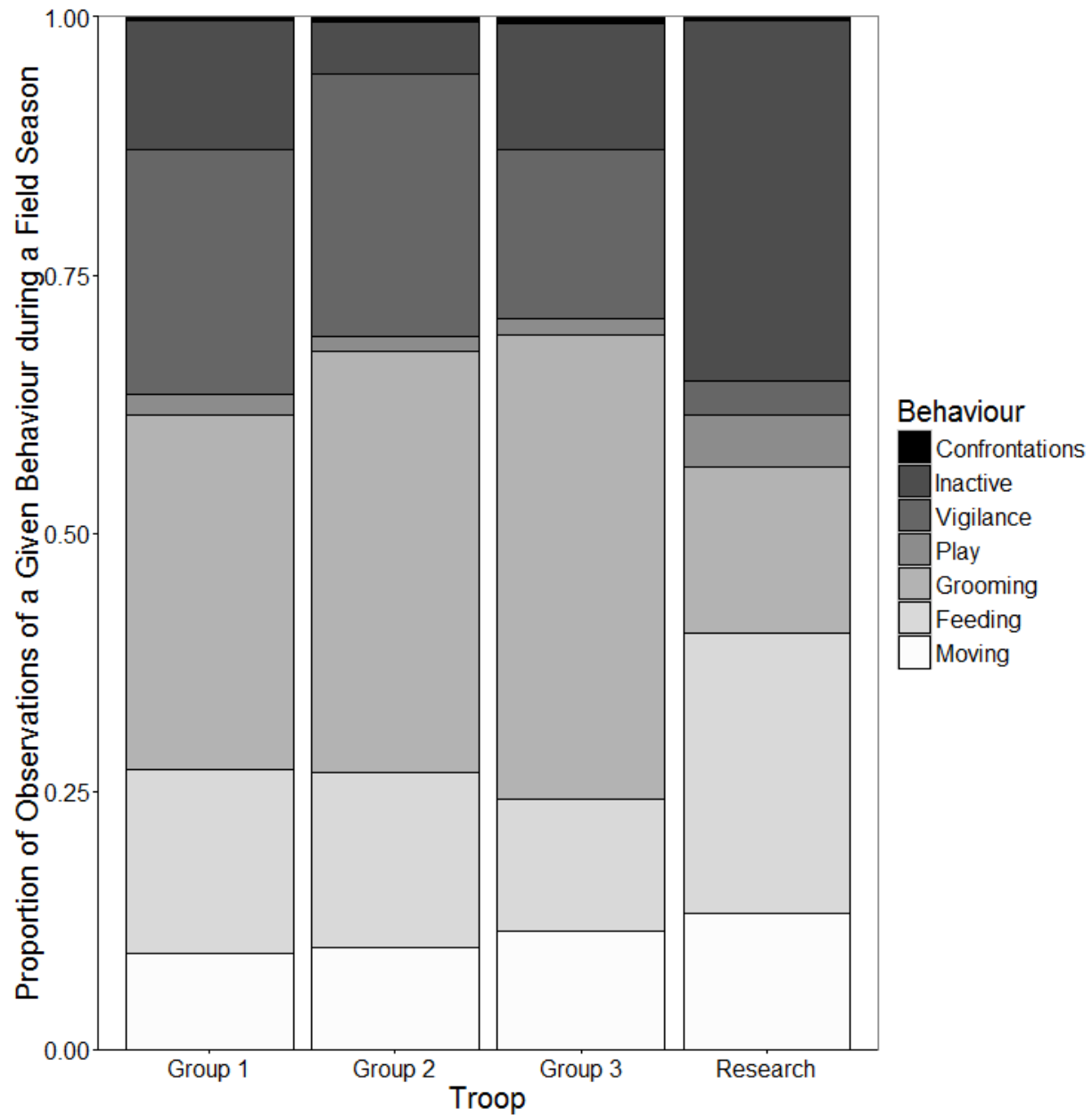
Figure 1.3. Map indicating how crowd size was recorded in the 2015 enclosure. The boxes labeled 1, 2, 3 and 4 indicate the four major visitor viewing area. If a macaque was positioned in the light grey shaded areas (bottom/left) during their observation period, visitors in area 1 and 2 would be counted. If an individual was positioned in the non-shaded area (top/right), visitors in area 3 and 4 would be counted. When observed in the dark shaded area (middle), visitors would be counted in either viewing areas 1 and 2 or in viewing areas 4 and 3, depending on the direction in which the animal was facing.

Figure 1.4. Mean activity budget (mean rate of observations with 95% confidence intervals) of the three Japanese macaque groups at the Zoo de Granby. Both group one and group two consisted of seven adult individuals observed during the 2014 field season. These two groups alternated being on display in the old enclosure on a weekly basis. Group three consisted of ten adult individuals (five from each of the two original groups) and was observed in the new enclosure during the 2015 field season. Means and confidence intervals for each group were generated from the least squares means of the model.

Figure 1.5. Overall activity budgets of the Japanese macaques groups at the Zoo de Granby during the 2014 (groups one and two) and 2015 (group three) summer field seasons compared to the activity budget of 24 wild individuals from the Western area of Yakushima (Hanya, 2004).

Figure 1.6. Overall activity budgets of the Japanese macaques groups at the Zoo de Granby during the 2014 (groups one and two) and 2015 (group three) summer field seasons compared to the activity budget of 32–41 research individuals housed in a large, vegetated outdoor enclosure at the Primate Research Institute (PRI), Kyoto University, Japan (Jaman and Huffman, 2008).
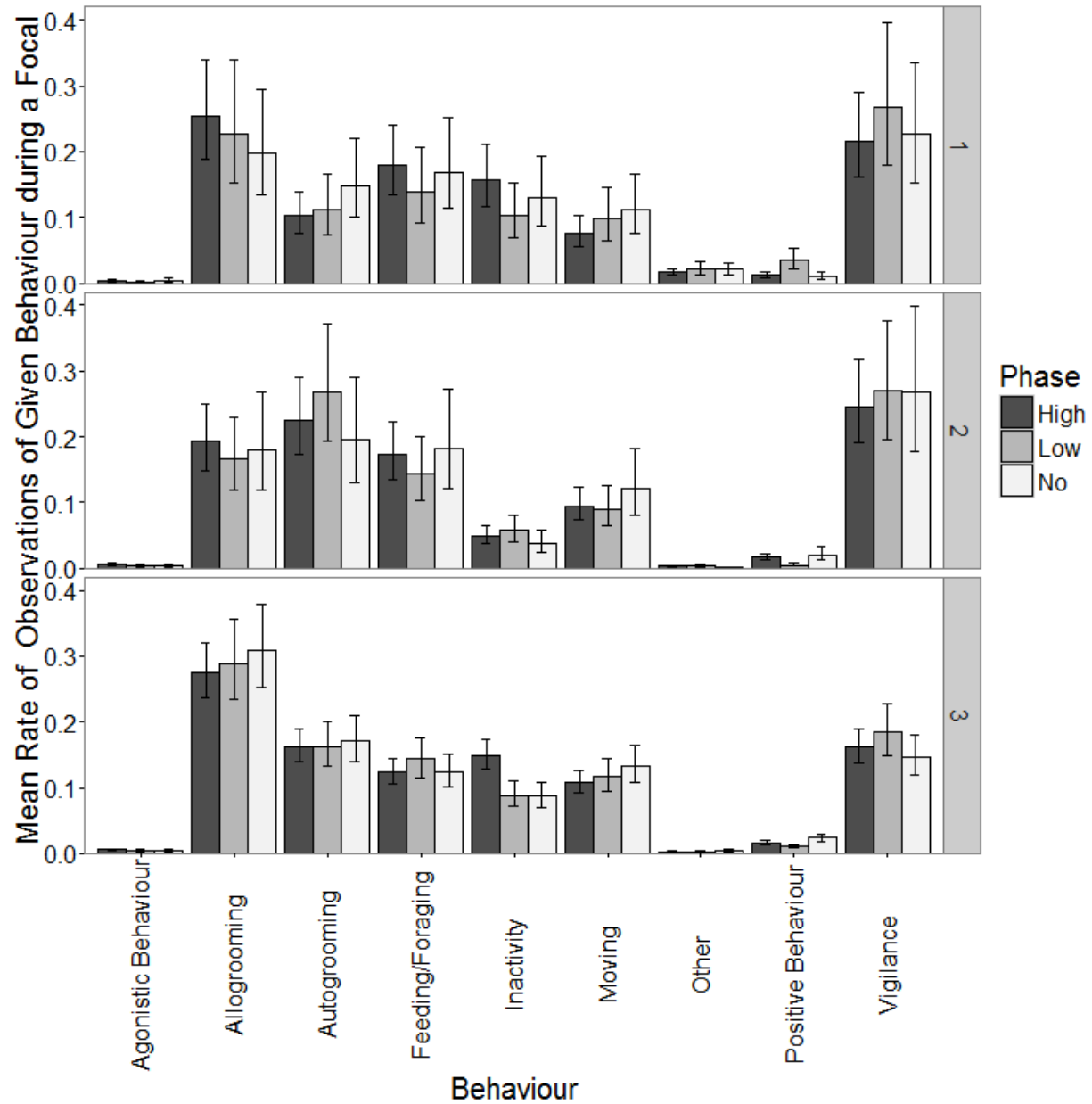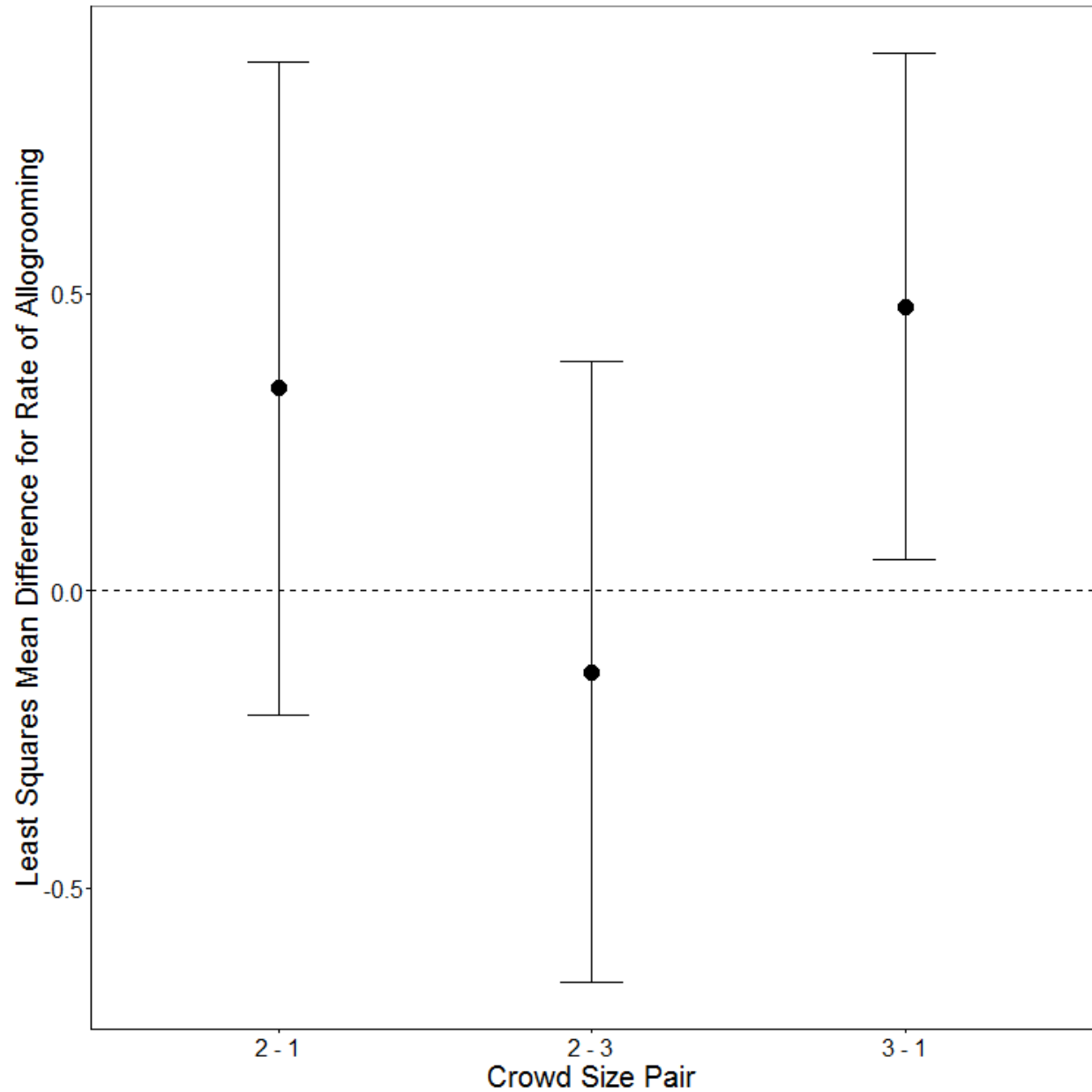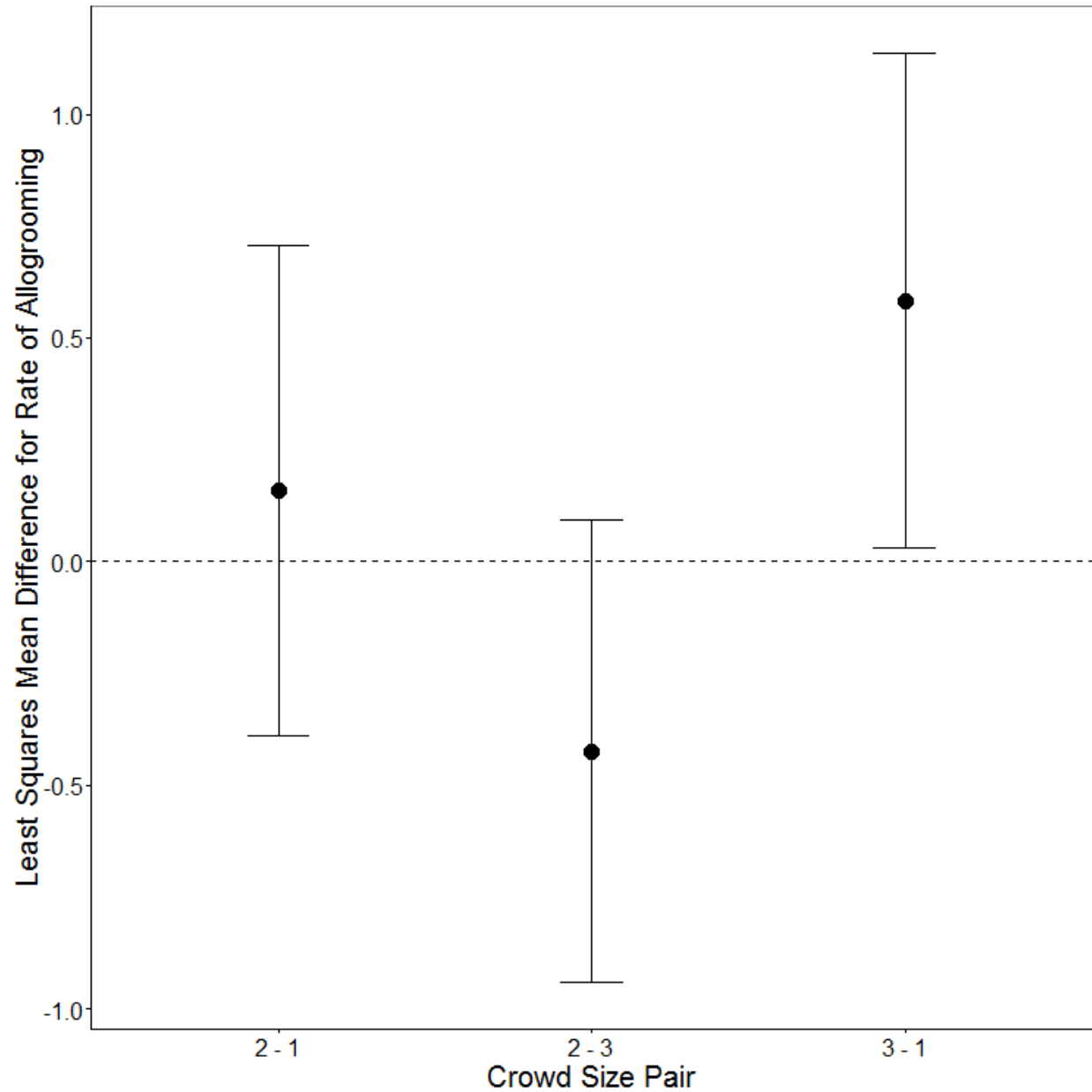
Figure 1.7. Mean rate of observations and 95% confidence intervals for each activity type during each phase for group one (top) and group two (middle) in the old enclosure, and group three (bottom) in the new enclosure. Means and confidence intervals were generated from the least squares means of the model.
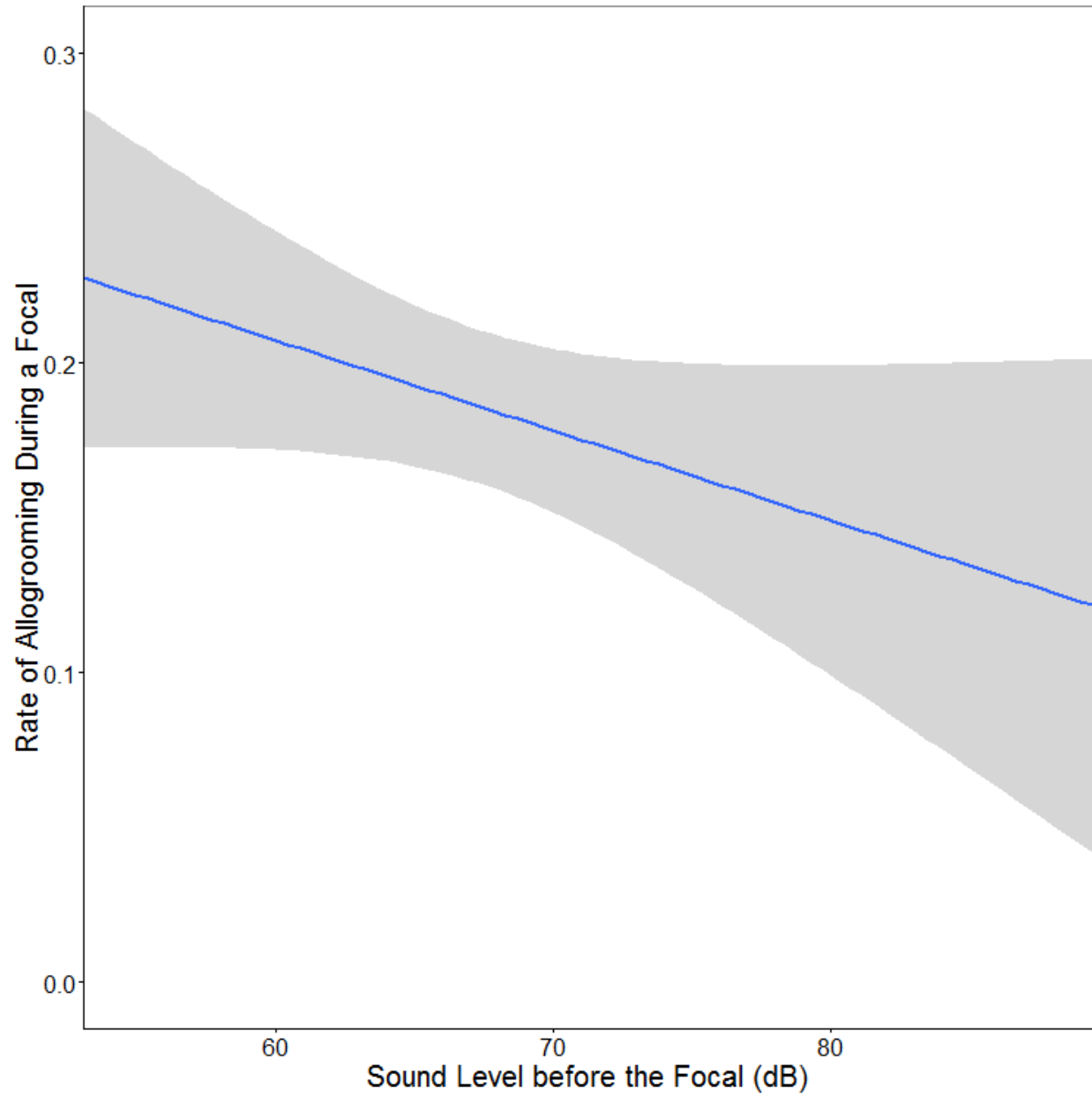
Figure 1.8. Pairwise differences between least squares means for rate of allogrooming in the three crowd sizes and their 95% confidence intervals in group one in the old enclosure (2014). Differences were calculated by subtracting the least squares means for the later crowd size indicated in the pair from the former.
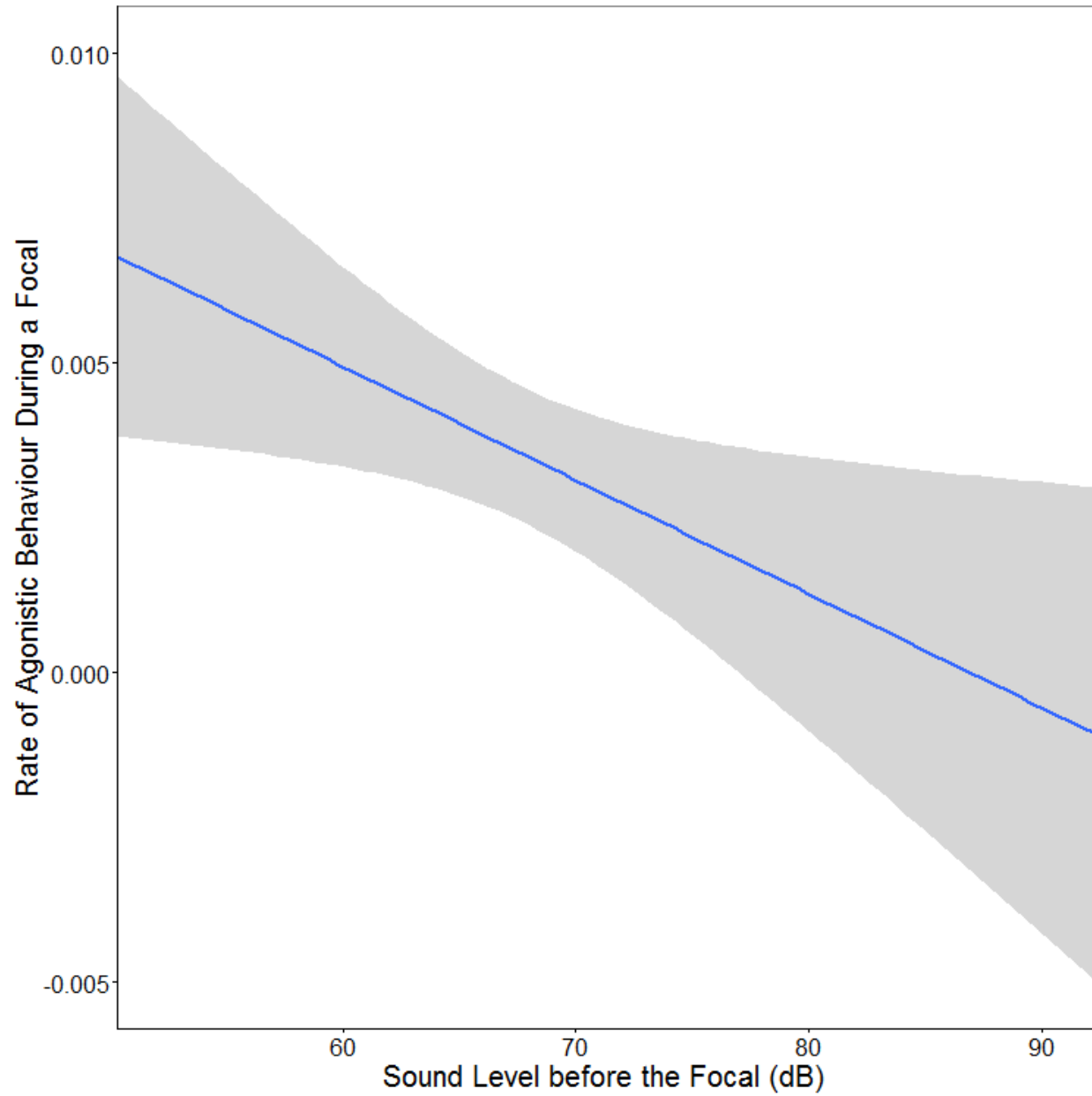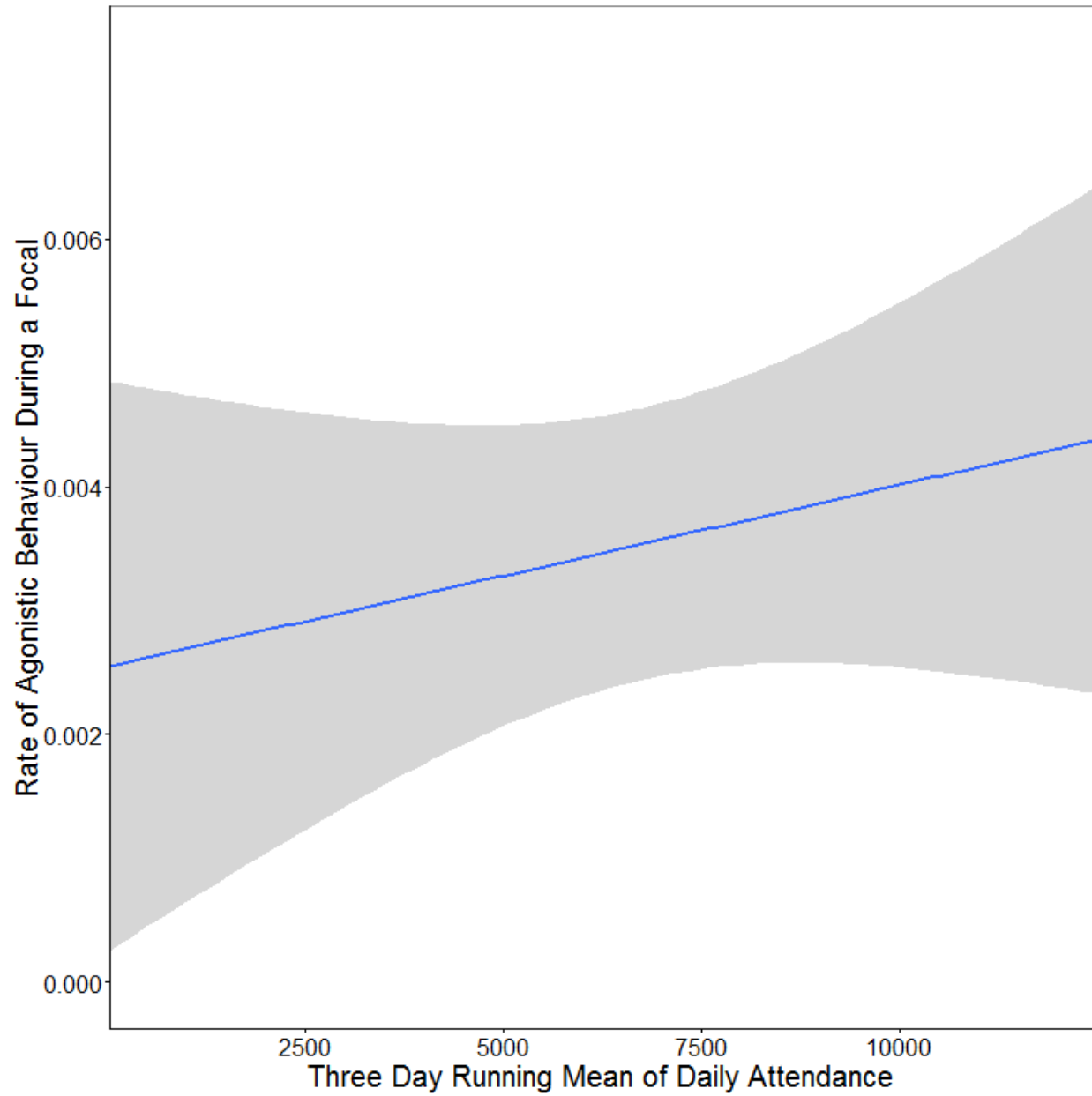
Figure 1.8. Pairwise differences between least squares means for rate of allogrooming in the three crowd sizes and their 95% confidence intervals in group two in the old enclosure (2014). Differences were calculated by subtracting the least squares means for the later crowd size indicated in the pair from the former.
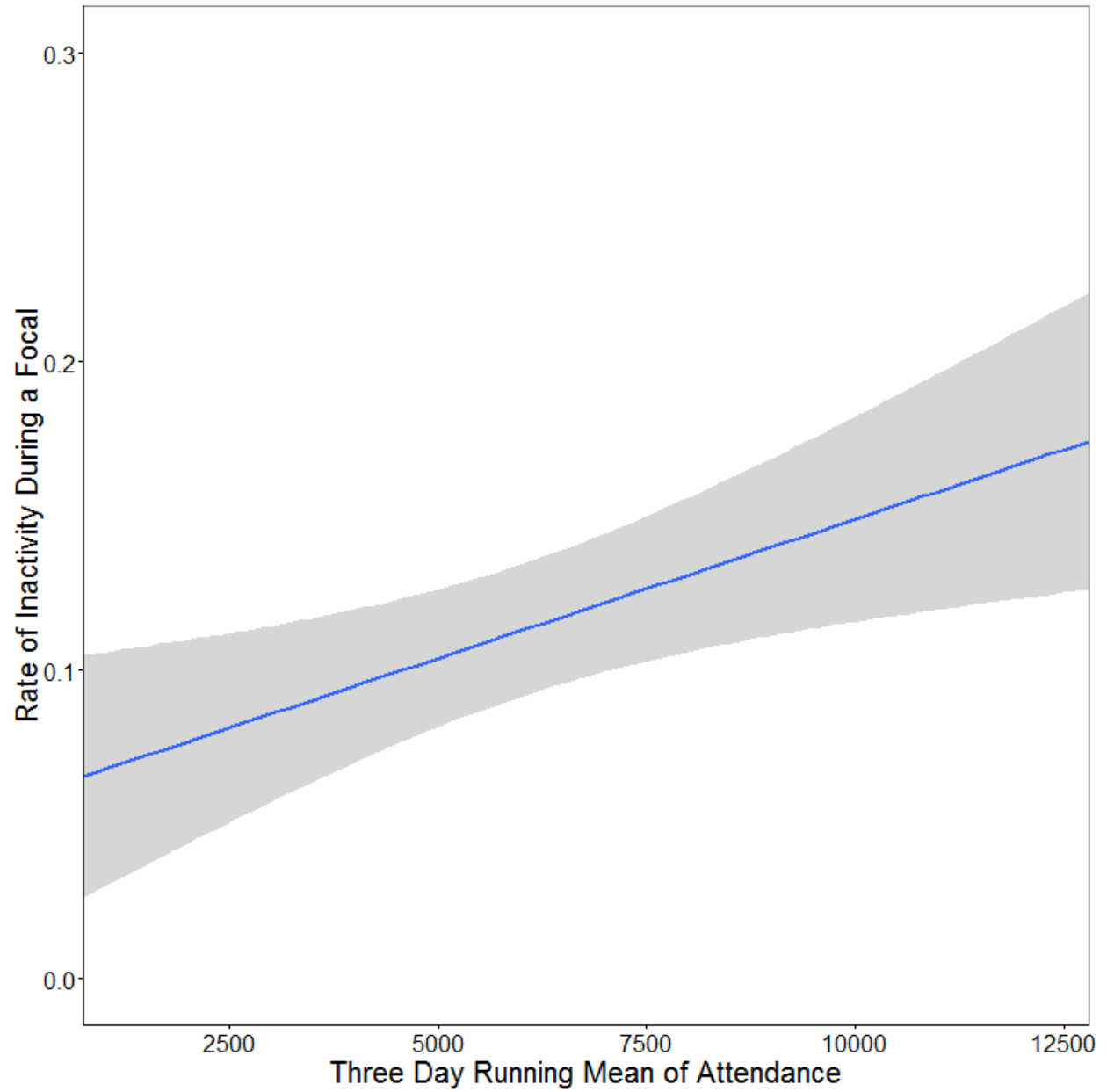
Figure 1.10. The bivariate trend (with standard error) between sound levels measured before a focal and the rate of allogrooming during a focal for group two in the old enclosure (2014).

Figure 1.11. The bivariate trend (with standard error) between sound levels measured before a focal and the rate of agonistic behaviour during a focal for group one in the old enclosure (2014).

Figure 1.12. The bivariate trend (with standard error) between the three day running mean of daily attendance and the rate of agonistic behaviour during a focal for group one in the old enclosure (2014).

Figure 1.13. The bivariate trend (with standard error) between the three day running mean of daily attendance and the rate of inactivity during a focal for group three in the new enclosure (2015).
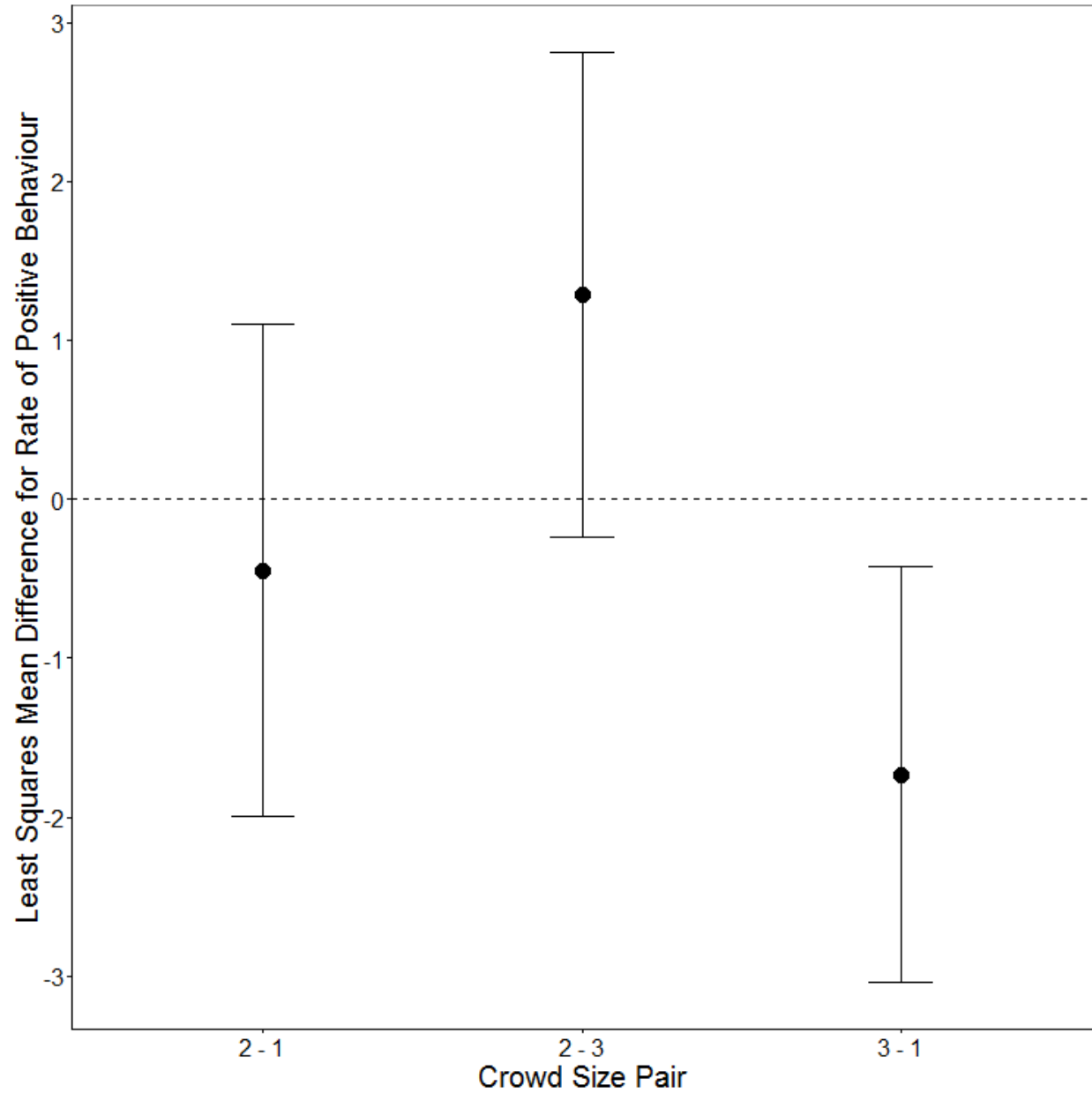
Figure 1.14. Pairwise differences between least squares means for rate of positive affect indicators in the three crowd sizes and their 95% confidence intervals in group one in the old enclosure (2014). Differences were calculated by subtracting the least squares means for the later crowd size indicated in the pair from the former.
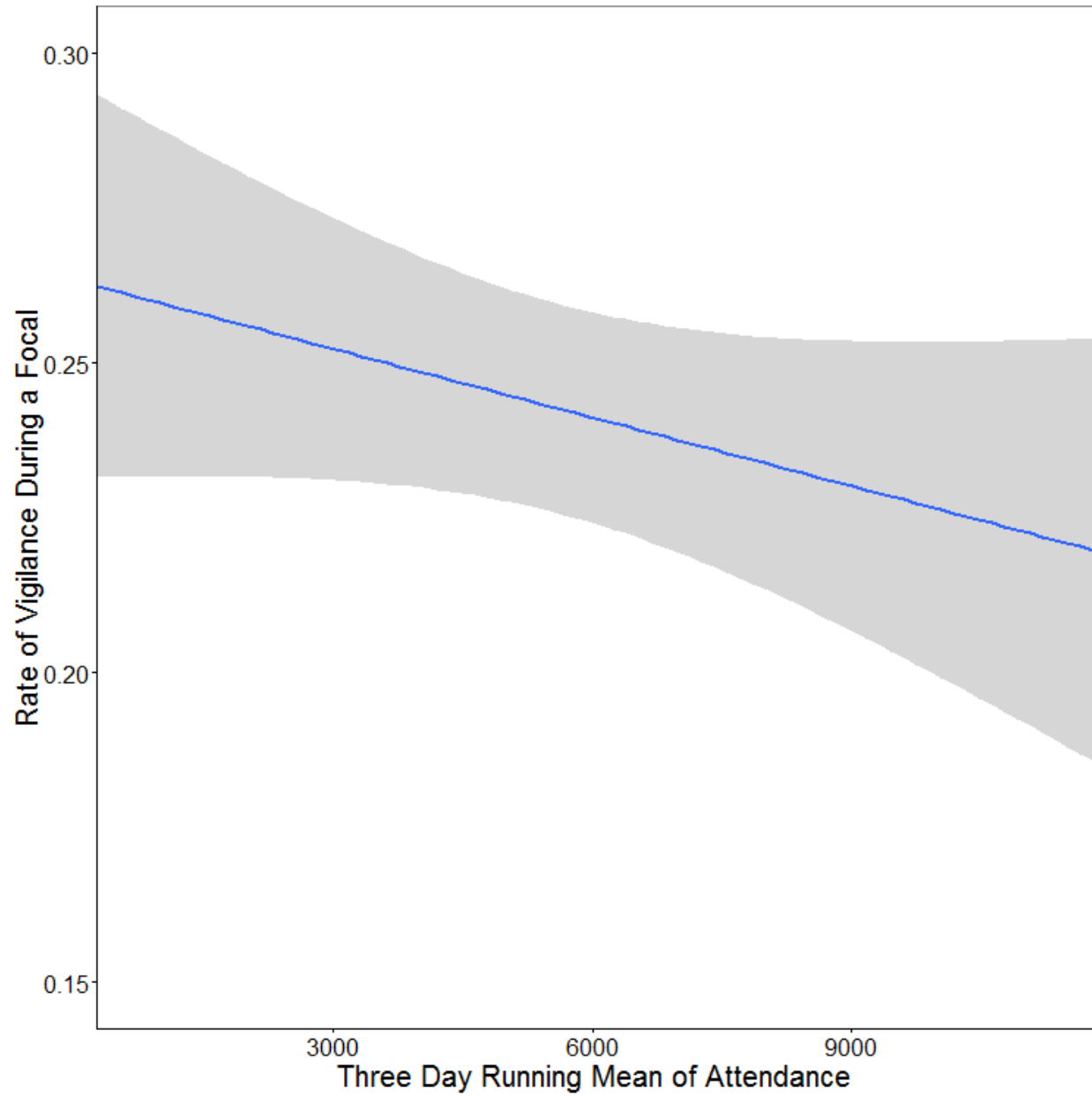
Figure 1.15. The bivariate trend (with standard error) between the three day running mean of daily attendance and the rate of vigilance during a focal for group two in the old enclosure (2014).
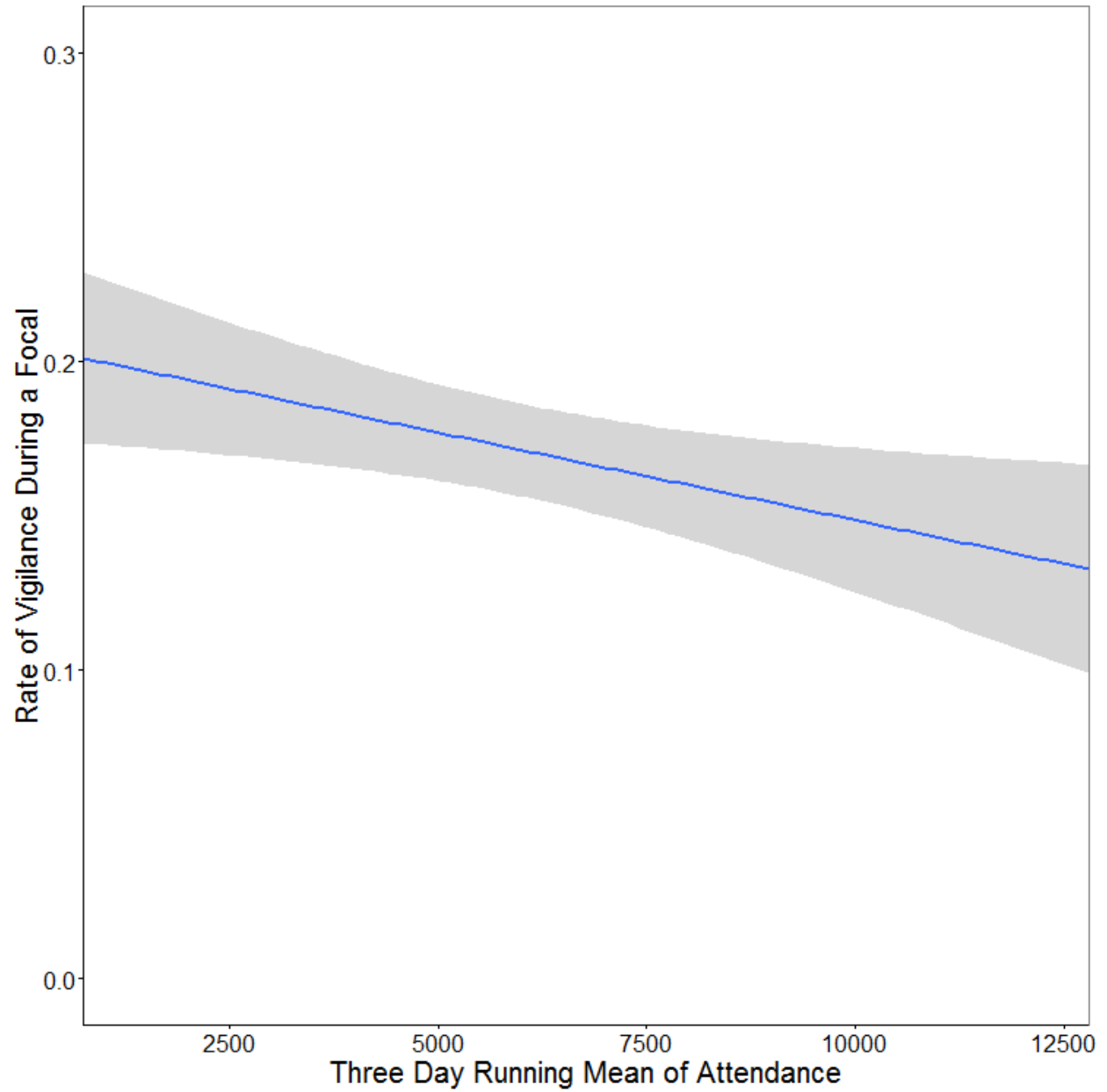
Figure 1.16. The bivariate trend (with standard error) between the three day running mean of daily attendance and the rate of vigilance during a focal for group three in the new enclosure (2015).
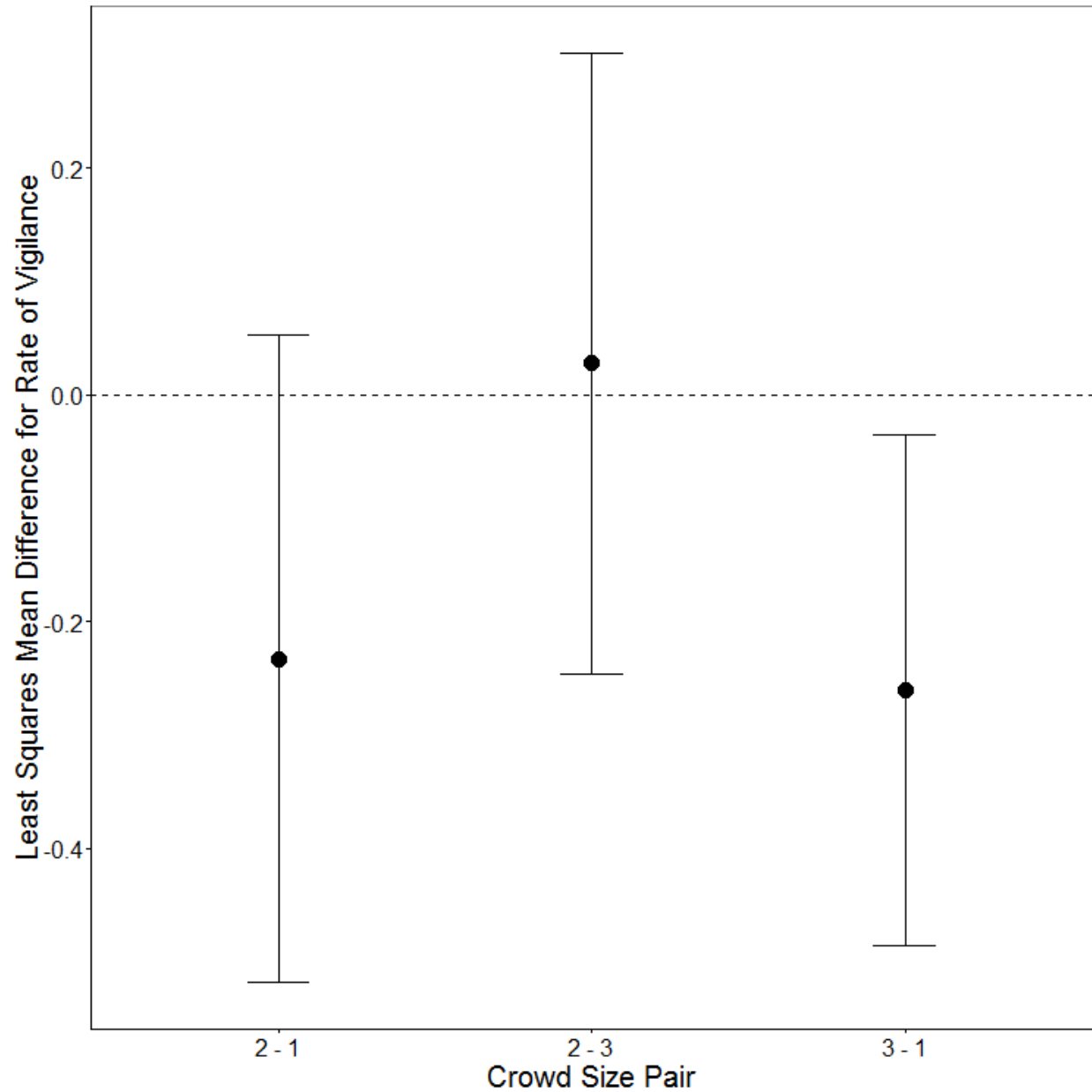
Figure 1.17. Pairwise differences between least squares means for rate of vigilance in the three crowd sizes and their 95% confidence intervals in group one in the old enclosure in the old enclosure (2014). Differences were calculated by subtracting the least squares means for the later crowd size indicated in the pair from the former.
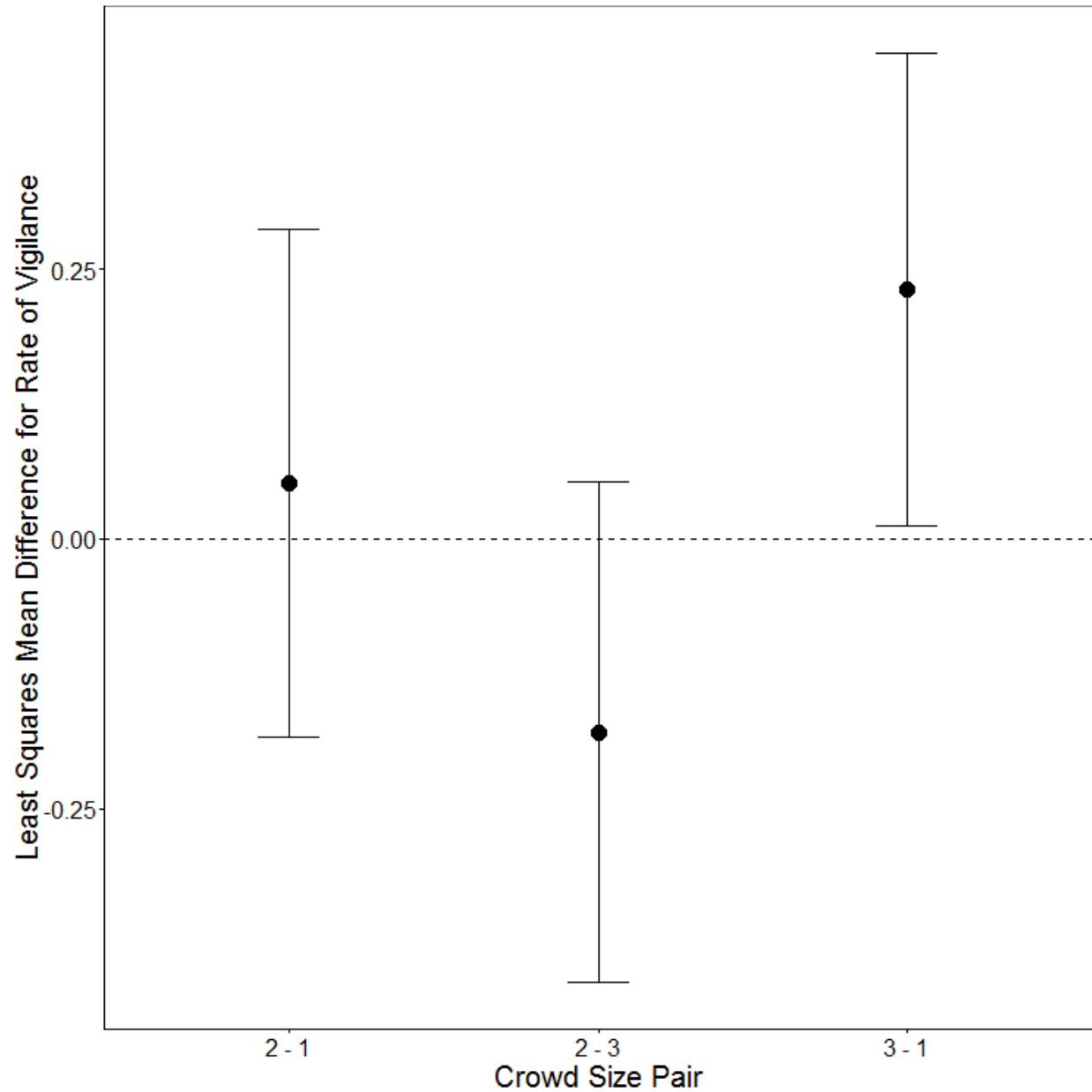
Figure 1.18. Pairwise differences between least squares means for rate of vigilance in the three crowd sizes and their 95% confidence intervals in group two in the old enclosure in the old enclosure (2014). Differences were calculated by subtracting the least squares means for the later crowd size indicated in the pair from the former
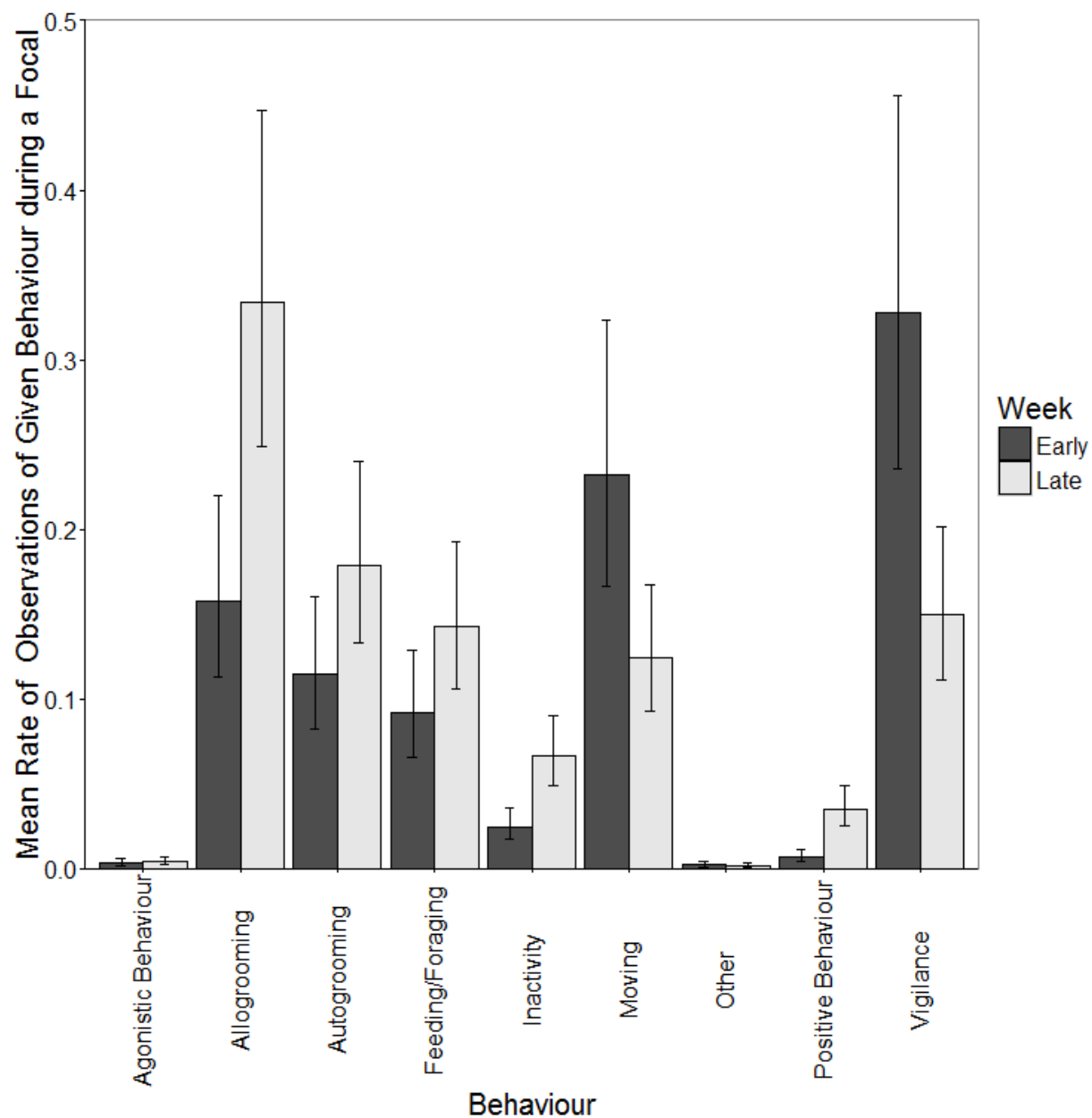
Figure 1.19. Mean rate of observations and 95% confidence intervals for each behaviour type during the early and late week in the new enclosure (2015). Means and confidence intervals were generated from the least squares means of the model.

Figure 1.20. Mean rate of observations and 95% confidence intervals for each behaviour type for each group during late August to October 2014 in the old enclosure (groups one and two) and during late August to September 2015 in the new enclosure (group three). Means and confidence intervals were generated from the least squares means of the model.

Figure 1.21. Mean proportion of observations of individuals in each of the four levels by each group, along with their 95% confidence intervals. L1 represents ground level to 1.5m off the group, L2 represents 1.5m to 3m off the ground, L3 represents 3m to 4.5m off the ground and L4 represents 4.5m off the ground to the top of the enclosure. Proportions were calculated by dividing the number of observation in a given level during a time period (AM/PM1/PM2) by the total number of observations in all levels during that time period. Means and confidence intervals were generated from the least squares means of the model.

Figure 1.22. Choropleth map of 2014 enclosure representing the mean proportion of observations of individuals in each of the horizontal areas by group one (a) and group two (b). Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period. Means were generated from the least squares means of the model.

Figure 1.23. Choropleth map of 2015 enclosure representing the mean proportion of observations of individuals in each of the horizontal areas by group three. Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period. Means were generated from the least squares means of the model.

Figure 1.24. Mean proportion of observations and 95% confidence intervals for each vertical level during each phase for group one (left), group two (middle) and group three (right). L1 represents ground level to 1.5m off the group, L2 represents 1.5m to 3m off the ground, L3 represents 3m to 4.5m off the ground and L4 represents 4.5m off the ground to the top of the enclosure. Proportions were calculated by dividing the number of observation in a given level during a time period (AM/PM1/PM2) by the total number of observations in all levels during that time period. Means and confidence intervals were generated from the least squares means of the model.

Figure 1.25. Choropleth map of the 2014 enclosure representing the mean proportion of observations of individuals in each horizontal area during the no visitor phase (a), the low visitor phase (b) and the high visitor phase (c) for group one. Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period Means were generated from the least squares means of the model.

Figure 1.26. Choropleth map of the 2014 enclosure representing the mean proportion of observations of individuals in each horizontal area during the no visitor phase (a), the low visitor phase (b) and the high visitor phase (c) for group two. Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period. Means were generated from the least squares means of the model.

Figure 1.27. Choropleth map of the 2015 enclosure representing the mean proportion of observations of individuals in each horizontal area during the no visitor phase (a), the low visitor phase (b) and the high visitor phase (c) for group three. Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period Means were generated from the least squares means of the model.
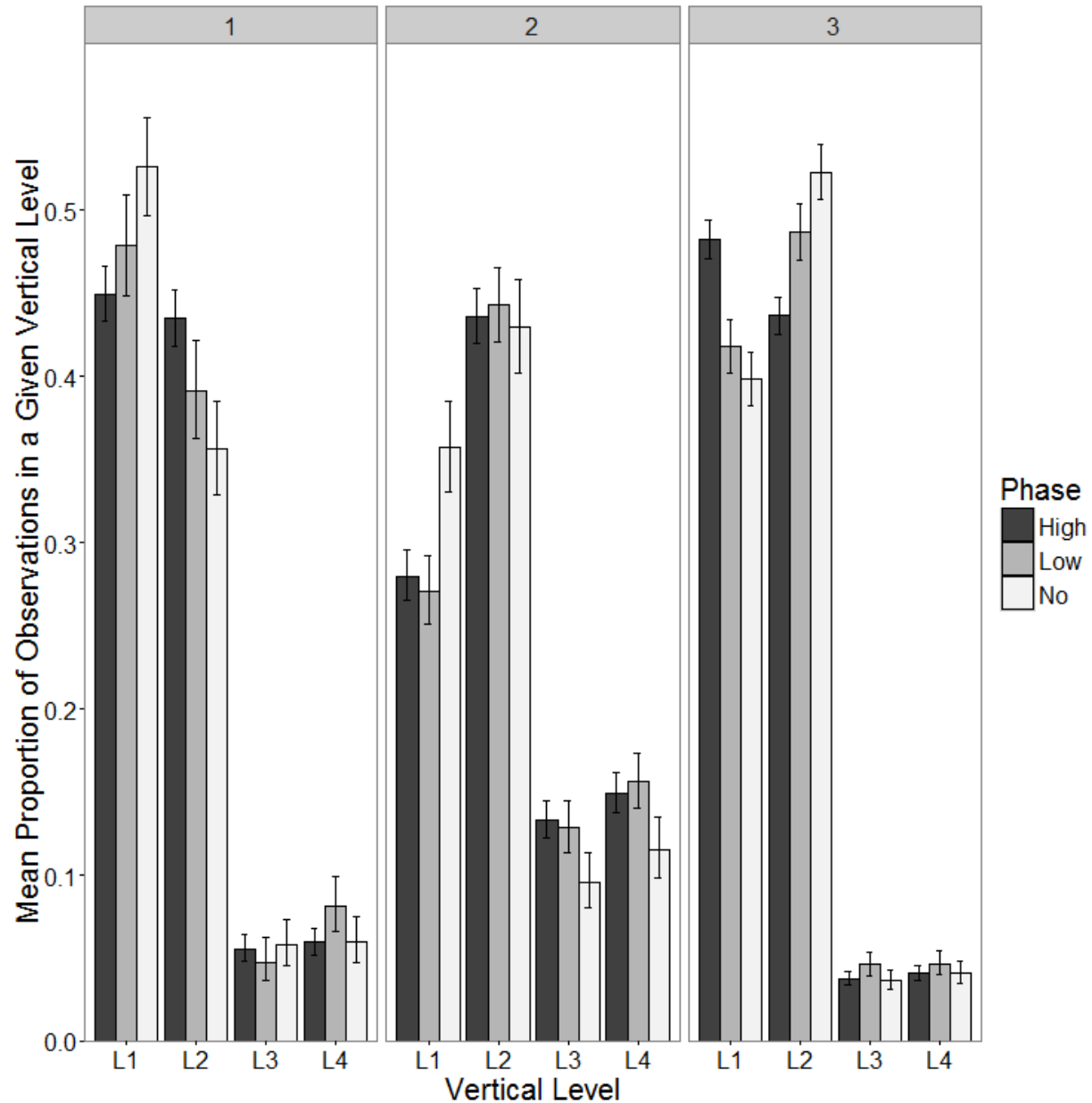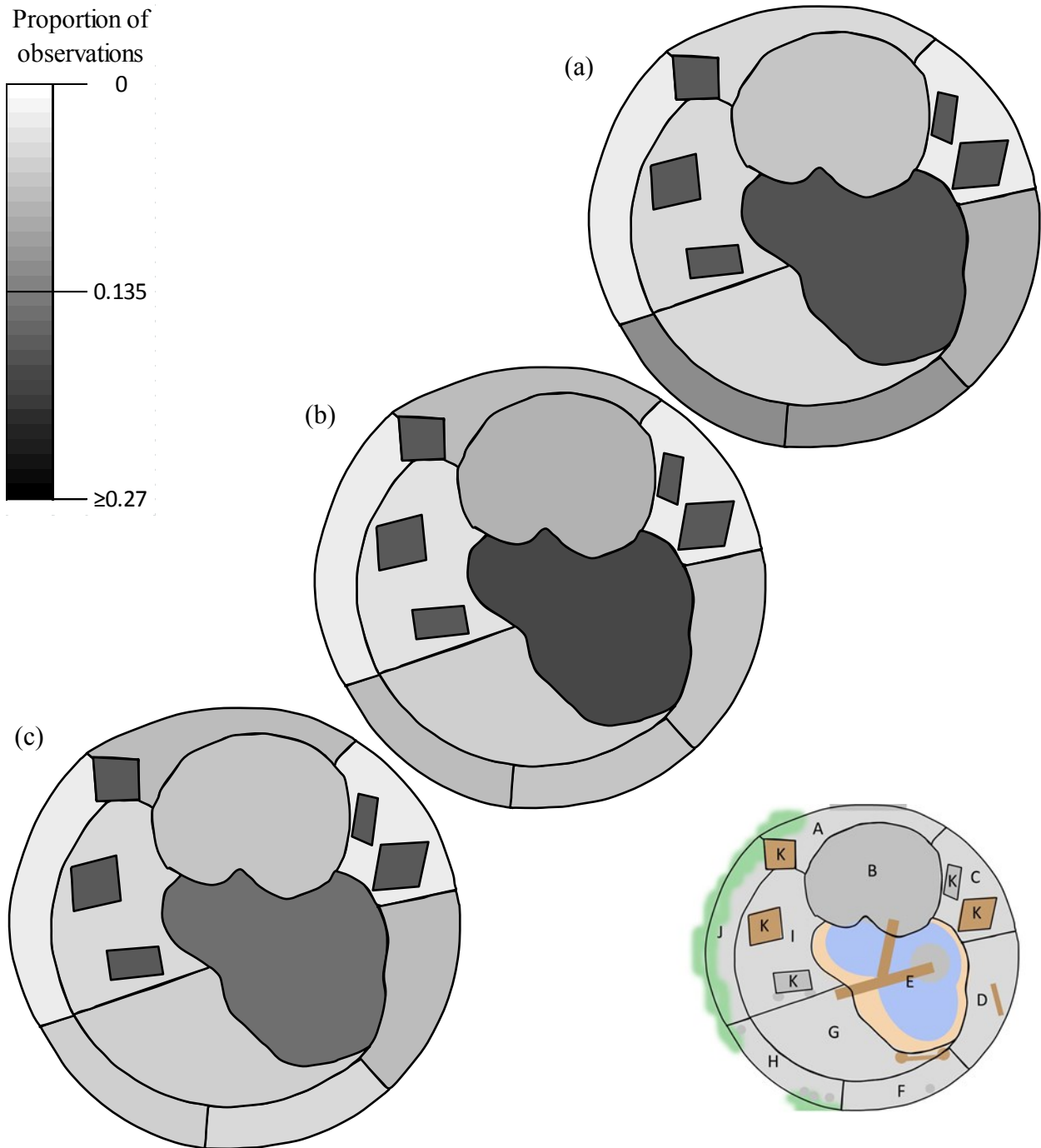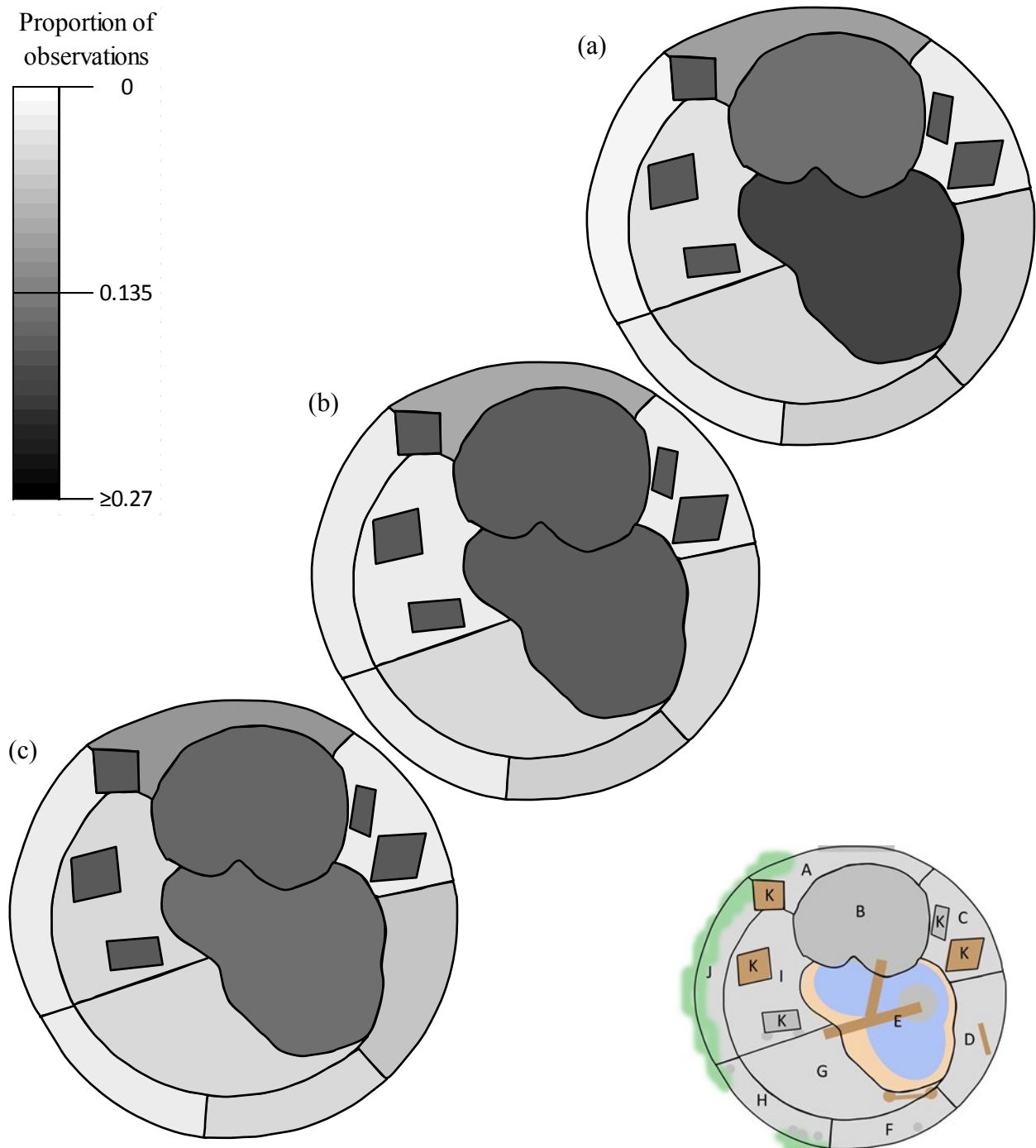
Figure 1.28. Mean proportion of observation and 95% confidence intervals for each vertical level during the early and late week of 2015. L1 represents ground level to 1.5m off the group, L2 represents 1.5m to 3m off the ground, L3 represents 3m to 4.5m off the ground and L4 represents 4.5m off the ground to the top of the enclosure. Proportions were calculated by dividing the number of observation in a given level during a time period (AM/PM1/PM2) by the total number of observations in all levels during that time period. Means and confidence intervals were generated from the least squares means of the model.
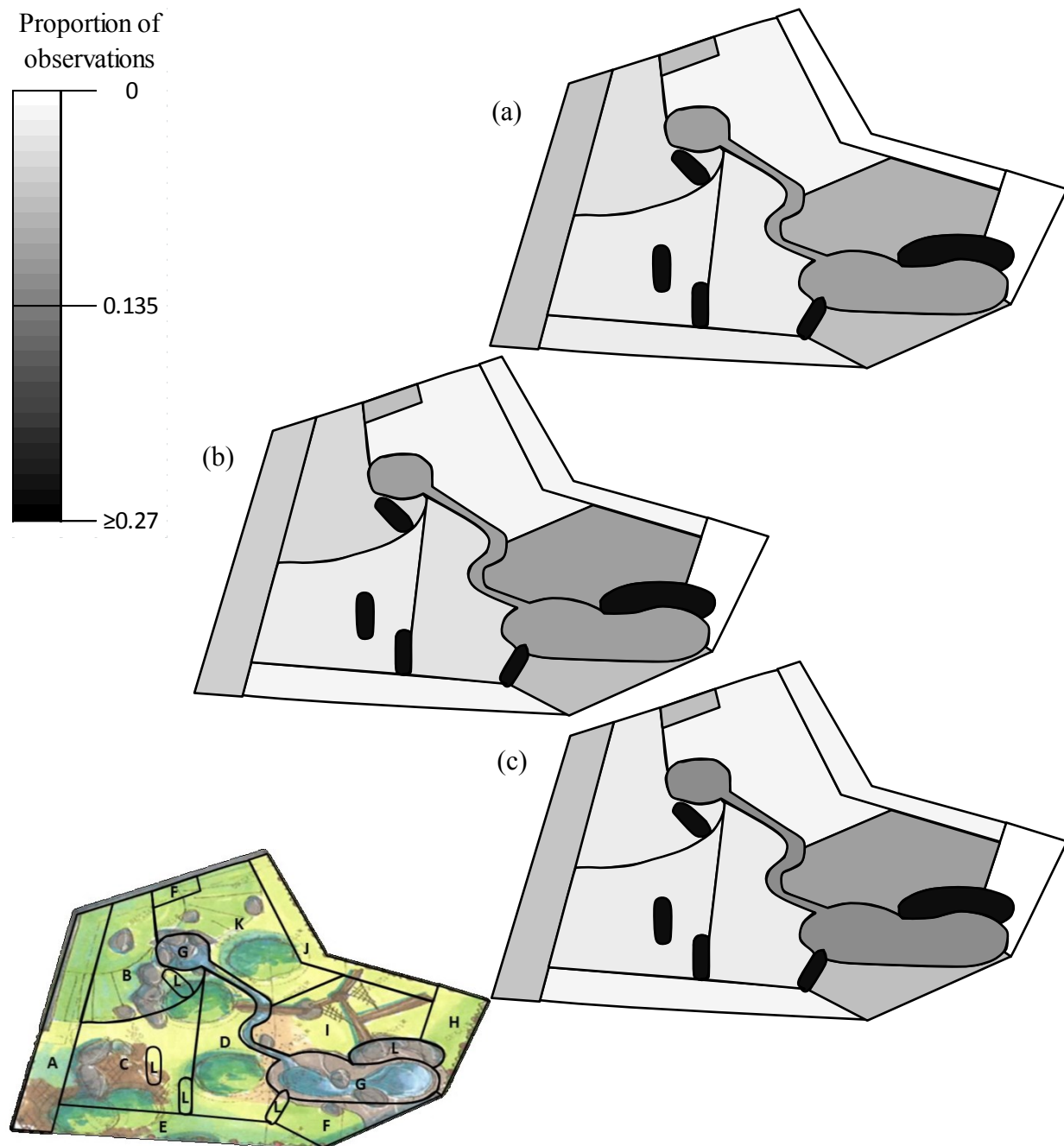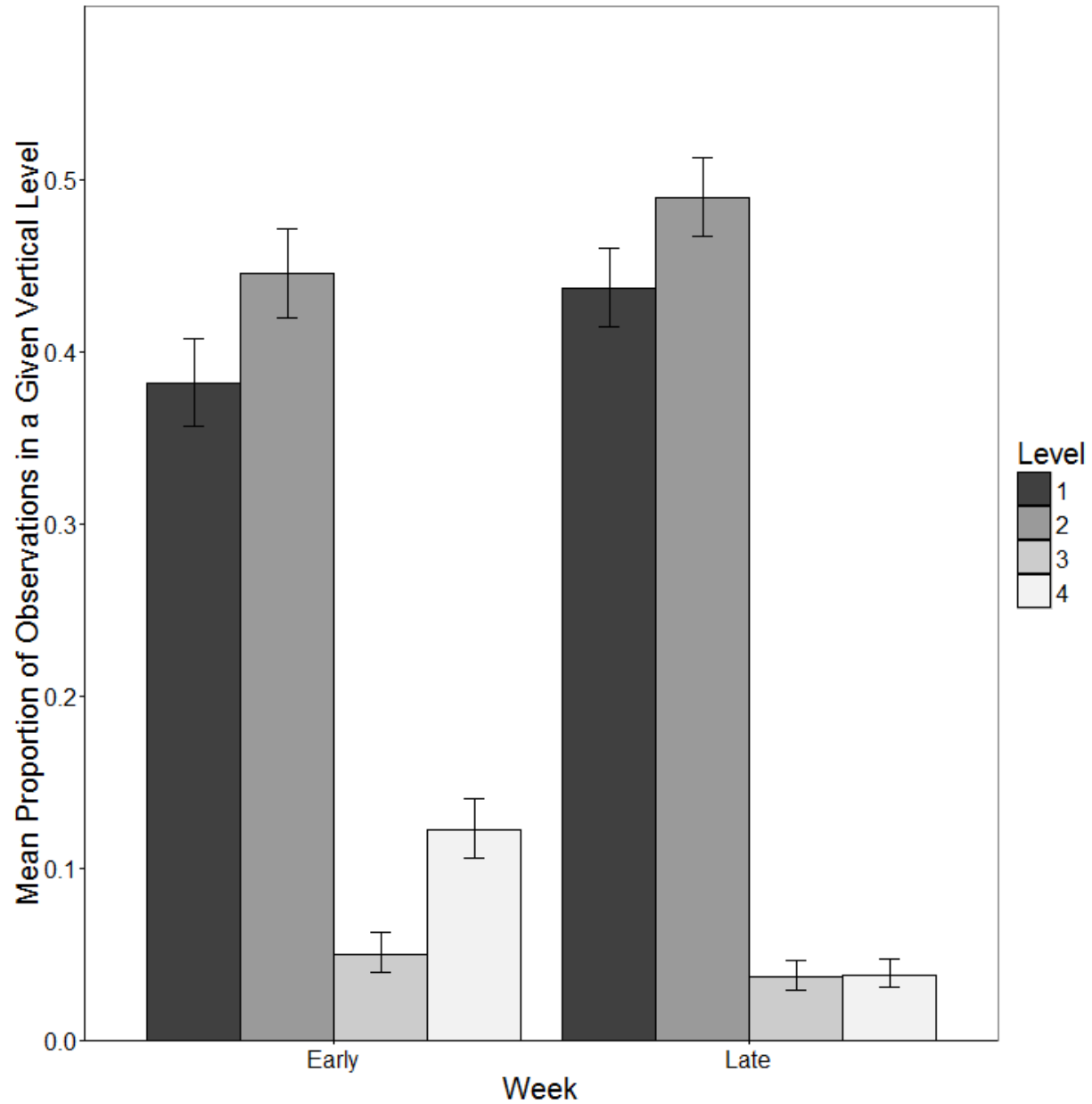
Figure 1.29. Choropleth map of the 2015 enclosure representing the mean proportion of observations of individuals in each horizontal area during the early week (a) and the late week (b). Proportions were calculated by dividing the number of observation in a given area during a time period (AM/PM1/PM2) by the total number of observations in all areas during that time period. Means were generated from the least squares means of the model.

Chapter 2:

Changes in the dominance hierarchy of captive female Japanese macaques as a consequence of merging two previously-established groups

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Abstract

Dominance hierarchies play an important role in reducing competition and aggression in social animals. In zoos, changes in group composition are often required due to management protocols, but these changes may have long lasting effects on dominance hierarchies, and, consequently, the wellbeing of the animals. We studied the changes in the female dominance hierarchy that occurred both during and after the formation of a group of ten adult Japanese macaques at the Zoo de Granby by combining members from two previously-established groups. There was no significant correlation between ranks before and after group formation, indicating a significant change in the hierarchy. Ranks in the newly formed groups did correlate with age of individual, while the mean rank of individuals added later in the group formation process was not different from those added earlier. Alliances between kin appeared to be important in determining rank; when the sister of the dominant female was removed, the hierarchy changed significantly once more. Zoo management must be aware of the consequences small changes in a social group can have when removing and transferring individuals in both primates and in other social species.

Introduction

      Dominance hierarchies exist in a wide diversity of social animals. When we refer to a dominance hierarchy, we often think back to the classic pecking-order, or the modified pecking-order, where the dominant individuals are determined through asymmetrical outcomes in agonistic interactions (Drews, 1993). Based on this definition, we assume that these animals are able to recognize each other and learn from previous experiences with another individual (Rowell, 1974). In this way, the establishment of a dominance hierarchy has a variety of functions such as providing leadership for a group and reducing aggression and injury by predicting outcomes of agonistic interactions (Rowell, 1974). This is why dominance hierarchies are common in gregarious animals, where there is increased competition for resources such as mates or food that may result in excessive conflict (Sterck et al., 1997).

      In wild Japanese macaques (*Macaca fuscata*), as in many other old-world monkeys, there exists a matrilineal dominance system. These systems are governed by two main "rules", proposed by Kawamura (1958): (1) adult females rank below and next to their mothers so that kin occupy adjacent ranks in the hierarchy, and (2) adult females rank above their older sisters. Though these rules may not always hold true, kin quite consistently occupy adjacent ranks in matrilineal primate species (Chapais, 1991). This is more due to the support individuals receive from their mothers and other kin, and not solely due to the innate privilege of being born to a high ranking mother (Chapais, 1991). In experimental trials, an individual consistently became dominant to individuals of the same age class if her mother or older sister was present regardless of her rank beforehand, and would subsequently fall in rank if her relative was removed and the relative of another, unrelated individual was added (Chapais, 1991). However, kin alliances are not the only factor that plays a role in the determination of rank within a group of macaques. Prior residency has been found to be an important predictor in both primates and other species, such as birds, with individuals who are added later in the group formation process achieving lower ranks than those added earlier (Bernstein and Gordon, 1980; Cristol et al., 1990; Rowell, 1974; Westergaard et al., 1999). Other factors, such as age, body size, competitive ability and the presence/absence of an alpha female have also been found to be important in determining ones place in a hierarchy (Lea et al., 2014; Sebastian, 2015)

In zoos, it is not uncommon for animals to be placed with unfamiliar, unrelated individuals, or for group composition to change dramatically in a relatively short time span. This may arise from a lack of space at the home institution or transfers may be made for the benefit of a regional or international population management program. These changes in group composition can disrupt alliances in social primates and have long lasting effects on their dominance hierarchies. There have been a number of studies on this subject in the wild and in research populations (Bernstein and Gordon, 1980; Honess et al., 2004; Westergaard et al., 1999), but the overwhelming majority of studies on group formation in zoo primates' focus on the formation of bachelor groups (i.e. all-male groups) or the introduction of completely unfamiliar animals (McDonald, 1994; Sha et al., 2013). In this study, we set out to quantify the changes in the female hierarchy that occurred when a group of ten adult Japanese macaques was established at Zoo de Granby by combining members from two previous groups as the individuals were being transferred to a new habitat.

Past research suggests that the manipulation of primate group composition in an experimental context generally results in changes in the hierarchy or the ranks of specific individuals (Bernstein and Gordon, 1980; Chapais, 1991; Honess et al., 2004; Westergaard et al., 1999). Studying ranks before and after group formation in a zoo context is a unique opportunity and allowed us to test the hypothesis that individuals' ranks in a dominance hierarchy change upon group formation and that these changes are due to either (1) familiarity with the environment or (2) individual age (and by proxy, physical health). If the former is true, we would expect that individuals added to the new group later in the formation process would have lower ranks than those added earlier in the process. If the latter is true, we would expect to see a correlation between age and rank, with younger individuals gaining higher ranks upon group formation and older individuals gaining lower ranks.

Methods

Subjects, Study area and Husbandry

We studied fourteen adult Japanese macaques which were originally housed in two groups of seven at Zoo de Granby, Granby, QC, until 2015. In April and May 2015, five individuals from each group were transferred to the indoor housing area of a newly constructed habitat (Appendix C) and introduced to form one group of ten individuals (group three). Individuals were removed from their original groups and introduced to each other based on a number of factors including ease of capture and transfer, previous dominance rank and perceived ease of introduction by the animal care staff. The transfers and introductions continued from April 20th to May 4th, 2015 (Appendix B).

Behavioural Observations

Data used in this analysis were collected July to October 2014, when the macaques were in their original groups, and July to September, 2015, with the new social grouping. Sampling was performed opportunistically (see Martin and Bateson, 2007) during ten minute focal sampling sessions, which were performed for an additional study. Data collection occurred between 9:30 and 18:00. When an agonistic behaviour was observed, notes were taken on the individual performing the behaviour, the type of behaviour (Table 2.1) and when possible, the individual towards which the agonistic behaviour was being performed was also recorded.

Statistical Analysis

Resolved agonistic interactions between females were used in the construction of the dominance hierarchies. These are interactions where a single individual in the dyad performs a submissive behaviour towards the other individual, such as withdrawing from the interaction or performing a submissive display (Chapais et al., 1991; Drews, 1993). In this scenario, the individual that did not display the submissive behaviour "won" the interaction and received a value of one for that dyad. Individuals who displayed the submissive behaviour "lost" and received a score of zero. Matrices were constructed for the females of each group using the sum of the values for each dyad. For group three, separate matrices were constructed for before and after August 15th, 2015, when there was an evident change in the hierarchy due to the temporary removal of one of the females. Males were excluded from the analysis due to low numbers in the

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Zoo de Granby groups. In addition males and females of matrilineal Cercopithecoidea species are rarely included in the same hierarchy in the previous literature (see Gust et al., 1991; Sebastian, 2015; Silk et al., 1981). Improved Landau's index of linearity (De Vries, 1995) was generated for each matrix and the rank of each individual was determined using the reordered matrices, all using Matman 1.1 for Windows (Noldus Information Technology 1998). In total, there were 80 resolved interactions in group one, 28 resolved interactions in group two, 70 in group three before the shift in hierarchy and 105 in group three after the shift.

In order to correlate ranks from groups of different sizes, standardized ranks were calculated for each individual using the formula $(N-R)/(N-1)$, where N is the number of individuals in the matrix and R is the numerical rank of the individual, with 1 being the highest possible rank (Robbins et al., 2005). Two Kendall's rank correlations were performed to assess the changes in the dominance hierarchy. The first correlated the standardized ranks of individuals in their initial groups (groups one and two) to their ranks in the new group (group three) before the shift on August 15th. The standardized ranks from group one and group two were pooled, therefore only one correlation was performed for both groups. As there were tied ranks in this analysis due to pooling the data, the p-value was determined using a normal approximation. The second correlated the ranks of individuals in group three before and after the shift on August 15th. Two additional Kendall's rank correlations were performed to determine whether a correlation existed between age and rank in group three both before and after the shift in hierarchy. We also performed a Mann Whitney U test to determine whether the mean standardized rank of the individuals added first to group three (Madjae, Magia, Iodine, Ionica, Iosa, Lullaby) was higher than that of the non-founder individuals (Shiwa, Miu, Zoe). All tests were performed in R 3.2.3 (R Core Team, 2015) at the 5% level of significance.

Results

Reordered matrices used to establish the ranks in the three groups of macaques are displayed in tables 2.2 through 2.5. The hierarchies for the females of both group one (h'=0.71) and group two (h'=0.80) were moderately linear, meaning the direction of the agonistic interactions were, for the most part, consistently one-sided. The hierarchy of the females in group three was moderately linear after the shift on august 15th (h'=0.65) but less so before the shift (h'=0.48)

A weak, but non-significant correlation was found between the pooled ranks of individuals in the 2014 groups and their initial rank in the 2015 group (τ=0.31, z=1.15, p=0.249; Figure 2.2). This was also true for the correlation between the ranks of individuals in group three before and after the shift in hierarchy (τ=0.33, T=24, p=0.260; Figure 2.3). There was a significant negative correlation between age and rank of individuals in group three before the shift (τ=-0.54, z=-1.99, p=0.046), but no significant correlation existed after the shift (τ =-0.20, z=-0.73, p=0.463; Figure 2.4). The mean rank before the shift was not significantly different between the founders of group three and the non-founders (W=11, p=0.714).

Discussion

The removal and transferring of animals in social groups is relatively common-place in zoos; the decision to do so is often made with the animals' well-being in mind, for example to reduce overcrowding, to transfer to a new exhibit, or to perform a medical procedure. However, the disruption of the social group can have prolonged effects on the hierarchy of the group and consequently on the physical and psychological health of the animals (Gust et al., 1991; Kaplan et al., 1983). We found that individuals' ranks changed dramatically following the formation of a new group from two previous groups. There were a number of reversals in the ranks of individuals who were in the same group before the merge. Most notable was the supplantation of Madjae over Miu, who was the dominant female in group one, and the fall of Zoe from the second highest ranking female in group two to below Iodine and Ionica in group three (Figure 2.5).

It is interesting to note that both Miu and Zoe were added to group three later in the formation process. Previous studies have shown that, in an experimental situation, a monkey that is familiar with its surroundings will often take precedence over a newly introduced individual (Rowell, 1974; Westergaard et al., 1999). This is not always the case, as Honess et al. (2004) found relatively few changes in hierarchy when a group was reintroduced in a new environment, but this study was performed with individuals who had all been housed together previously. Order added may be more important when introducing unfamiliar individuals, as those added earlier will have time to form social relationships than those added later. This hypothesis has been supported in experimental trials on rhesus macaques (*Macaca mulatta*), where alpha and beta males consistently became subordinates when introduced to a new group, but regained their high rank relatively easily when reintroduced to their original group (Bernstein and Gordon, 1980). As our group formation consisted of merging two existing groups and not introducing completely unfamiliar individuals, a non-founders previous experience and social relations with the founder individuals may have helped them attain a higher rank. However, it is also possible that the low number of non-founders (3) versus founders (6) confounded this analysis

The observed reversals in ranks were likely due to the strong alliance that was formed between Madjae, Shiwa and Ionica (older sister, younger sister and mother, respectively). Lower ranking females are often able to overcome higher ranking females when in the presence of their

kin (Chapais, 1991). This alliance may also explain why Shiwa, who was the second last individual added to the group, was able to achieve such a high rank. It is quite remarkable that these family members were quick to form an alliance despite Madjae being housed in separate group from Shiwa and Ionica for approximately seven years. Though Miu's mother, Iosa, was present in the new group as well, her advanced age and deteriorating physical condition may have prevented her from becoming involved in the majority of aggressive interactions. In both our group and in previous studies, age appears to be a significant variable in determining rank, with younger adult females being more likely to challenge older individuals and move upwards in the hierarchy (Silk et al., 1981; Takahata, 1991). However, it is likely that this only true for adult individuals as Chapais (1991) found that females under the age of three were often outranked by an older subordinate individual (85.7% of dyads) when other macaques were not present; individuals over the age of three were not (0% of dyads).

The importance of this kin alliance was further illustrated by the dramatic change in hierarchy that occurred on August 15th, when Shiwa was temporarily removed from the group due to an intense aggressive interaction between her and Madjae. Without Shiwa present, Madjae had little support when confronted by Iodine and Magia, and consequently dropped from the highest ranking position to the third highest ranking position, below these two older females (Figure 5). As rank was no longer correlated with age after this shift, it seems clear that although it can play a role in determining an individual's rank in the formation of hierarchies and can influence their likelihood of overthrowing an already stable hierarchy, it may be less important than the presence of kin and other allies. Oates-O'Brien et al. (2010) also found some support for the significance of alliances in rhesus macaques, where matrilineal overthrows were more likely to occur when high ranking females were removed from the group.

It is important that a social animal be housed with others of its species, as isolation can result in severe indicators of poor welfare such as self-biting, stereotypic pacing and floating limb behaviour (Lutz et al., 2003; Mallapur and Choudhury, 2003); however, captivity can also be stressful for social-housed animals as groupings are often different than that which would occur naturally and there is limited space to flee during aggressive interactions (Morgan and Tromborg, 2007). Having an unstable hierarchy increases the risk of social stress and can result in decreases in health and welfare, as is apparent from both previous research (Gust et al., 1991;

Kaplan et al., 1983; Sapolsky, 2005), and from the deterioration in coat condition anecdotally observed in this study (Appendix D).

It is evident that changes in the hierarchies of social animals, especially primates, are to be expected when combining groups of individuals that were previously housed separately. Our results suggest that age my play a role in determining an individual's rank in a newly formed group but there was no strong evidence that order added affected rank acquisition. We believe that much of the observed results may have been due to the fact that the macaques at the Zoo de Granby were familiar with or related to members of the opposite group. Even after multiple years of separation, individuals from species that form strong kin or non-kin alliances, such as chimpanzees or rhesus macaques, may recognize each other upon reintroduction and these renewed alliances could allow them to overcome previously dominant individuals. The removal of one of these individuals could then result in further hierarchy changes and social stress. Zoo management must be conscious of the potential consequences of removing and transferring individuals and thoroughly evaluate the decision before doing so.

Tables and Figures

Table 2.1. The ethogram of agonistic behaviours, derived from de Waal et al (1976)

Activity	Definitions
Submissive Behaviour	Agonistic behaviours indicating fear or submission. The specific behaviour that is observed will be further classified into the following subcategories: **Flight (F)** - Fast withdrawing locomotion from another individual **Flight-intention (FI)**: Postures or movements expressing a tendency to flee such as crouching or shrinking. **Submission (S):** submissive facial expressions such as teeth-bearing When the behaviour is directed towards another individual, identity of both individuals involved in the behaviour will be recorded.
Dominant Behaviour	Agonistic behaviour of an aggressive or dominant nature. The specific behaviour that is observed will be further classified into the following subcategories: **Threat (T) -** Facial expressions and postures that convey agonistic intentions. Two dominance behaviours that are not mentioned in de Waal et al. (1976) but that were observed in the preliminary observations, branch shaking and leaping, will also be included in this category **Chase (C)** - Quick and brusque movements towards another individual **Physical Assault (PA)** – Varying degrees violent physical contacts. When the behaviour is directed towards another individual, identity of both individuals involved in the behaviour will be recorded for each event

Table 2.2. Reordered matrices showing the number of resolved agonistic encounters between dyads in group one (old enclosure). Individuals along the horizontal axis are the "winners" of interactions and those along the vertical axis are the "losers". The order of individuals in the axes represents the linear hierarchy of individuals for that group, with those at the top of the vertical and on the left of the horizontal being the lowest ranking and those at the bottom of the vertical and the right of the horizontal, the highest ranking.

ID	Chilly[1]	Lullaby	Magia	Madjae	Iosa	Miu
Chilly[1]	*	8	1	4	0	3
Lullaby	0	*	5	0	0	6
Magia	0	0	*	5	4	8
Madjae	0	0	0	*	1	24
Iosa	0	0	0	0	*	11
Miu	0	0	0	0	0	*

1. The individual indicated was not involved in the formation of the new group

Table 2.3. Reordered matrices showing the number of resolved agonistic encounters between dyads in group two (old enclosure). Individuals along the horizontal axis are the "winners" of interactions and those along the vertical axis are the "losers". The order of individuals in the axes represents the linear hierarchy of individuals for that group, with those at the top of the vertical and on the left of the horizontal being the lowest ranking and those at the bottom of the vertical and the right the right of the horizontal, the highest ranking.

ID	Iodine	Ionica	Iopolda[1]	Zoe	Shiwa
Iodine	*	2	7	0	7
Ionica	0	*	0	3	4
Iopolda[1]	0	0	*	0	2
Zoe	0	0	0	*	2
Shiwa	0	0	1	0	*

1. The individual indicated was not involved in the formation of the new group

Table 2.4. Reordered matrices showing the number of resolved agonistic encounters between dyads in group three (new enclosure), before the shift in hierarchy that occurred on August 15th, 2015. Individuals along the horizontal axis are the "winners" of interactions and those along the vertical axis are the "losers". The order of individuals in the axes represents the linear hierarchy of individuals for that group, with those at the top of the vertical and on the left of the horizontal being the lowest ranking and those at the bottom of the vertical and the right the right of the horizontal, the highest ranking.

ID	Lullaby	Zoe	Iosa	Ionica	Iodine	Magia	Miu	Shiwa	Madjae
Lullaby	*	1	0	0	1	0	1	0	0
Zoe	0	*	0	0	1	12	11	7	1
Iosa	0	0	*	0	0	2	1	1	0
Ionica	0	0	0	*	0	4	1	0	0
Iodine	0	0	0	0	*	0	1	4	2
Magia	0	0	0	0	0	*	0	2	0
Miu	0	0	0	0	0	0	*	4	9
Shiwa	0	0	0	0	0	0	0	*	4
Madjae	0	0	0	0	0	0	0	0	*

Table 2.5. Reordered matrices showing the number of resolved agonistic encounters between dyads in group three (new enclosure), after the shift in hierarchy that occurred on August 15th, 2015. Individuals along the horizontal axis are the "winners" of interactions and those along the vertical axis are the "losers". The order of individuals in the axes represents the linear hierarchy of individuals for that group, with those at the top of the vertical and on the left of the horizontal being the lowest ranking and those at the bottom of the vertical and the right the right of the horizontal, the highest ranking.
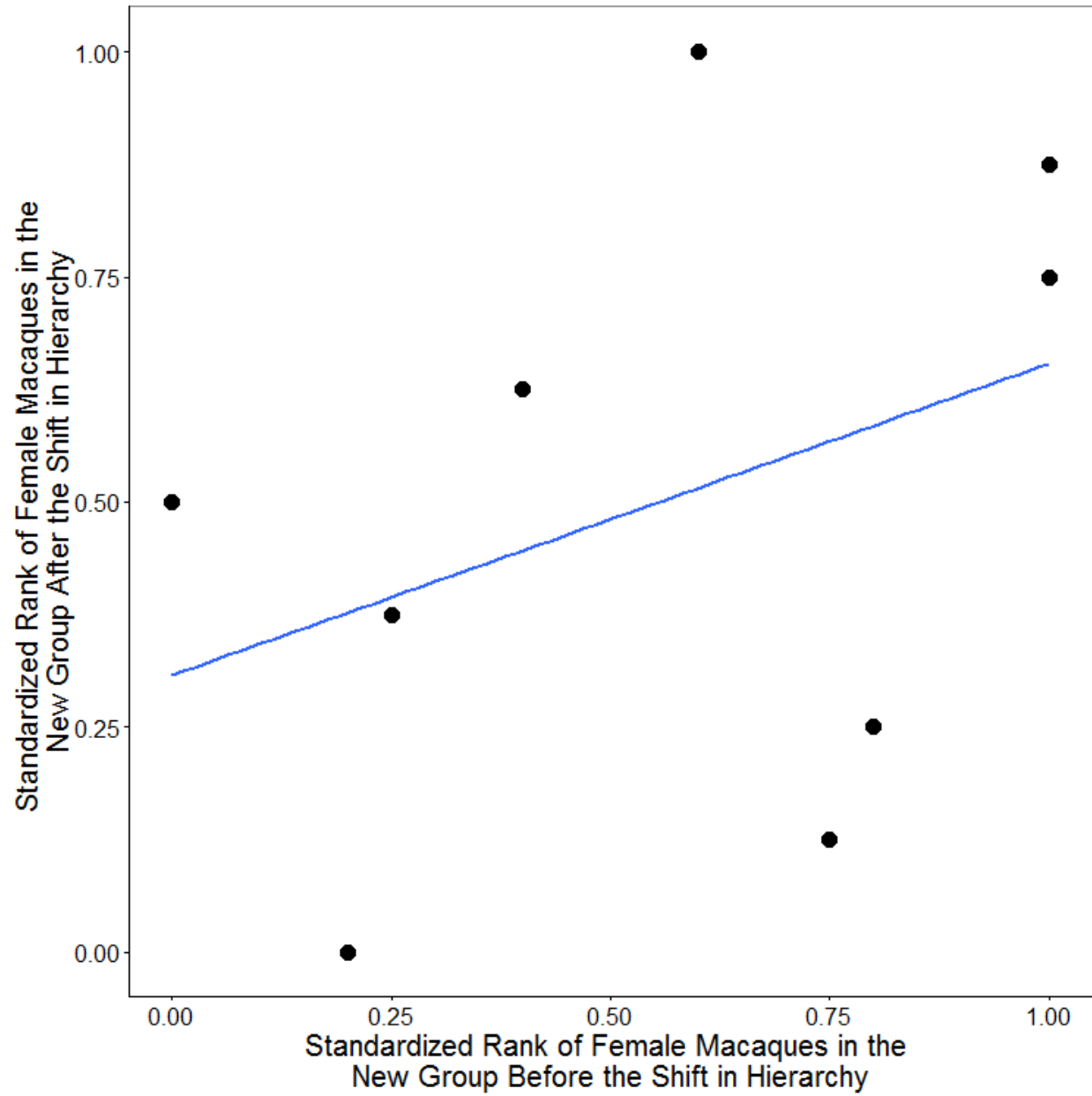
ID	Ionica	Zoe	Lullaby	Miu	Iosa	Shiwa	Madjae	Magia	Iodine
Ionica	*	0	0	8	1	2	1	3	1
Zoe	0	*	0	18	0	12	2	12	3
Lullaby	0	0	*	0	0	3	0	0	1
Miu	0	0	0	*	0	5	6	0	2
Iosa	0	0	0	0	*	0	0	0	0
Shiwa	0	0	0	0	0	*	2	2	3
Madjae	0	0	0	0	0	0	*	1	16
Magia	0	0	0	0	0	0	0	*	1
Iodine	0	0	0	0	0	0	0	0	*

Figure 2.1. Correlation between the standardized rank of the female Japanese macaques at the Zoo de Granby in their old groups (groups one and two) and in the new group (group three), before August 15th.

Figure 2.2 . Correlation between the standardized rank of the female Japanese macaques at the Zoo de Granby in the new group (group three) before and after the shift in hierarchy on August 15th.

Figure 2.3. The trend between the standardized rank of the female Japanese macaques in group three at the Zoo de Granby and their age (as of June 2014), both before the shift in hierarchy on August 15th (left) and after the shift (right).

Figure 2.4. A visual representation of the changes that occurred in the dominance hierarchy of the female Japanese macaques at the Zoo de Granby in 2015. The old groups are the groups which were observed in 2014 (group one and two) and the new group is the group observed in 2015 (group three). The shift in hierarchy in group three occurred on August 15th, 2015.

General Conclusions

With the advancement of zoos has come an increased sense of responsibility towards the zoo animals' welfare. It is no surprise that the behaviour and welfare of animals is one of the most prominent research subjects to be undertaken in zoos (Melfi, 2005; Stoinski et al., 1998). However, due to the apparent taxon bias in the data, there are still many questions left to be answered for a number of under-studied species (Melfi, 2009). For this thesis, we tested a series of hypotheses using the Japanese macaques at Zoo de Granby as a model. Our results suggested that the activity budget of zoo-housed Japanese macaques does differ from that of wild and research populations, and that this difference could potentially be due to the presence of visitors. Minor visitor effect was observed in all three groups, but the behaviours analyzed did not suggest a negative or positive impact of visitor presence. It did, however, support the use of multiple visitor-related variables when studying this phenomenon, and the potential presence of group- and enclosure-based differences in visitor effect. The increasing acceptance of visitor effect as a multifaceted phenomenon is apparent in the literature (Choo et al., 2011; Sellinger and Ha, 2005) and future research should continue in this direction. Behavioural reactions of the Zoo de Granby macaques to very specific visitor conditions, such as the presence of visitors with food, presence of young children and interactions through viewing windows, were only noted anecdotally in this study and not analyzed, we believe these would be variables worth investigating for Japanese macaques, as their effects on other species have been supported quantitatively (Choo et al., 2011; Clark et al., 2012)

The data from this thesis also suggested that the macaques did habituate to the new enclosure but did not necessarily reap any welfare benefits within the three to five months of being transferred to a larger, more complex enclosure. This may be due to confounding variables, such as insufficient habituation time, as previous research has found that primates vary in the length of time they require to adjust to a change in habitat (Ogden et al., 1990; Ross et al., 2011b). However, it is likely that the large change in hierarchy both before and during the field season in the new enclosure played an important role in the behaviour which was observed. It has been hypothesized that captivity is more stressful for social animals because there is less opportunity for subordinates to avoid dominant individuals (Creel, 2001). In both wild and captivity, lower ranking individuals in stable, linearly-organized societies experience more signs

of stress than their dominant counterparts; however, this changes when hierarchies are unstable and dominant individuals are being challenged and leads to greater physical and psychological stress for these high ranking individuals (Sapolsky, 2005).

High levels of aggression may occur only within the first few hours of a group formation in captivity; however physiological indices of stress, such as high cortisol levels and decreased immune function, may persist for anywhere from 24 hours to nine weeks (Gust et al., 1991; Sapolsky, 2005). Social stress can even impact physical health of primates, such as the increased hardening of the coronary arteries (coronary artery atherosclerosis) observed in socially stressed *Macaca fascicularis* (Kaplan et al., 1983). Through the alopecia rating taken during this study between March 2015 (before new group formation) and February 2016, we noted that individuals who experienced more drastic changes in rank were also recorded to, at least temporarily, have worse coat conditions than before their change in hierarchy. This occurred with Miu and Zoe early in group formation, and Shiwa, Madjae and Ionica after the shift in hierarchy on August 15th, 2015 (Appendix D). Individuals such as Shiwa and Iodine were even observed actively pulling and eating the hair of other individuals in 2015, an act that is generally performed by high ranking individuals towards lower ranking individuals as a form of aggression (Reinhardt et al., 1986). Overall, the macaques did not experience the improvement in coat condition that was hoped for with the transfer to the new enclosure, further supporting the idea that the stress of an unstable hierarchy may have masked the positive benefits of the new enclosure during this study. A lengthier study would be required to fully evaluate changes in behaviour and hair regrowth once the new group has attained a stable hierarchy and are fully habituated to the new enclosure, and to evaluate what these changes mean for the groups welfare.

In this instance, the Zoo de Granby decided to form a new group of individuals with the hopes that the individuals would benefit from their new environment. However, in the first chapter, we found no strong indication of an increase in welfare the months after the transfer, only an increase in allogrooming, likely due to the social tension in the newly formed group. As shown in this study, even the removal of one key individual can result in large shifts in the hierarchy, which may potentially decrease the animals' welfare. Hence, zoo management must be wary when manipulating social groups, in both primates and in other social species; the costs and benefits to both the individual and the group as a whole must be considered. We would also

like to stress the importance of accounting for confounding variables, such as changes in the social group and habituation, when performing research and when planning the translocation of animals to new environment. Longer, more extensive research protocols should be implemented both before the construction of a new habitat and after the translocation of animals to a new habitat to maximize our understanding of this process.

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Appendix A
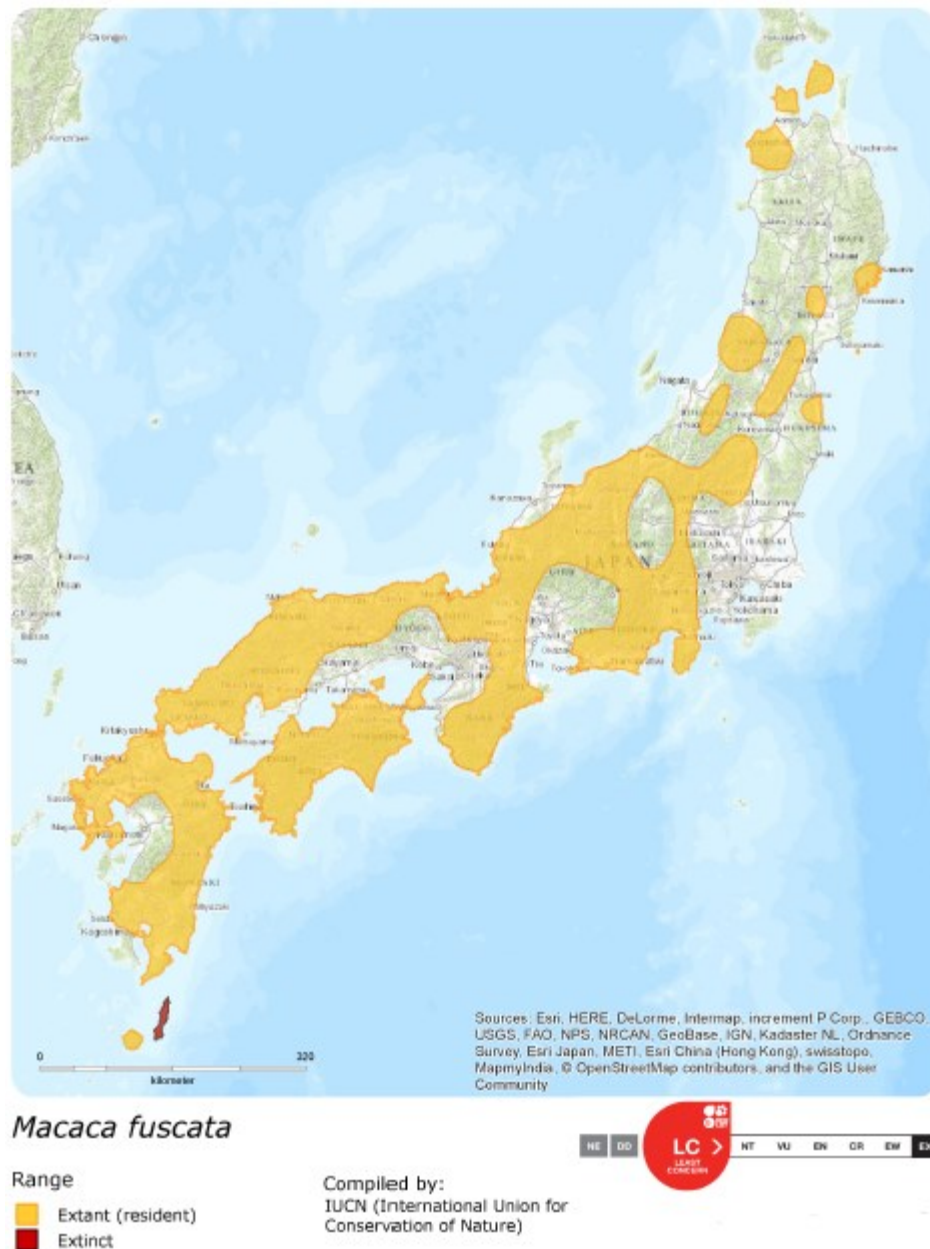
Figure 3.1. The range map of wild Japanese macaques, according to IUCN red list (Watanabe and Tokita, 2008). Japanese macaques are widespread across many of the islands of Japan and have only been extirpated from Tane Island.

Appendix B

Table 3.1. Information on the fourteen Japanese macaques currently housed at the Granby Zoo.

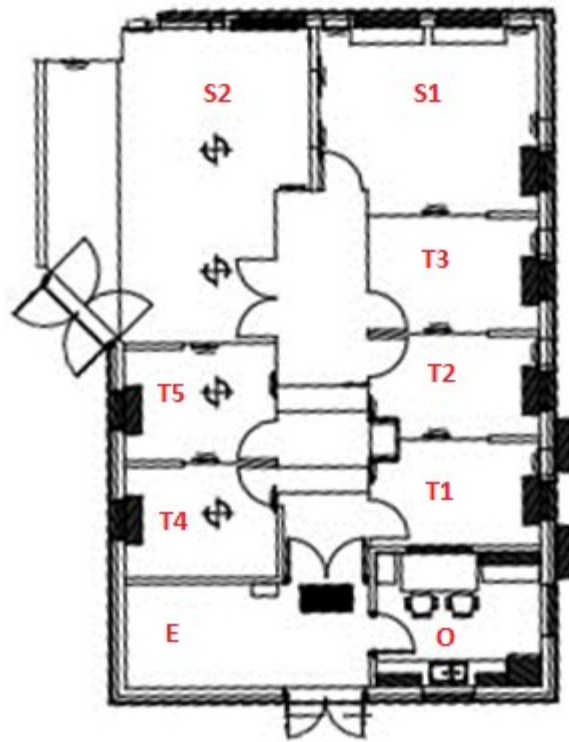| ID	Name	Group	Sex	Age as of June 2014	Date Added to Group Three	Family Relations
M93199	Iosa	1,3	F	20	April 22nd, 2015	Mother of Miu
M96112	Magia	1,3	F	17	April 22nd, 2015	Mother of Remon
M93198	Chilly	1	F	20	NA	NA
M96110	Lullaby	1,3	F	18	April 22nd, 2015	Mother of Zoe
M06017	Remon	1	M	7	NA	Son of Magia Half-brother of Miu, Shiwa and Madjae (Paternally)
M05014	Madjae	1,3	F	8	April 22nd, 2015	Daughter of Ionica Full-sister of Shiwa Half-sister of Miu and Remon (paternally)
M06011	Miu	1,3	F	8	May 4th 2015	Daughter of Iosa Half-sister of Madjae, Shiwa and Remon (paternally)
M98139	Iodine	2,3	F	15	April 22nd, 2015	NA
M93200	Mago	2,3	M	20	April 22nd, 2015	NA
M94129	Ionica	2,3	F	19	April 22nd, 2015	Mother of Madjae and Shiwa
M97130	Iopolda	2	F	17	NA	NA
M96111	Linus	2	M	17	NA	NA
M06027	Shiwa	2,3	F	7	May 1st, 2015	Daughter of Ionica Full-sister of Madjae Half-sister of Miu and Remon (paternally)
M02019	Zoe	2,3	F	11	April 29th, 2015	Daughter of Lullaby

Figure 3.2. Front view of the 2014 Japanese Macaque on-display enclosure. The enclosure consisted of a chain link dome measuring 8.5m in radius and was equipped with climbing branches, three wooden dens and two stone dens, an artificial stone hill and a water feature with waterfall and pool. The viewing area consisted of approximately two thirds the perimeter of the enclosure and there was approximately 1.5m of space between the enclosure the viewing area, maintained by a second chain-link barrier fence.

Figure 3.3. Front view (top) and back view (bottom) of the new Japanese macaque enclosure, completed in May 2015. The enclosure measures 665m^2 and includes dens with heat lamps, a climbing structure, a stream and a thermal pool. There are four main viewing areas, one separated from the enclosure by a Plexiglas window, the others separated by fencing.
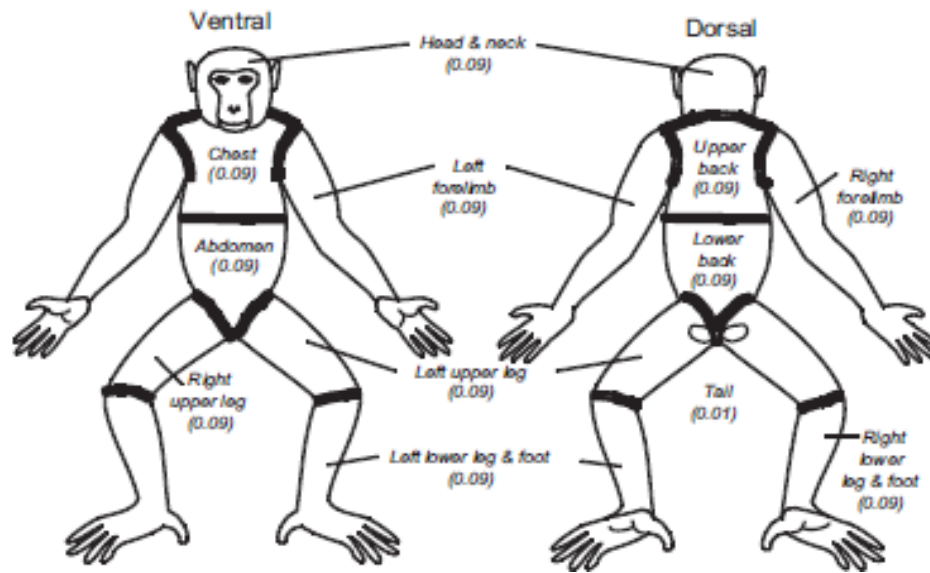
Figure 3.4. A schematic drawing of the indoor housing area of the new Japanese macaque enclosure at the Zoo de Granby. S1 and S2 are large "day rooms" that have doors connecting to the outdoor enclosure and Plexiglas windows through which visitors can view the macaques. They are separated by a concrete wall with a large, metal sliding door. T1 through T5 are smaller transfer rooms. They are separated from each other and from the day rooms by paint-coated metal-grid fencing and connected by a small, sliding fence doors. T4 and T1, and T5 and T2 are also connected by an enclosed, overhead catwalk. Area E is the entrance to the building and storage area and area O is the office a prep-room.

Appendix D

The coat condition rating system implemented at the Zoo de Granby between March 2015 and February 2016 was that created by Bellanca et al. (2014). Rating were taken on five occasions, once before the transfer to the new enclosure and the formation of the new group (March 31st, 2015), twice after the formation of the new group but before the shift in hierarchy (May 26th and July 29th, 2015) and twice after the shift in hierarchy (September 15th, 2015 and February 23rd, 2016). All ratings were performed in the indoor and off-display sections of the habitats. Coat condition was recorded by shading affected areas (bald areas of greater than 2.5cm^2, excluding wounds, scars and other naturally hairless areas) on printouts of a generic primate body plan and percent of body parts affected was calculated as indicated in figure 3.4. The tail was excluded; therefore the maximum percent of the body that could be affected was 99%. This method proved to be relatively effective in cataloguing general changes in coat condition, however several drawback to performing it in the zoo environment were encountered, such as the occasional inability to get a complete view individuals in the back and corners of the habitat, or the challenge of recording accurately for quick-moving individuals. Ratings for all individuals are available in table 3.2.

Table 3.2. The percent body surface affected by alopecia recorded on each of the five sampling, for the fourteen Japanese macaques at the Zoo de Granby. Of these fourteen individuals, Linus and Remon were never introduced into the new group. Iopolda was only introduced into the new group prior to the February 23rd, 2015 sampling date. "NA" represents points were no rating was performed for an individual, as they were not present for medical or other reasons. A box indicates individuals who experienced a large decrease in coat condition following a fall in rank.

Individual	March 31st, 2015	May 26th, 2015	July 29th, 2015	September 15th, 2015	February 23rd, 2015
Iodine	60	54	63	72	81
Ionica	18	NA	0	45	18
Iosa	63	54	45	36	63
Lullaby	72	54	36	21	18
Madjae	18	54	9	63	NA
Magia	27	72	54	63	72
Miu	27	81	45	45	45
Shiwa	63	36	18	18	63
Zoe	36	72	36	45	27
Chilly	45	36	54	45	27
Iopolda	63	NA	NA	NA	27
Linus	36	NA	NA	NA	NA
Mago	81	81	81	72	54
Remon	0	NA	0	0	0

Figure 3.4. Diagram from Bellanca et al. (2014) showing the generic primate body plan on which coat condition was recorded and associated values for each section. The body is separated eleven major sections: head and neck, left arm, right arm, chest, abdomen, upper back, lower back, left upper leg, left lower leg, right upper leg, right lower leg. Each of these sections comprises approximately 9% of the body surface. The twelfth section, the tail, makes up the final 1%. The percent values for all the affected areas are summed in order to determine the total percent of body surface affected.