

**Welfare Assessment Through Foraging: Understanding the Animals' Points of
View**

BY

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THESIS

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This thesis is dedicated to my wonderfully supportive husband and overall favorite person, Brian, and my writing buddy in utero, Baby Smith. Thank you, my baby boy, for providing me with a hard deadline to finish, and an excuse to take frequent snack-breaks!

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SMTS

CONTRIBUTION OF AUTHORS

Chapter I is an introductory chapter highlighting my path to graduate school, and the development of my research ideas and questions.

Chapter II is a published manuscript (Sandra M. Troxell-Smith, Michal J. Tutka, Jessica M. Albergo, Deebika Balu, Joel S. Brown, John P. Leonard. (2016) “Foraging decisions in wild versus domestic *Mus musculus*: What does life in the lab select for?” *Behavioural Processes* 122: 43-50), where I implemented the research study, compiled and analyzed the data, and wrote the manuscript. Michal Tutka and Jessica Albergo both conducted a portion of the research project. Deebika Balu assisted with animal transportation and husbandry. My advisor, Dr. Joel Brown, provided guidance with study design, implementation, statistical analysis and writing. Dr. John Leonard generated and provided the two laboratory mouse strains for the study, and provided writing guidance.

Chapter III describes a study at the Brookfield Zoo where I assess the environmental preferences of individual okapi (*Okapia johnstoni*) using food patches.

Chapter IV describes a foraging patch study at the Lincoln Park Zoo designed to: a) quantify the efficacy of patch use studies as an enrichment opportunity, and b) determine the spatial and foraging preferences for zoo-housed Parma wallabies (*Macropus parma*), and Patagonian maras (*Dolichotis patagonum*).

Chapter V is a published manuscript (Sandra M. Troxell-Smith, Lance J. Miller. (2016) “Utilizing Natural History Information for Zoo Animal Management: A Case Study with Okapi (*Okapia johnstoni*).” *Journal of Zoo and Aquarium Research* 4: 38-41), where I implemented and conducted the research, compiled and analyzed the data, and wrote the manuscript; Dr.

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Chapter VI describes a study that determined how animal personality influences the foraging and habitat choices of the brushtail possums (*Trichosurus vulpecula*) in the Ku-Ring-Gai Chase National Park, NSW, Australia.

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LIST OF ABBREVIATIONS

AZA	Association of Zoos and Aquariums
BLUPs	Best Linear Unbiased Predictors
BZ	Brookfield Zoo
C	Metabolic cost
GUDs	Giving-up Density
H	Harvest Rate
IRD	Initial Resource Density
LOC	Landscape of Comfort
LPZ	Lincoln Park Zoo
MANOVA	Multivariate Analysis of Variance
MOC	Missed Opportunity cost
NSF	National Science Foundation
P	Predation cost
SMRH	Small Mammal and Reptile House
SEM	Standard Error of Mean
UIC	University of Illinois at Chicago
USyd	University of Sydney, Sydney, Australia

SUMMARY

In this dissertation, I use foraging ecology and patch-use theory to develop a quantifiable metric to understand animal environmental preferences and decision-making in laboratory, zoo, and wild species. Chapter I describes my journey toward and through my dissertation. Each of my five remaining dissertation chapters investigates a novel application and integration of foraging ecology and animal behavior, and suggest opportunities for the improvement of animal welfare efforts through inclusion of foraging ecology principles.

Chapter II utilizes foraging ecology principles to determine effects of domestication on the problem-solving and foraging strategies of laboratory vs. wild-caught house mice (*Mus musculus*). Domesticated laboratory strains adopted more energy-efficient foraging strategies, and responded more favorably to foraging challenges than their wild counterparts. This study advanced existing literature regarding how the domestication process influences problem-solving and resource acquisition in laboratory species, and provided an excellent opportunity to mentor two undergraduates, both of whom are co-authors on this manuscript.

Chapter III describes a study at the Brookfield Zoo to assess the environmental preferences of individual okapi (*Okapia johnstoni*). Based on intensity of foraging in experimental patches, I found that individuals greatly varied their response to and utilization of the same exhibit space. This study indicates that individuals within a species differ in their assessment of captive environments, and thus do not perceive these captive spaces identically. I conclude that individual behavioral differences must be incorporated into animal management and welfare decisions.

Chapter IV describes a foraging patch study at the Lincoln Park Zoo designed to: a) quantify the efficacy of patch use studies as an enrichment opportunity, and b) determine the spatial and foraging preferences for zoo-housed Parma wallabies (*Macropus parma*), and

Patagonian caviar (*Dolichotis patagonum*). Food patches reliably revealed environmental preferences for each species. Food patches also increased foraging time and decreased the frequency of inactive and/or stereotypic behaviors for both species. Both behavioral outcomes are key goals for zoological institutions, further demonstrating the utility of implementing the food patch technique as a method to assess and improve captive animal welfare.

Chapter V further describes an additional collaboration with the Brookfield Zoo that examined the effects of implementing social separation (visual barriers) on stereotypic behavior in okapi. Visual barrier installation drastically reduced okapi stereotypic behavior, suggesting that captive social situations have large impacts on animal welfare. Solitary species in the wild may require additional management considerations in captivity, namely more natural social scenarios.

Chapter VI extended my behavioral ecology research from captive to wild animal populations. Working with free-ranging brushtail possums (*Trichosurus vulpecula*) in the Ku-ring-Gai Chase National Park, NSW, Australia, I determined how animal personality influences the foraging and habitat choices of the brushtail possums. I trapped and established personality metrics for 12 possums, which were then released and monitored to determine individual differences in food quality preference and response to environmental risk. This work supplements current understanding regarding how individual personality traits influence environmental and ecological choices, particularly in a species of management interest.

I. INTRODUCTION

A. Overview

As a behavioral ecologist, I implement studies based in foraging ecology and patch-use theory to understand animal environmental preferences, and how those preferences influence animal decision-making. The overarching goal of my dissertation is to test, apply, and demonstrate the utility of foraging ecology and the patch-use/giving-up density (GUD) approach as a metric for assessing the environmental perceptions and welfare of captive and wild animals. Each of my five dissertation chapters investigates a novel application and integration of foraging ecology and animal behavior, and suggests opportunities for the improvement of animal welfare efforts.

In my second chapter, I apply foraging ecology in the laboratory to compare the foraging strategies and aptitudes of three strains of house mice (*Mus musculus*): a typical domestic laboratory strain, a genetically modified “mutant” strain (Balu et al. 2012), and a wild-caught strain. My third and fourth chapters investigate the benefits of implementing foraging ecology in zoos. In particular, my third chapter uses foraging ecology to understand the environmental perceptions of okapi (*Okapia johnstoni*) at Brookfield Zoo, Brookfield, IL, and my fourth chapter investigates the perceptions and preferences of both Parma wallabies (*Marcopus parma*) and Patagonian cavies (*Dolichotis patagonum*) at the Lincoln Park Zoo, Chicago, IL. My fifth chapter stemmed from my work with the okapi at the Brookfield Zoo, Brookfield, IL. In collaboration with Dr. Lance Miller, we explored the effect of manipulating visual barriers with the goal of reducing stereotypic behavior in an adult female okapi. Finally, for my sixth chapter, I traveled to Sydney, Australia to work with Dr. Clare McArthur and her lab. We studied free-ranging common brushtail possums (*Trichosurus vulpecula*), and investigated how different

personality traits (boldness, activity, exploration, and docility) influence the foraging decisions of possums.

B. My graduate journey

My fascination with exotic animals began when I was very young. Growing up, I distinctly remember traveling with the family down to SeaWorld in Ohio (when there was an Ohio location). We sat in the splash zone during the Shamu and Friends show, and I remember being fascinated with the orcas and how they interacted with their trainers. I was forever transformed, and knew that I wanted a career working with animals.

It is ironic that SeaWorld has since become the target for animal rights groups after the *Blackfish* documentary was aired on CNN in 2013. Public perception and opinion on housing animals, particularly cetaceans, in captivity changed practically overnight (Pierce 2015), and has spurred many animal advocacy groups to argue for the immediate release of animals and closing of SeaWorld and other captive facilities (Huggan 2016). While such extreme measures are highly impractical from a variety of standpoints, the conversation surrounding captive facilities, animal ethics, and welfare has certainly changed. How different my life would have been had my parents avoided zoos or SeaWorld while I was a child. Even worse, what would happen for generations of children if these facilities were shut down, as growing public opinion currently suggests. I want my children to have the opportunity to enjoy experiences and learn about the natural world through zoos and aquariums. The ability to travel to far-off places to see animals in their natural habitat, like an okapi in the Ituri forest in the Democratic Republic of Congo (Bodmer and Rabb 1992), is financially unrealistic for the majority of people. And while some argue that children can learn just as much about animals through watching the Discovery Channel and Animal Planet, direct personal experiences and connections with animals are

second to none to creating lasting impact. Moreover, resorting to forcing children to stay inside to experience and learn about the world around them is only furthering the growing disconnect between humans and nature. However, it also goes without saying that providing captive animals with the best care possible (both physically and emotionally) is of the utmost importance. As the need to understand and improve welfare for captive animals continues to grow, particularly in the face of a harsh public climate, my passion and desire to contribute grows with it.

Like many children, I thought that a career with animals would lead to either being a veterinarian or a zookeeper. Then I enrolled in an Animal Behavior class during my undergraduate studies at Michigan State University. The course instilled a passion for animal behavior beyond just caring for animals. This led to an internship at the Toledo Zoo (Toledo, OH), which was my first experience working directly in a zoological setting. I learned about operant conditioning and enrichment. I had found my calling. Not only was I able to express my creative side by developing new enrichment activities for animals, but I learned that doing so could have a direct impact on the animals I worked with. Enrichment activities provided an opportunity for animals to engage in an activity outside their normal routine. This internship led me to pursue a series of additional internships and experiences where I was able to learn and study animal behavior and welfare. Ultimately, this path led to graduate school.

It wasn't until I met Dr. Joel Brown, however, that I realized I could take my interests in animal enrichment and welfare a step further. In learning, discovering, and exploring the principles of foraging theory and patch-use, I immediately recognized the potential for foraging theory to augment current enrichment techniques. Food patches could provide an enrichment activity in and of themselves by providing additional foraging opportunities for captive animals (though this hadn't been directly studied yet). And, when properly applied and calibrated, food

patches can quantify foraging effort, foraging location preferences, and other aspects of the animal's perceptions of its exhibit and well-being. Though these ideas had been studied extensively in wild populations, I was somewhat flabbergasted to find that these techniques had yet to catch on in captive populations.

Upon entering the Brown lab, I had the pleasure of meeting two of Joel's former grad students (Drs. Jo-Elle Mogerman and Jennifer Howell-Stephens) that also shared an interest in applying foraging ecology to zoological institutions. Their dissertation work (Mogerman 2011, Howell-Stephens 2012) provided valuable background and inspiration for my research. I not only benefited from and expanded upon the groundwork they laid for my own studies, but their successful previous collaborations with the two zoos in the Chicago area made it significantly easier for me to eventually pursue my own research in both locations. Before initiating my own zoo studies, however, I had the opportunity to get my feet wet using foraging theory principles in another captive population: laboratory mice.

C. Patch-use in the laboratory

In my first year of graduate school, while still formulating ideas for possible zoo population studies, I wanted to gain direct experience with giving-up densities and food patch methodologies. Fortunately, Joel had recently learned of a new mouse strain developed in the laboratories of Drs. John Leonard and Jennifer Schmidt. The mouse strain contained a transgenic mutation affecting its ability to learn and generate memories appropriately (Balu et al. 2012). This mutation was also thought to influence anxiety and risk-taking behaviors. I, along with two excellent undergraduate assistants (Michal Tutka and Jessy Albergo), used patch use to understand the effects of domestication on the foraging preferences and strategies of the transgenic strain compared to wild-caught mice, and the progenitor laboratory strain (Chapter

II). Jensen & Gustafsson (1997) and Gustafsson et al. (1999) have previously suggested that wild animals utilize more energetically costly behavioral strategies compared to their domestic counterparts (i.e. domestic animals expend less energy performing behaviors than wild animals). I was interested in pursuing this hypothesis to determine if the wild-caught mouse strain foraged in a more energetically costly manner than domestic strains. I was also interested in investigating whether domestic strains continue to take a cost/benefit approach to foraging, or if generations of *ad libitum* access to food has resulted in less sophisticated foraging strategies. Foraging choices were quantified using giving-up densities (GUDS; Brown 1988) in three different foraging scenarios: (1) individuals were given a choice of foraging in covered or open microhabitats, and in patches of varying initial resource density; (2) individuals were exposed to calls of aerial predators and control calls throughout a foraging bout to assess differences in perceived predation risk; and (3) individuals were presented with foraging trays that contained altered seed locations to assess resource detection abilities.

Results from this study provided interesting insight into the possible effects of the domestication process. All strains revealed a significant preference for foraging under cover as opposed to in the open. Both domestic strains exploited patches of varying quality according to optimal foraging expectations, whereas the wild strain surprisingly did not. Predator calls did not influence foraging of any strain. Altering seed location within a patch revealed that basic laboratory mice were able to detect food resources better than either the mutant or the wild strains. Overall, both domestic strains foraged to a greater degree, and responded to foraging challenges more efficiently than the wild mice. These results support the hypothesis suggested by Jensen and Gustafson (1997), as the domestic mice performed more energy-efficient foraging behaviors and strategies compared to their wild counterparts. It is also

important to note that residual fear responses were retained by both the laboratory and mutant mouse strains despite generations of safety in captivity.

Overall, by utilizing foraging ecology in the laboratory, I found interesting effects of captivity on foraging behavior and decision making. Implementation of patch use studies in the laboratory can reveal long-term, and possibly more biologically relevant, differences among mouse strains than those found in typical acute behavioral tests currently employed by laboratories. This study advanced existing literature regarding how the domestication process influences problem-solving and resource acquisition in laboratory species, and provided an excellent opportunity to mentor two undergraduates, both of whom are co-authors on the resulting published manuscript. For my future zoo studies, the mouse work provided me with “mini-exhibit spaces” and the opportunity to show how food patches can be used to reveal the perceptions of “captive” animals.

D. Zoo Foraging Ecology: Okapi, cavies, and wallabies

1. Foraging ecology as a welfare framework

Optimal foraging theory explores how animals balance the costs and benefits associated with foraging decisions (MacArthur and Pianka 1966). Because resources are typically distributed in patches throughout the environment, patch use theory can be used to examine how an animal makes decisions in reference to a particular food patch. Charnov's (1976) Marginal Value Theorem predicts that an animal will leave a patch when the resource payoff falls below the average rate of return for the surrounding area. How an animal interprets the payoff will be dependent on how it perceives both the reward from the resource, and the costs associated with foraging. Brown (1988) identifies these costs as metabolic expenditure, foregoing alternative fitness-related opportunities, and perceived exposure to forms

of risk or discomfort while foraging in a given patch. These costs are quantified in the equation $H = C + MOC + P$, where H equals the quitting harvest rate, C equals the metabolic expenditure of foraging in the patch, MOC equals missed opportunity costs, and P equals perceived or actual predation risk. When an animal's quitting harvest rate (H) equals the sum of the other components, the benefits of foraging no longer exceed the costs in that patch, causing the animal to “quit” and move on. The amount of food left over in a patch (the Giving-up density -- GUD) can be used as a surrogate for the quitting harvest rate and is ultimately a direct reflection of how an animal perceives its environment (Brown 1988).

The ecological significance of each of the equation's components in a captive situation likely differ from those experienced in the wild. For instance, in the wild, missed opportunity costs (MOC) typically include mating related activities, providing for young, and searching for shelter (Brown 1988). In a captive environment, however, most of these variables are highly controlled by humans. In many zoos, animal breeding is carefully planned and only preselected animals are introduced for breeding purposes (Swaisgood 2007), rendering searching for a mate unnecessary. Similarly, shelters for inclement weather are readily provided for captive species and may already be an existing part of the exhibit structure, consequently removing the need to create or search for a safe area. Therefore, the captive environment essentially controls for an animal's missed opportunity costs (Mogerman 2011).

In the wild, the energetic costs of foraging (C) take into account the various search and handling times associated with acquiring different food items. In zoos, an animal's diet is strictly regulated and monitored by nutritionists and veterinarians, and may be considerably different from what animals consume in nature (e.g. differences in size and texture, and highly regulated nutritional content; Morgan and Tromborg, 2007). Many times the staple diet will be consistent,

with seasonal variation in supplemental fruits and vegetables. Therefore, the overall food handling and processing times in captivity are relatively stable from day to day. Also, zoo diets are typically provided on a predictable schedule and in a predetermined location(s) (Watters 2009), which significantly reduces the search time and effort that animals in captivity experience. Thus the energetic costs for animals foraging in captivity are also largely consistent.

The final component, perceived or realized predation risk (P) is similarly affected by the captive scenario. Intuitively, predation risk takes into account the need to respond to and avoid detection by predators (Brown 1988). Zoos and other captive facilities, however, typically strive to minimize exposure to actual predation risk for animals in their care, and thus realized predation risk is generally negligible. However, unlike the other equation elements, perceived predation risk may still be experienced by animals in captivity. For instance, exhibits of prey and predator species may be close together, allowing for olfactory, auditory, and sometimes visual exchange between animals. Predator/prey odors can also be incorporated into enrichment regimes and activities (see Wells, 2009 for a review). It has even been suggested that visitors may represent a form of stress, and perhaps risk, to zoo animals (Davey 2007, Morgan and Tromborg 2007). So, while it is unlikely that zoos would intentionally house predators and prey in the same exhibit space, exhibits may still contain areas and elements that resident animals perceive as threatening and/or risky.

Since MOC and C are inherently controlled for in captive situations, areas of perceived risk (P) can be easily quantified. When provided with a number of patches that are equal in quality, an animal in a safe or comfortable situation/environment will exploit a food resource more thoroughly than an animal in a risky or uncomfortable environment (Whelan and Maina 2005). Therefore, extensive patch use (i.e. low GUD) can be used as a proxy to detect areas

where the forager feels safe and comfortable, whereas low patch use (i.e. high GUD) can indicate that the forager feels unsafe or uncomfortable (Brown 1988). In the wild, these procedures have been used to develop species-specific “landscapes of fear” (i.e. an environmental “map” of areas of preference and aversion; Brown et al., 1999; Laundré et al., 2010). Similar quantification methods can be used in captivity to develop an animal’s “landscape of comfort”/LOCs within their own exhibit, and can provide additional behavioral benefits to captive foragers.

2. **Putting theory into practice**

Following the completion of the foraging experiment with the mice, I felt confident enough to apply the techniques to my true passion: zoo populations. Inspired by Jo-Elle’s initial work with LOCs at the Brookfield Zoo, I was interested in taking zoo foraging ecology to the next level and explore additional applications. For example, how do animals perceive their exhibit, and can zoos incorporate these insights into exhibit modifications to improve an animal’s perceptions? Are food patches actually an effective form of behavioral enrichment, and if so, how effective are they? Can the presence of food patches influence visitor perceptions of an exhibit? I addressed these questions in two studies utilizing: (1) okapi (*Okapia johnstoni*) at the Brookfield Zoo (Chapter III), and (2) two Patagonian cavies (*Dolichotis patagonum*), and two Parma wallabies (*Macropus parma*) at the Lincoln Park Zoo (Chapter IV).

At the Brookfield Zoo, the okapi are a species of interest due in large part to the extreme stereotypic behavior demonstrated by the adult female, Augusta. Stereotypic behaviors have been defined as invariant and repetitive behaviors that seem to have no immediate function (Mason 1991), and a large portion of the captive okapi population has been noted to perform some form of stereotypic behavior (Bennett et al. 2015). At the time of the study, Augusta had recently developed a severe stereotypic head-rolling behavior that was of management concern.

Staff hoped exposure to more enrichment and foraging opportunities would reduce the incidence of the stereotypic behavior. An adult male, Hiari, was added to the study to provide a comparison to Augusta's behavior and preferences. While the male, Hiari, also demonstrated stereotypic mouthing and licking behaviors, zoo staff did not consider his stereotypies to be as concerning as Augusta's. This unique situation provided a valuable opportunity to evaluate the efficacy of food patches as an enrichment tool, and in particular, their potential to reduce stereotypic behaviors. This study lasted two summers (2013 and 2014), during which I was able to develop a successful food patch design. This allowed me to evaluate both Hiari's and Augusta's behavior and environmental preferences in two different exhibit yards. Based on intensity of foraging in experimental patches, I found that the two okapi varied in their response to and utilization of the same exhibit space. Further, food patches resulted in increased time spent foraging and within exhibit movement compared to traditional foraging methods, suggesting food patches provide a valuable behavioral enrichment opportunity. I concluded that individual behavioral differences must be incorporated into animal management and welfare decisions, and that food patches and GUDs can provide the tools necessary to evaluate these differences while providing valuable behavioral benefits to the forager.

At the Lincoln Park Zoo (Chapter IV), The Patagonian caviies and Parma wallabies were selected for several reasons. While they did not display any concerning stereotypic behaviors, both species received very limited enrichment opportunities. Food was typically presented at set times throughout the day, and was simply placed in two different trays within the exhibit. As such, zoo staff expressed great interest in experimenting with additional foraging opportunities for these species. Secondly, the cavy and wallaby exhibits were directly adjacent to each other at the zoo, which greatly facilitated adding an additional comparison on the effect that food patches

may have on visitor interest. Moreover, both species occupied relatively small exhibits that used exhibit tiering and topography to add visual interest, but the effect these exhibit modifications had on animal preferences was unknown. Finally, patch use studies on wild Patagonian caviés (Sombra 2011) and Bennett wallabies (*Macropus rufogriseus rufogriseus*; While and McArthur 2006) have been performed providing a valuable foundation regarding how these species may respond to food patches in captivity. Food patches reliably revealed environmental preferences for each species that were similar to their foraging responses in the wild. For both species, food patches also increased foraging time and within exhibit movement, and decreased the frequency of inactive behaviors, which suggest that food patches provide an effective behavioral enrichment technique. Finally, there appeared to be a trend toward increased visitor number and interest at the exhibits when food patches were provided, but these results were not significant. Future studies are needed to more thoroughly investigate the impact food patches may have on visitors.

E. Visual barriers and stereotypic behavior

Following the completion of the okapi food patch study, conversations with Dr. Lance Miller led to the development of an additional investigation involving Augusta and her stereotypic behavior. In this study (Chapter V), I examined the effect of placing visual barriers between the okapi holding areas on Augusta's performance of stereotypic behavior. As okapi are solitary in the wild (Hart and Hart 1988, Bodmer and Rabb 1992, Hart 2013), they are unlikely to have frequent visual contact with conspecifics. In zoos, however, limited space options can drastically impact how individuals are housed. Even if facilities have the space available to singly-house individuals, which would mimic the "wild" social scenario, these individuals may retain the ability to see conspecifics in other exhibit areas. The visual stimulus of a conspecific

without being able to directly interact could become frustrating, as natural social behavior cannot be expressed. Over time, such situations might lead to stereotypic behaviors (Mason et al., 2007). Using behavioral observations, I monitored Augusta's stereotypic behavior prior to and after the establishment of visual barriers in her holding area. The barrier completely blocked her visual access to the neighboring male okapi. I also distributed surveys to animal care staff, asking them to rate their impressions of changes in Augusta's behavior after visual barriers were established. Results from both the observations and surveys suggest that visual barrier installation drastically reduced Augusta's performance of stereotypic behavior, further demonstrating that captive social scenarios can have large impacts on animal welfare. Furthermore, construction of visual barriers can provide a low-cost solution for zoological facilities facing housing issues for solitary species.

F. Brushtail possum personality and foraging decisions

Like many of my lab mates, I had an interest in adding to my skill set and performing actual field work. But due to limited experience with "roughing it" while growing up, I was not entirely confident in how I would fare in a completely remote location. Following the completion of my zoo foraging ecology studies, I was awarded a NSF Graduate Research Opportunities Worldwide fellowship (GROW). The fellowship presented an amazing opportunity. However, this also meant leaving my family and living abroad for several months, which caused more than a little hesitation. Nevertheless, Joel and I discussed several possible collaborators, and due in part to my life-long desire to visit Australia, we approached Dr. Clare McArthur at the University of Sydney. After some trial and tribulation to secure funding and many conversations with Clare, I was off to Australia. There were many long nights spent in the middle of the park, and a few car hiccups (including learning how to drive a manual vehicle on the opposite side of

the road!), but the experience was unforgettable. It was the best possible scenario to gain field experience while retaining my own room, toilet, hot shower, and bed to return to! From a professional perspective, I gained international colleagues, learned valuable new statistical and methodological techniques, and had the experience of successfully performing field work. On a personal level, I made some incredible life-long friends as I experienced life in a different country.

My research in Australia investigated how individual animal personality influences foraging decisions, particularly the tradeoff between food quality and risk (Chapter VI). Animal personality traits (including boldness, exploration, activity, and aggressiveness; Réale et al. 2007) are known to have ecological implications for survival (Bremner-Harrison et al. 2004) and reproductive success (Both et al. 2005), and thus are inherently linked to individual fitness (Réale et al. 2007, Smith and Blumstein 2007). Important ecological activities, like foraging, are known to be influenced by personality traits. For example, bold captive fallow deer (Bergvall et al. 2011) and free-ranging grey mouse lemurs (Dammhahn and Almeling 2012) were found to forage more thoroughly in “risky” areas than shy individuals. While some GUD studies have evaluated the impact that individual energetic (Brown 1992, Olsson et al. 2002, Berger-Tal et al. 2010) and physiological state (i.e. ectoparasite load; Webster et al. 2007, Raveh et al. 2011) has on foraging decisions, most ignore individual variation in favor of evaluating population-level responses. Even fewer have directly evaluated the impact that individual animal personality may have on GUDs (Bedoya-Perez et al. 2013, Mella et al. 2015).

The goal of this study was to evaluate the influence of animal personality on decisions relating specifically to the tradeoff between nutrition and predation risk. Working with free-ranging brushtail possums (*Trichosurus vulpecula*) in the Ku-Ring-Gai Chase National Park,

NSW, Australia, I determined how animal personality influences the foraging choices of the brushtail possums. Along with several other students from Clare's lab, I trapped and established three personality metrics (boldness, docility, and exploration) for 12 possums. Those individuals were then released and monitored as they approached and ate at experimental foraging stations (created by me and Dr. Valentina Mella) to determine individual differences in preference for food quality and response to environmental risk. Each foraging block contained three above-ground "safe" feeders, which contained a different diet of varying nutritional quality (low, medium, high). The block also included a risky feeder with the high-quality diet to determine the nutritional quality at which possums were willing to switch from safe to risky feeders.

Of the 12 possums with established personalities, six visited the foraging stations. At a population level, possums biased their foraging toward "safe" feeders containing high quality food. They then preferred high-quality risky feeders, followed by safe, lower-quality feeders. At an individual level, the proportion of time spent at each of the four feeders differed, suggesting that individual personality plays a role in complex foraging decisions. Furthermore, individuals also varied in the proportion of time they spent in key behaviors (vigilance, foraging, and searching for food) at the feeders, further indicating that individual approach to foraging varies. In particular, the personality trait *activity* appears to explain some of the individual variation in these behavioral results. By linking established personality metrics to foraging decisions made at the experimental stations, this work will ultimately supplement current understanding regarding how individual personality traits influences important environmental and ecological choices.

G. Concluding remarks

The experience that I have gained throughout my Ph.D. studies has only furthered my desire to see foraging theory officially incorporated into animal welfare regimes, particularly for

captive animals. The potential to apply giving-up densities and patch use with the goal of understanding and improving captive animal care is still largely untapped. While I was able to evaluate patch-use as a tool to reveal landscapes of comfort, evaluate their efficacy as an enrichment technique, and begin to investigate its possible effect on visitor interest in the exhibit, any one of these aspects could have been pursued in much greater detail. For instance, the next logical step is to use animal LOCs to determine and implement opportunities for exhibit design and improvements, which I did not have the opportunity to pursue myself during my studies. For the okapi, this could have included evaluating density of canopy cover from one tree to the next to determine whether canopy quality influenced patch preference. In addition to canopy cover, alterations to the fenceline where Augusta experienced high GUDs (i.e. improved shade cloth quality, less permeable visual barriers, etc.) could have been used to determine whether her foraging aversions were the result of a visual issue. For the male okapi, aversions suggested a possible avoidance of the visitor area, but whether this was a result of actual human visitors, or the topography and layout of the exhibit itself remains largely unclear. In the case of the cavies and wallabies, given the LOC's demonstrated by both species, and the previous patch use studies in wild individuals, I would suggest switching the animals' exhibits. The cavies prefer open sightlines, and the wallaby exhibit is largely free of obstructions. The wallabies prefer foraging close to cover. The present cavy exhibit provides greater cover. After learning that one of the wallabies and one of the cavies has passed away since the conclusion of the LPZ study, I also believe that providing additional foraging opportunities through food patches is more important than ever, particularly for the social cavy. Much expense and expertise goes into exhibit design, but what is lacking are tools to assess, in advance, the preferences of the animals themselves. Just about everyone's opinion is included, save for the animals themselves!

Beyond using LOC's to improve exhibit spaces, I highlight several additional applications of patch-use studies for captive species in Chapter IV that could have a genuine impact on revealing and understanding aspects of animal welfare. For example, longitudinal patch-use studies could provide an opportunity to effectively quantify changes in animal foraging as they happen, and thus can reveal changes in physical health, seasonal differences, and social hierarchies in real time. Increasing foraging opportunities via food patch studies can also impact stereotypic behavior, as increasing foraging time leaves less time for other abnormal behaviors. Overall, patch-use studies are an effective and low-cost method to reveal nuanced information about animal perceptions, directly from their perspective. I sincerely hope to be in a position in the future where I can pursue applications of captive patch-use and GUDs more extensively.

Finally, throughout my graduate studies I have discovered that I am passionate about engaging young scientists in behavioral ecology research. Throughout my dissertation, I was able to advise several undergraduates as they used foraging theory to further explore their own interests, which was an incredibly rewarding experience. Currently, I volunteer at the Holly Middle School (Holly, MI) where I work with the Ecology class as they develop their own LOC foraging study with white-tailed deer in the field behind the school. Their creativity and genuine interest has demonstrated that patch-use theory and techniques are accessible to even 7th and 8th graders, and that such studies can be incorporated into the science curriculum at a young age to demonstrate ecosystem and ecological interactions in action. I ultimately see myself as a research scientist at a University or zoological facility. Yet, I certainly hope to continue outreach efforts with K-12 students in my future career.

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II. FORAGING DECISIONS IN WILD VERSUS DOMESTIC *MUS MUSCULUS*: WHAT DOES LIFE IN THE LAB SELECT FOR?

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S. M. Troxell-Smith, M. J. Tutka, J. M. Albergo, D. Balu, J. S. Brown, J. P. Leonard. Foraging decisions in wild versus domestic *Mus musculus*: What does life in the lab select for? *Behavioural Processes*, 129; 43-50: 2016. doi:10.1016/j.beproc.2015.10.020.

A. Abstract

What does domestication select for in terms of foraging and anti-predator behaviors? We applied principles of patch use and foraging theory to test foraging strategies and fear responses of three strains of *Mus musculus*: wild-caught, control laboratory, and genetically modified strains. Foraging choices were quantified using giving-up densities (GUDs) under three foraging scenarios: (1) patches varying in microhabitat (covered vs. open), and initial resource density (low vs. high); (2) daily variation in auditory cues (aerial predators and control calls); (3) patches with varying seed aggregations. Overall, both domestic strains harvested significantly more food than wild mice. Each strain revealed a significant preference for foraging under cover compared to the open, and predator calls had no detectable effects on foraging. Both domestic strains biased their harvest towards high quality patches; wild mice did not. In terms of exploiting favorable and avoiding unfavorable distributions of seeds within patches, the lab strain performed best, the wild strain worst, and the mutant strain in between. Our study provides support for the hypothesis that domestic animals have more energy-efficient foraging strategies than their wild counterparts, but retain residual fear responses. Furthermore, patch-use studies can reveal the aptitudes and priorities of both domestic and wild animals.

B. Introduction

Animal domestication may reverse or relax selection for traits sculpted by natural selection in the wild (Price 1984, 1999). Wild animals continually respond to fluctuating climatic conditions and uncertainty in the availability, distribution, and variety of foods. This enhances predator avoidance and increases the efficiency of acquiring resources, mates, and shelters. Domestic animals, on the other hand, are generally maintained under relatively static and controlled environments with reduced predation risk (Augustsson & Meyerson 2004; Hale 1969). Moreover, the process of domestication typically involves some form of artificial selection for specific morphological and behavioral characteristics (Driscoll, Macdonald & O'Brien 2009, Hare, Wobber & Wrangham 2012, Mignon-Grasteau et al. 2005). Hence, the combination of artificial selection and a static environment may simplify an animal's behavioral repertoire (Hale 1969), resulting in less sophisticated responses to environmental stimuli (Budiansky 1994, Hemmer 1990). Alternatively, some studies have suggested that removal of environmental uncertainty and predation risk may select for domestic animals to develop extremely efficient foraging strategies, (Andersson, Nordin & Jensen 2001, Gustafsson et al. 1999, Jensen & Gustafsson 1997).

Foraging theory provides a practical framework to study how animals perceive and respond to heterogeneity in predation risk and resource abundances, and how these perceptions may have changed during the domestication process. In nature, resources typically vary in time and space. Foragers must decide when to give up on depleting a food patch prior to moving on to another patch, or to another activity. The amount of food left over in a depletable, experimental food patch after a foraging bout (the giving-up density, or GUD; Brown 1988) provides a metric for how an animal perceives its environment. GUDs have frequently been

used as environmental assessments of food preferences, predation risk, and foraging aptitudes for wild species, including rodents (Arthur, Pech & Dickman 2004, Brown 1988, Kotler, Brown & Knight 1999, Powell & Banks 2004, Ylonen et al. 2002). GUD studies in domestic species are rare, with work limited to laboratory rats (Arcis & Desor 2003) and domestic goats (Shrader et al. 2008, Shrader et al. 2012).

The house mouse (*Mus musculus*) is an ideal organism for understanding the influence of domestication on foraging choices and responses to predation risk. As ubiquitous human commensals, wild house mice are exposed to selection pressures from predation risk and food scarcity and unpredictability. They should exhibit sophisticated behaviors in response to food and safety. Their domestic counterpart, the common laboratory mouse (Yang et al. 2007), has been bred and maintained in captivity for generations (in many cases, since the early 1920's; Beck et al., 2000) with *ad libitum* access to food and relative safety. Comparison of foraging strategies within these strains of *M. musculus* can therefore provide unique insight in how domestication and captivity may influence foraging aptitudes and perceptions. Additionally, because some strains of laboratory mice have been genetically manipulated, house mice provide an opportunity to examine how these manipulations influence foraging.

Here, we examine how domestication has affected the foraging decisions and environmental perceptions of three strains of house mouse: 1) wild-caught, 2) a standard lab strain, and 3) a genetically modified “mutant” strain with a mutation affecting its ability to learn and generate memories appropriately (Balu, Schmidt & Leonard 2012). We applied the GUD approach to compare the behavioral sophistication of these strains to cues of predation risk and heterogeneity in the patchiness of seed resources. Does domestication result in a lack of foraging aptitudes, or alternatively, more energy-efficient foraging strategies? We

tested the following predictions and hypotheses, presenting alternative hypotheses where appropriate:

-Risk/Fear: Wild mice should demonstrate strong fear responses and exhibit higher GUDs in response to patches lacking cover (relative to those under cover), and higher GUDs in response to auditory cues of predation (relative to control cues). The standard lab strain should demonstrate less change in GUDs in response to cover or auditory cues, and the genetically modified mutant strain should respond the least.

- Initial Resource Density: Wild mice should be capable of differentiating between patches of varying quality, as this permits more efficient food harvest. Wild mice should bias foraging effort towards rich rather than poor patches (equalize GUDs between rich and poor patches). Proportion of food harvested should increase with initial patch richness. If domestication has selected for a reduction in foraging aptitudes, then the lab strain should be less responsive to patch richness (i.e. will not detect richness differences). If the mutant mouse is further rendered “clueless”, it should respond the least (highest difference in GUDs between rich and poor patches). Alternatively, domestication may have selected for more energy efficient foraging strategies resulting in higher foraging performances than the wild mice.

-Seed Location: Optimal foraging predicts that foragers should bias their harvest towards favorable arrangements of seeds within a patch while avoiding unfavorable arrangements (Brown & Mitchell 1989). We would expect wild mice to have lower GUDs in patches where seeds are concentrated in just half of the substrate, or have higher GUDs in patches where seeds are concentrated at the bottom of the patch. The difference in GUDs between favorable and unfavorable seed arrangements should be less for the lab strain and least for

the mutant strain if the domestic strains have become inept. Alternatively, the opposite may occur if domestication has selected for higher foraging efficiencies.

C. Methods

1. Study animals

The house mouse (*Mus musculus*, Linnaeus 1758) is an abundant human commensal throughout the Chicago region (Hoffmeister & Mohr 1972). Wild male and female *Mus musculus* were humanely captured using Sherman traps (7.6 cm x 8.9 cm x 22.9 cm; H.B. Sherman Traps, Inc.) from three locations within and around the city of Chicago. One trapping location was on the greenhouse grounds of the University of Illinois at Chicago (UIC) campus, the second was the garage of SMTS, and the third was a sun porch of JSB. All wild caught mice would therefore likely have exposure to both indoor and outdoor pressures, including human hazards, pets, and natural avian and terrestrial predators. Traps were checked and emptied daily. Captured wild mice were transported to a laboratory at UIC where they were placed individually into experimental arenas (see *Experimental Set-Up*). Wild mice that were <10g were released.

Domestic *Mus* of both sexes came from ongoing research in the lab of JPL, UIC. All mice were fully grown (≥ 90 days old) and were on a C57BL/6 background. Here, the term background describes the original inbred laboratory strain from which our lab strains were derived. Our baseline control strain (hereafter “lab” strain) represented the control progenitor of the mutant strain. The genetically modified strain had a transgenic mutation that lowers anxiety. Specifically, these *Grin2adeltaPKC* mice (hereafter “mutant” mice) have site directed mutations in two Serines and two Tyrosines in the C-terminus of the NR2A subunit of the NMDA receptor (Balu, Schmidt & Leonard 2012). These sites, and homologous sites in

Grin2b, may help regulate changes in synaptic strength that underlie learning, memory, and anxiety-related behaviors (Balu, Schmidt & Leonard 2012, Liao et al. 2001).

2. **Experimental set-up:**

In accordance with the Institutional Animal Care and Use Committee (protocol no. 12-017), experiments were conducted in an indoor laboratory at UIC. All mice were housed individually in ventilated plastic arenas: a clear, plastic 85L Rubbermaid container (88.9cm x 42.5cm x 32.7cm) with a transparent cover. Each arena contained a nest box with shredded paper towel for bedding, *ad libitum* water, four foraging trays, and two shelves (each 35cm x 31.8cm x 15.2cm). Foraging trays were placed under and on top of each shelf (Figure 1A). Commercially available millet seed was provided in the trays *ad libitum* until experiments began, and was also provided *ad libitum* in between experiments. Carrots and greens were provided as a dietary supplement, but in limited amounts so as to not constitute a significant calorie source for the mice. Prior to our study, we performed a pilot experiment to ensure that all of the house mice can be successfully maintained on a millet diet supplemented with greens and carrots (unpublished data). During experiments, seeds were provided as described below. All mice were allowed to acclimate to their arenas for at least a week prior to the commencement of the experiment. Animals remained in arenas between experiments, and arenas were sanitized after the conclusion of each study. The room had a 12:12 light-dark cycle, and room temperature was kept at $22 \pm 1^{\circ}\text{C}$. All experiments occurred between August and November 2012.

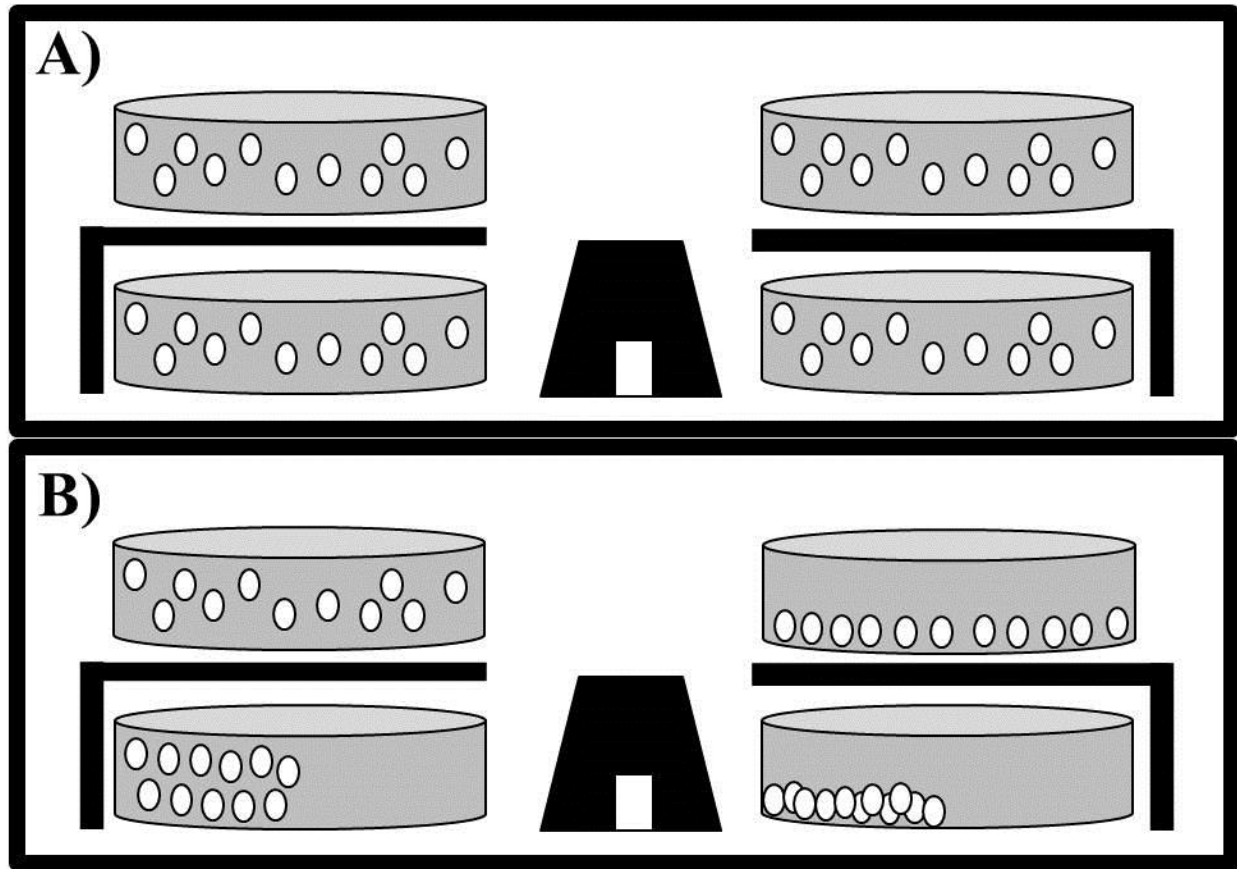


Figure 1. A) Graphic of basic experimental arena set-up containing a central nest box (black) and four food patches. Each food patch is depicted as a gray saucer with sand and millet seeds (white ovals) mixed randomly throughout the patch. Shelves (black structures) separated “covered” and “open” trays. Each tray is situated either on top of shelf (open), or below shelf (cover). Millet seed density and seed location within patch varied according to individual study protocol. B) Graphic of seed orientation within a tray for Study 3. Random/Full (R,F) orientation tray- top left; Bottom/Full (B,F) orientation tray- top right; Random/Half (R,H) orientation tray-bottom left; Bottom/Half (B,H) orientation tray-bottom right.

Animals were kept in the arenas for a total of five weeks, and were cycled through each of the three studies in the following order: Study 1, Microhabitat and Initial Resource Density; Study 2, Auditory Cues of Predation Risk; and Study 3, Altered Seed Location within patches. All mice were weighed prior to placement in arenas, and again at the conclusion of the experiment to determine whether weights were maintained throughout the experiment. Animals were placed in a container, then on to a balance accurate to the nearest 0.1g. Mice were humanely euthanized via CO₂ at the conclusion of the study.

3. Data collection

We used GUDs to quantify and compare the foraging prowess and preferences of our three mouse strains. To generate GUDs, food was mixed with an inedible substrate to create diminishing returns to harvest when foraging (Brown 1988). Following standard GUD procedures for wild rodents (Kotler 1997, Leaver & Daly 2003, Mohr et al. 2003, Morgan & Brown 1996), foraging trays consisted of plastic saucers (25cm in diameter) filled with millet seeds mixed into 1L of sifted sand. Resulting sand depth in tray was approximately 2.5 cm. The amount of millet and mixing procedures varied with experiment. In each study, animals freely foraged in patches for 24 hours. Foraging trays were removed and sieved every 24 hours to recover the uneaten millet seeds from the sand. The seeds were cleaned of debris and weighed to provide the GUD. Following sieving, trays were restocked with millet and placed back into the arenas.

4. Study 1: Microhabitat and initial resource density

For this study, we used six mutant (five male, one female), 11 lab (eight male, three female), and 13 wild mice (eight male, five female). Mouse numbers between strains varied based on availability – mutant and lab strains were generally limited by availability from

the breeding colony. We tested for effects of microhabitat (preference for foraging in the open or under cover), and initial resource density (hereafter IRD; 1g or 2g of millet per patch). Each arena contained two, 2g millet patches and two 1g millet patches, which were placed under the shelves (cover microhabitat) or on top of the shelves (open). As a fully crossed design, if the patch on top of a shelf was a 2g patch, the corresponding patch underneath the shelf was a 1 g patch, and orientation on the opposite shelf was reversed. Patch orientation was switched daily to control for any side bias. Data were collected from each mouse for 4 days. We then converted raw GUD measurements into proportions using the following equation: $((\text{IRD-GUD})/\text{IRD})$ to investigate foraging strategies of each strain.

5. **Study 2: Auditory cues of predation risk**

We used six mutant (five male, one female), 11 lab (eight male, three female), and 14 wild (eight male, six female) mice for this study. Arena set-up was identical to Study 1: foraging patches consisted of either 1g or 2g of millet seeds (altered IRD) mixed randomly into 1L of sand. As described above, foraging patches were oriented in a fully crossed design. Mice were exposed to one of four different auditory cues during a 24 hour foraging bout: red-tailed hawk (*Buteo jamaicensis*), great horned owl (*Bubo virginianus*), crickets (*Gryllus pennsylvanicus*), and humans (*Homo sapiens*). Hawk and owl species were chosen for their known predation on *Mus musculus* (Artuso et al. 2014, Hoffmeister 2002, Preston & Beane 2009), and for their prevalence in the Chicago, Illinois area. Predator cues were retrieved from the Cornell Lab of Ornithology (<http://www.birds.cornell.edu>). Human speech may be viewed benignly or as a threat, and cricket chirps served as a benign control cue, and were retrieved from YouTube (www.youtube.com). Using speakers placed at opposite ends of the lab, a single cue was played for 15-30 sec every half hour throughout the 24 hour foraging period. Cue order was

decided via Latin Square design. Data were collected from each mouse for 8 days, resulting in 2 days of each cue per mouse.

6. Study 3: Altered seed location

We used four mutant (three male, one female), seven lab (seven males), and 12 wild (seven male, five female) mice for this portion of the study. Arena set-up was similar to Study 1, except each foraging tray contained 2g millet seeds, and the distribution of seeds within a tray was varied. Seed position within a tray fell into all four combinations of two levels of vertical dispersion and two levels of horizontal dispersion. Vertical dispersion could be across the full depth profile of the tray (R), or isolated to the bottom half of the sand's depth profile (B). Horizontal dispersion could be across the full surface of the tray (F), or isolated to one half of the tray's surface (H). Therefore, Random/Full (R, F) trays had the millet mixed randomly vertically and horizontally throughout the 1L of sand. Bottom/Full (B, F) trays had all seeds evenly dispersed horizontally, but isolated to the lower 500mL of sand. Random/Half (R, H) trays had all seeds evenly dispersed vertically, but isolated to 500mL of sand on one side of the tray. Bottom/Half (B, H) trays had all seeds isolated to the bottom 250mL of sand on one half of the tray (Figure 1B.). These tray configurations resulted in a fully-crossed, two- factorial experimental set-up. Individual patch orientation within the arena was decided via Latin Square design. Data were collected from each mouse for 8 days.

7. Analysis

All data were analyzed using partially-hierarchical ANOVAs under the general linear models of SYSTAT 13 (SPSS Inc., Chicago, IL, U.S.A.). Individual mice were nested within strain for all analyses, thus avoiding pseudo-replication for between strain comparisons while providing maximum statistical power for within mouse effects. We initially tested for and

included the effects of mouse sex. In this case, individual mice were nested within strain and sex. However, as there were no significant main or interaction effects with sex in Study 1, and Study 3 only had males available for the lab strain, we removed sex from further analyses for those two studies. In Study 2, full analysis of sex for all strains was not possible due to the mutant strain having just one female (5 males to 1 female). We therefore tested for treatment effects without sex, and tested for sex by re-running the analysis with just the lab and wild strains, and found a main effect of sex along with several interactions. We report both the initial full analysis with all three strains (Analysis 2A), and a secondary analysis (Analysis 2B) which allowed us to more closely examine sex differences between the lab and wild strains. The remaining factors in Study 1 and 3 were fully crossed with mouse and strain, and so could be tested over the full model's error degrees of freedom. Due to experimental designs, effects of microhabitat could be analyzed in each of the three studies. Initial Resource Density (IRD) effects were analyzed in Study 1 and Study 2, and the vertical and horizontal seed dispersion treatments replaced IRD in Study 3 as independent variables. GUD was used as the dependent variable in all analyses, and proportion of food harvested was also used as the dependent variable to further analyze for effects of IRD in Study 1. We used days of each experiment as replicates rather than repeated measures since the GUD measurement on one day does not perforce dictate the GUD of the next. We also satisfy conditions of sphericity (von Ende 1993).

D. Results

1. Study 1: Microhabitat and initial resource density

Mean GUDs for all strains, not just the wild strain, were significantly lower under cover compared to the open microhabitat ($F_{1, 715} = 13.9, P < 0.001$) (Figure 2). The domestic strains exhibited significantly lower GUDs than the wild mice ($F_{2, 27} = 34.9, P < 0.001$).

Contrary to initial hypotheses, there was no significant interaction between strain and microhabitat ($F_{2, 715} = 0.8, P > 0.05$). Lab and mutant strains retain a fear response and preference for cover. GUDs increased significantly with initial resource density ($F_{1, 715} = 244.6, P < 0.001$). The increase in GUDs with initial resource density was significantly more striking for the wild mice than for the lab strains (interaction of strain with IRD: $F_{2, 715} = 57.2, P < 0.001$). Finally, there was a significant effect of individual within strain, indicating individual variation among mice in their GUDs independent of strain ($F_{27, 715} = 7.0, P < 0.001$).

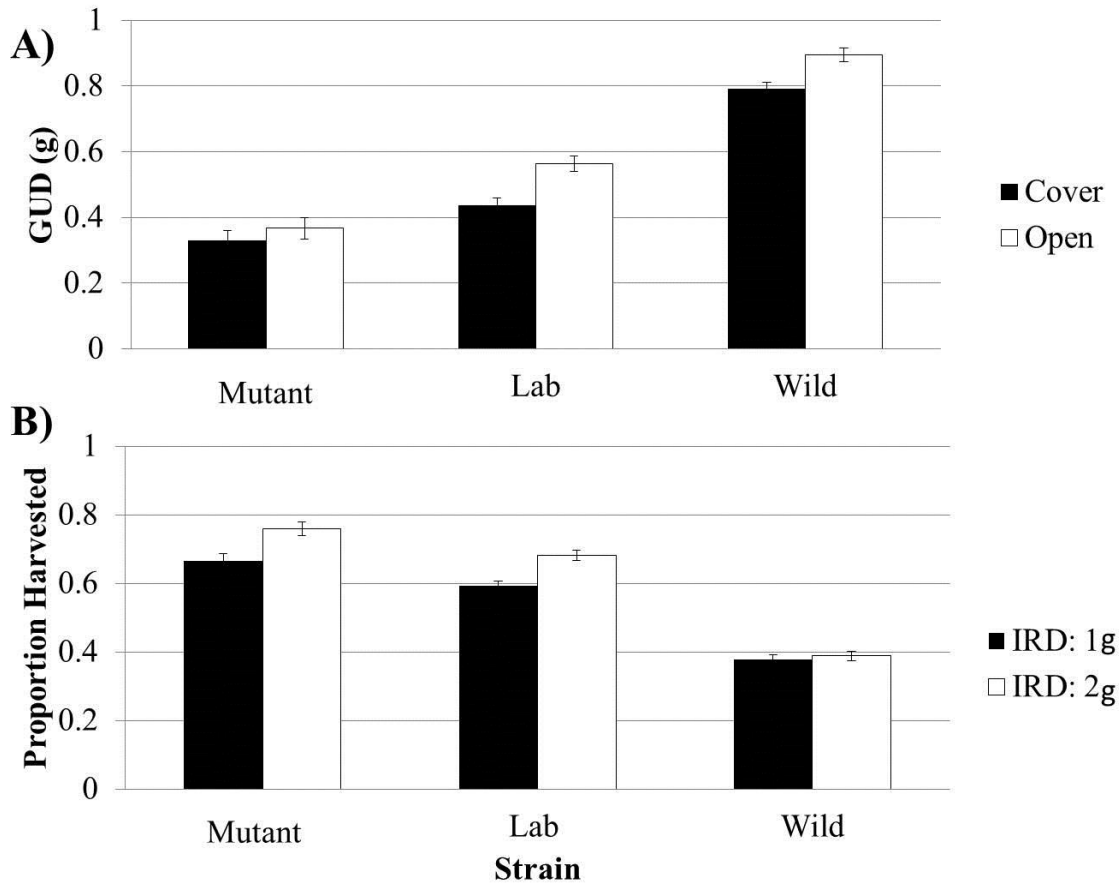


Figure 2. GUDs and proportion of food harvested in the microhabitat and IRD study. A) Pooled mean GUD (\pm SEM) for the three mouse strains in the cover (gray) vs. open (white) patch orientations. Both microhabitat (cover/open) ($P < 0.001$) and mouse strain ($P < 0.001$) had significant effects on GUDs. B) Pooled mean (\pm SEM) proportion of seeds harvested for each mouse strain in the 1g (gray) and 2g (white) patch orientations. There was a significant interaction of mouse strain and Initial Resource Density (IRD) ($P < 0.001$) on GUDs.

In terms of proportion of seeds harvested from food patches, both lab strains of mice biased their foraging towards rich patches and depleted a larger proportion of food from the 2g

patches than the 1g patches. The wild mice depleted approximately the same proportion from each patch (Figure 2). We plotted mean GUDs for each strain on depletion trajectory curves (IRD^{-1}), and found that the wild mice most closely follow a “fixed time” strategy, whereas both the mutant and lab strain trend toward a “fixed quitting harvest” strategy (Abu Baker & Brown 2009, Brown & Morgan 1995; Figure 3).

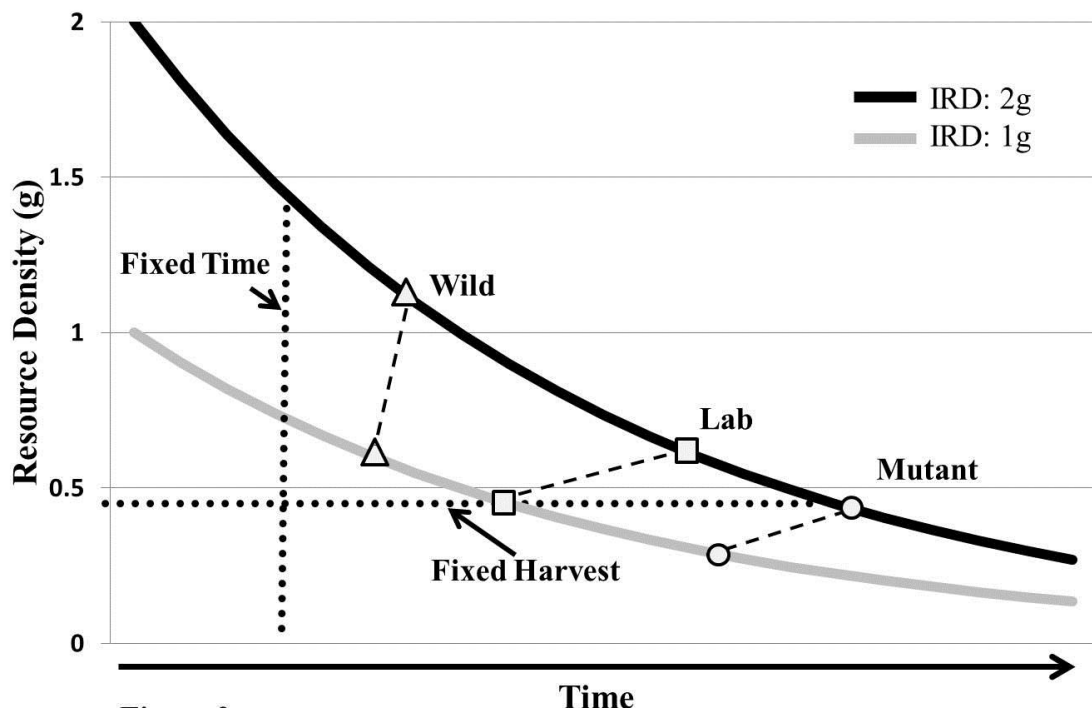


Figure 3. Mean GUDs plotted on depletion trajectories (Initial Resource Density; IRD^{-1}) for the three strains of mice. Mean GUD \pm SEM for Mutant (circles): 1g = 0.323 ± 0.026 ; 2g = 0.458 ± 0.035 ; Lab (squares): 1g = 0.4 ± 0.02 , 2g = 0.62 ± 0.036 ; Wild (triangles): 1g = 0.616 ± 0.014 , 2g = 1.212 ± 0.024 . Fixed time and fixed harvest strategies are presented as circle dashes, and are adapted from Brown and Morgan (1995).

2. **Study 2A: Auditory cues of predation risk (full analysis)**

As in Study 1, strain, initial resource density and microhabitat all significantly influenced GUDs ($F_{2, 28} = 21.2, P < 0.01$; $F_{1, 932} = 576.0, P < 0.001$; and $F_{1, 932} = 15.5, P < 0.001$ respectively). As before, GUDs were lower under cover than in the open, the two lab strains had lower GUDs than the wild mice, and GUDs increased with initial resource density. The two lab strains were again more successful than the wild strain at biasing activity towards the richer food patch (interaction of strain with IRD: $F_{1, 932} = 90.3, P < 0.001$). Contrary to our hypotheses, there was no effect of noise on mean GUD ($F_{3, 932} = 1.0, P > 0.05$). We also found no significant interaction between noise and mouse strain ($F_{6, 932} = 0.6, P > 0.05$) (Figure 4). As in Study 1, GUDs varied significantly between individual mice within strains ($F_{28, 932} = 19.0, P < 0.001$).

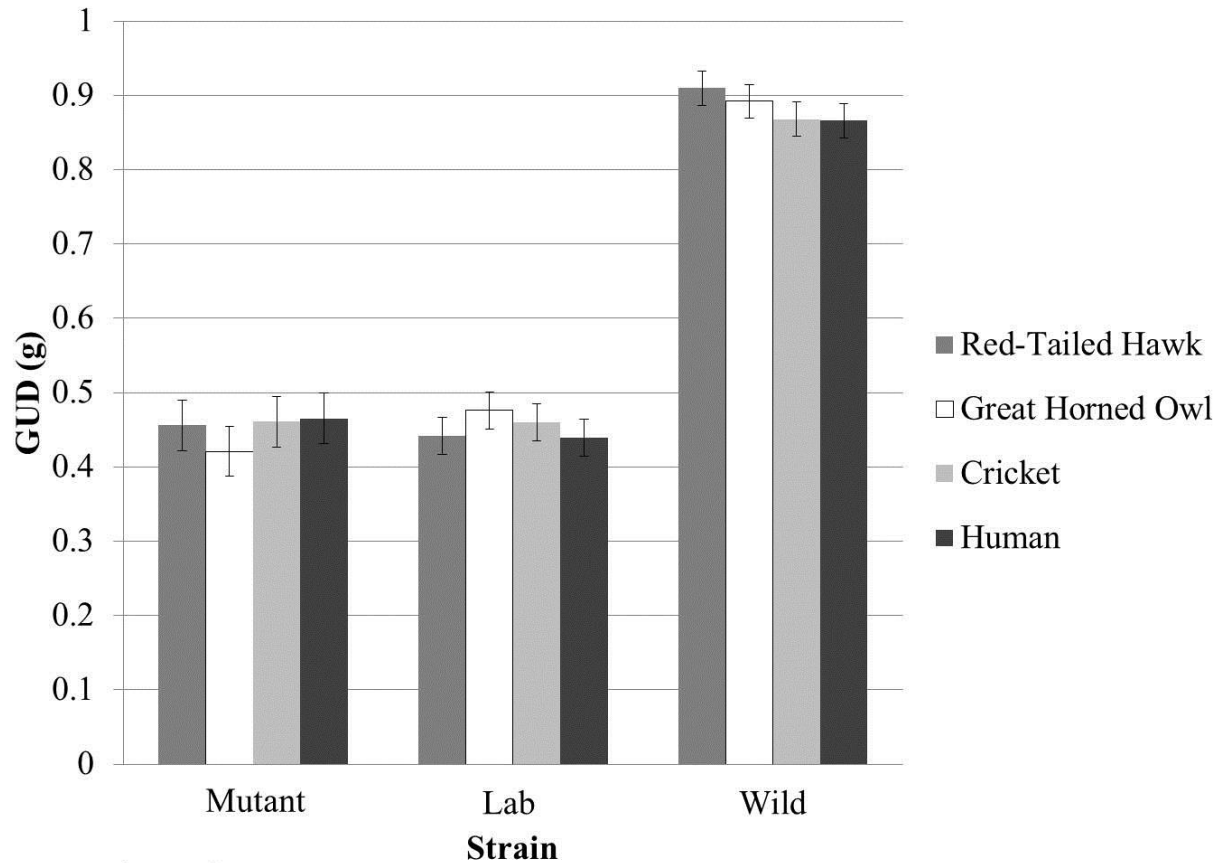


Figure 4. Mean (\pm SEM) GUD for each mouse strain in response to each auditory cue: red-tailed hawk (medium gray), great horned owl (white), cricket (light gray), and human (dark gray). There was no significant effect of auditory cues on mouse foraging ($P < 0.40$).

3. Study 2B: Auditory cues of predation risk (sex differences)

Unlike in the first experiment, sex contributed to a number of statistically significant effects. All statistically significant effects remain as reported in the previous analysis. Males had significantly lower GUDs (0.62g versus 0.79g) than females ($F_{1,21} = 6.7$, $P = 0.015$).

A significant interaction effect of mouse sex and initial resource density ($F_{1,753} = 21.3$, $P < 0.001$) revealed that males were better at biasing their foraging towards the rich patches. A significant interaction effect of mouse sex and microhabitat revealed that females were more averse to the open microhabitat than males ($F_{1,753} = 20.9$, $P < 0.001$ respectively). The interaction of strain and sex was not significant ($F_{1,21} = 4.05$, $P > 0.05$).

4. Study 3: Altered seed location

As in the prior two studies, domestic strains had significantly lower GUDs than the wild mice ($F_{2,20} = 64.1$, $P < 0.001$). While GUDs continued to be lower in the covered than open microhabitat, this effect was not significant ($F_{1,701} = 3.3$, $P > 0.05$). As predicted, seed dispersion influenced GUDs. In terms of vertical dispersion, as expected, GUDs were significantly higher when seeds were on the bottom (B) than when randomly dispersed (R) ($F_{1,701} = 1,936.4$, $P < 0.001$, respectively). In terms of horizontal dispersion, GUDs were not significantly different when seeds were dispersed in just half the surface (H) than the full surface (F) ($F_{1,701} = 2.8$, $P > 0.05$). As before, GUDs varied significantly between individual mice within strains ($F_{20,701} = 4.2$, $P < 0.001$).

There were significant strain by horizontal dispersion and strain by vertical dispersion interactions ($F_{2,701} = 8.5$, $P < 0.001$; $F_{2,701} = 44.1$, $P < 0.001$ respectively, Figure 5), indicating that the strains differed in their ability to assess the varied seed locations. There was no significant interaction between horizontal (Full/Half) and vertical (Random/Bottom) seed dispersions ($F_{1,701} = 1.8$, $P > 0.05$). The progenitor lab strain was most successful at taking advantage of favorable dispersions while avoiding unfavorable ones, whereas the wild-caught mice were least successful in biasing their foraging toward favorable dispersions.

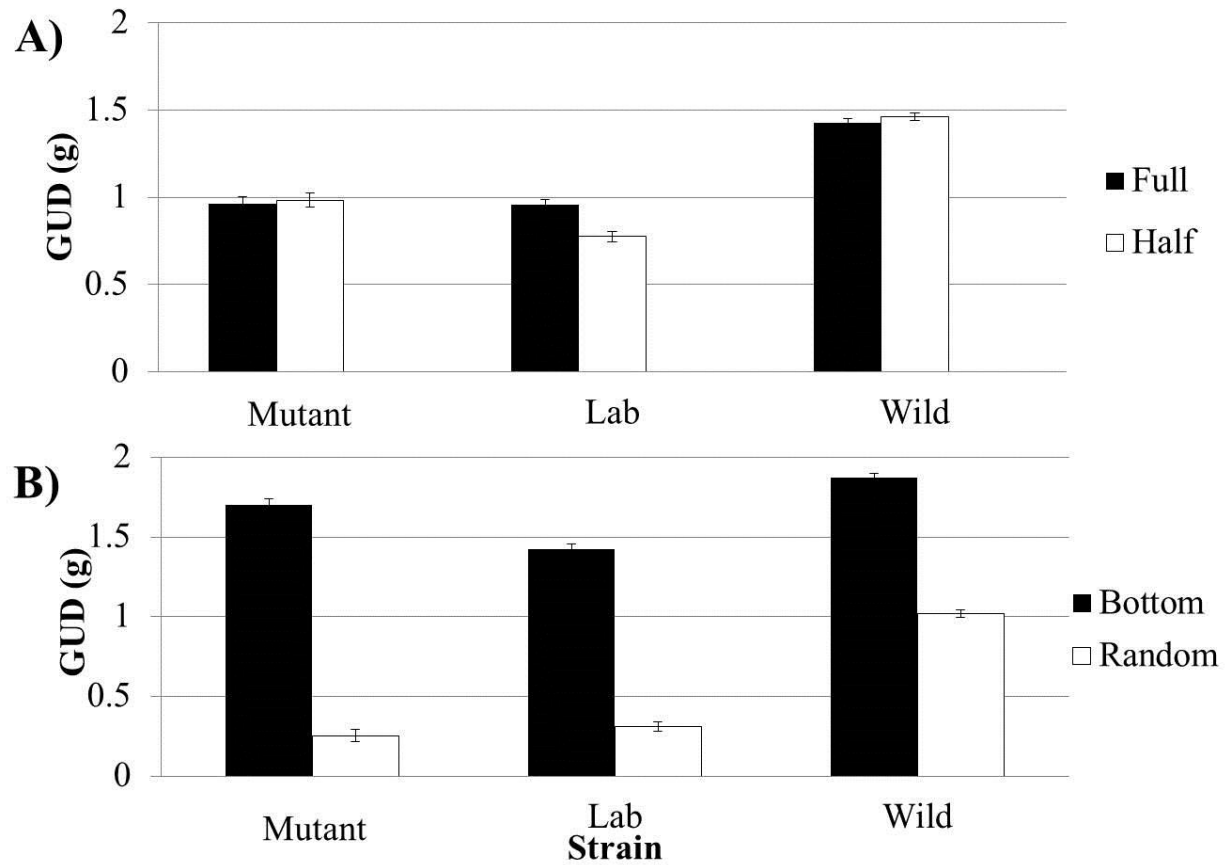


Figure 5. GUDs in the altered seed location experiment. A) Mean (\pm SEM) GUD for each mouse strain in patches with seeds in full (gray) and half (white) seed orientations. There was a significant interaction between mouse strain and the full/half seed orientation on GUDs ($P < 0.001$). B) Mean (\pm SEM) GUD for each mouse strain in patches with seeds in bottom (gray) and random (white) seed orientations. There was a significant interaction between mouse strain and the random/bottom seed orientation ($P < 0.001$).

E. Discussion

Using a giving-up density approach, we compared the patch use behavior of two domestic and one wild strain of house mice, *Mus musculus*. The domestication and simplicity of lifestyle of the domestic strains did not seem to compromise their foraging aptitudes. Quite the opposite, our results support our alternative hypotheses. In terms of biasing foraging towards rich and favorable patches and away from unfavorable ones, the standard lab strain performed best, the genetically modified mutant strain in between and the wild mouse worst. As expected, wild-caught mice perceived cover as the safer microhabitat compared to the open, but this preference was retained and shared by both domestic strains. Despite previous studies suggesting that wild rodents (Hendrie, Weiss & Eilam 1998) and laboratory mice (Hendrie & Neill 1991) respond to auditory cues of predation, the auditory cues of predation risk used in this study had no effect on the patch use of any of the three strains. It is possible that the mice habituated to the auditory cues provided during study, or the behavioral responses of the mice to the calls were too acute to be detected by GUDs alone.

The perception of higher levels of risk in open patches rather than covered is consistent with numerous studies of diverse rodent species that show lower GUDs in “bush” compared to “open” microhabitats in the wild (Brown & Alkon 1990, Jacob & Brown 2000, Kotler et al. 2002, Mohr et al. 2003, Valone & Brown 1989, Ylonen et al. 2002). Despite generations of domestic breeding and artificial selection, both domestic mouse strains responded as strongly to microhabitat as the wild-caught mice. These results coincide with Arcis & Desor (2003), who found that laboratory rats prefer foraging in a covered patch compared to an exposed patch. Associating environmental structure with predation strongly drives foraging decisions in wild animals (Thorson, Morgan & Brown 1998), and it appears that these preferences remain in these domestic strains as well.

Wild mice performed poorly in response to patches varying in quality. When presented with patches of high or low initial food abundance, astute foragers should harvest a higher proportion of food from rich rather than poor patches (Abu Baker & Brown 2009, Brown & Morgan 1995, Mitchell & Brown 1990, Valone & Brown 1989), and should harvest more from patches that contain a more favorable arrangement of seeds per unit area (e.g. micropatch partitioning; Schmidt and Brown, 1996). In our study, the proportion of food harvested by the wild mice was independent of initial patch quality. Thus, the wild mice exhibited a less energy-efficient “fixed time” foraging strategy (Brown & Morgan 1995). Furthermore, the wild mice did not take advantage of having all of the seeds concentrated into half of the patch’s surface area, and overharvested the patches when all seeds were in the bottom half relative to the two domestic mouse strains.

Regardless of the foraging experiment, the wild mice always had higher GUDs than the domestic strains. Despite harvesting less food overall, the wild mice did explore and forage from all available patches in each study. From this paradox, we conclude that the wild mice minimized activity while the domestic strains may have had overall higher activity levels including time spent simply exploring or wandering the enclosure. It is also possible that the wild mice may simply have lower mass specific metabolic rates compared to the domestic strains; however our data do not allow us to directly address this possibility.

The poor aptitudes of the wild mice in response to patch qualities may result from apprehensive foraging. Kotler et al. (2002) measured the ability of wild gerbils to micropatch partition within an aviary. The gerbils demonstrated low performance on challenging foraging tasks as a result of redirecting attention from foraging to predator detection in a manner that lowers predatory risk, feeding rate and overall foraging aptitudes (Brown, Kotler & Bouskila

2001, Dall, Kotler & Bouskila 2001). While we have no direct behavioral measurements on activity or apprehension (e.g. vigilance, exploration, etc.) in this study, wild mice did appear to spend a large amount of time in their nest boxes while humans were present, whereas the domestic strains were frequently active and outside of their nest boxes in the presence of the researchers (SMTS, personal observation). Augustsson & Meyerson (2004) and Augustsson et al. (2005) noted a similar situation: wild mice performed less active behaviors and displayed greater avoidance of open areas in common laboratory tests when compared to two domestic laboratory strains (C57BL/6 and BALB/c). We believe that the highly confined, unfamiliar arena environment of the present study may have heightened the apprehension level of the wild mice. Despite this possibility, the performance of the wild-caught mice still provides a valuable reference point for evaluating the performance of the domestic strains.

In general, the domestic strains performed better on each foraging task than initially anticipated. For example, both strains biased their foraging toward richer patches. By using a “fixed-quitting-harvest rate” patch use strategy, these strains maximized the rate of energy intake while minimizing foraging time (Brown & Morgan 1995). They also preferred patches with seeds aggregated into one half of the tray, and biased effort away from patches where the seeds were buried in the bottom of the tray, indicating that strong aptitudes for resource detection still exist despite generations of access to *ad libitum* food.

Several previous studies that have reported sex differences in behavioral performance of laboratory mouse strains (Ennaceur et al. 2006, Rodgers & Cole 1993, Võikar et al. 2001), and indeed, we also detected several differences in Study 2. Overall, males had significantly lower GUDs than females, were better at biasing behavior towards the rich patch, and were more willing to harvest the risky “open” microhabitat than females. Together, these results suggest

that the male mice were less fearful than females (for both lab and wild strains). These results accord with several previous studies indicating that male C57BL/6 laboratory mice were more willing to explore novel situations and objects, or were less risk averse, than both their female (Frick & Gresack 2003) and wild strain (Augustsson & Meyerson 2004) counterparts .

Given the key role of NMDA receptor phosphorylation in synaptic plasticity (Wang et al. 2014), the mutations in the mutant strain could influence a variety of responses and motivations related to learning, memory, and foraging aptitudes. For example, this mutant strain was found to demonstrate less anxiety-related behavior compared to its wild-type laboratory counterpart (Balu, Schmidt & Leonard 2012). Our present study however revealed surprisingly few differences in foraging abilities and performance between the mutant strain and its progenitor strain. The progenitor lab strain biased foraging toward the more favorable half patches, whereas the mutant strain did not. This difference in foraging aptitude suggests that the mutation may negatively influence resource detection abilities. Additionally, the mutant mice had significantly lower overall GUDs than the lab strain. The mutation may increase food motivation directly or indirectly through increased activity and energy expenditure. It is also possible that the mutation may result in behavioral differences in foraging approach, which cannot be detected with the GUD technique alone (Bedoya-Perez et al. 2013). Direct behavioral observations or video recordings could be used to evaluate these possible differences.

These results contribute to the discussion on whether domestic animals have lost aptitudes or have evolved shifted aptitudes. The demonstrated differences in foraging strategy between the wild and domestic strains strongly suggest that our domestic strains have maintained efficient foraging strategies, despite generations of being housed in captivity. These results are consistent with studies comparing the foraging strategies of wild vs. domesticated fowl

(Andersson, Nordin & Jensen 2001) and wild-type crossbred vs. domestic pigs (*Sus scrofa*) (Gustafsson et al. 1999). In both studies, the wild breeds were “out-foraged”, and exhibited less efficient and effective foraging strategies than their domestic counterparts. For example, the wild breeds spent less time at each food patch, and moved more frequently between patches without consuming more food to compensate for the additional travel. As humans provide food directly for domestic animals and often select for larger body size (as in the examples with the pig and fowl), it is likely that energy-demanding foraging behaviors are passively selected against as generations progress (Andersson, Nordin & Jensen 2001), resulting in an “adaptive laziness”. Thus, domestic animals could gain a fitness advantage by utilizing more efficient behavioral strategies compared to animals in the wild (Jensen & Gustafsson 1997). Part of this adaptive laziness may include heightened aptitudes for detecting and responding to foraging opportunities, even as energetically costly fear responses are lost.

Our results suggest future studies. For example, the video recording of activity patterns and behavioral responses could complement the GUDs. Video analysis could answer questions regarding differences in foraging times and approaches between strains, and might have identified whether auditory predation cues resulted in differences in behavioral response (but not overall foraging effort). Addition of olfactory cues of predation may result in more definitive behavioral responses to risk compared to auditory cues. Repeating our study with all strains housed in a more natural, wild environment may result in different foraging performances for all strains. Wild mice may perform better as a result of larger, more familiar surroundings, but the domestic strains may respond very differently. As domestic laboratory mouse strains are already known to differ in behavioral responses to common laboratory tests (Crawley 1996, Crawley et al. 1997, Podhorna & Brown 2002, Rodgers & Cole 1993), testing additional strains (C57BL/6,

CD1, BALBc, A/J, etc) and transgenic strains could reveal novel differences in foraging approaches and fear responses.

Results from our study also demonstrate the value of incorporating the GUD approach into laboratory behavioral testing regimes. Most traditional laboratory tests used to quantify fear and anxiety responses of animals, such as the light-dark box, elevated plus maze, and open field test, are based on acute behavioral responses to mostly novel stimuli. Therefore, several factors can influence and possibly confound results (Belzung & Griebel 2001). Indeed, there is debate on whether results from these tests are truly representative of fear/anxiety experienced by an animal, or if results simply reflect a “forced” response to novelty (Misslin & Cigrang 1986). In contrast, the study set-up presented here allows for highly repeatable, controlled manipulations within the animal’s housing environment for a prolonged period of time. By allowing our strains to forage freely in food patches, we were able to reveal novel influences of domestication on baseline apprehension levels, fear responses and foraging preferences that would not have been possible with traditional laboratory tests of fear and anxiety. We therefore suggest that GUD experiments can complement and enhance existing laboratory research methods for laboratories interested in fear, anxiety, and foraging aptitudes. We further suggest that examining and quantifying foraging decisions as a behavioral titration, rather than acute behavioral responses resulting from exposure to a novel stimuli or environment, may enhance laboratory and domestic animal studies by supplying researchers with unique and biologically relevant reflections and assessments of risk.

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III. FORAGING ECOLOGY AND WELFARE ASSESSMENT OF TWO OKAPI (*OKAPIA JOHNSTONI*) AT THE BROOKFIELD ZOO

A. Abstract

Applying principles of foraging ecology to zoo-housed animals can positively influence animal behavior, and assist with evaluating exhibit space and design. In this study, we implemented zoo foraging ecology by measuring giving-up densities (GUDs) in food patches to address several welfare-related questions with captive okapi (*Okapia johnstoni*). Our objectives were to: (1) determine whether food patches can reveal how the individual animals perceive their exhibit space (i.e. areas of preference and aversion; landscapes of comfort); and (2) determine whether implementation of food patches could reduce performance of repetitive behavior. We established 24 food patches throughout the 929 m² outdoor exhibit, determined each okapi's landscape of comfort, and evaluated the effects of the presence or absence of these food patches on okapi behavior. While the male okapi foraged food patches to lower GUDs than the female, both revealed landscapes of comfort that were unique to each individual. Food patch presence did not significantly lower the proportion of time spent in repetitive behavior, but did significantly increase the proportion of time that each animal spent actively foraging and animal movement throughout the exhibit space. We conclude that utilizing foraging ecology with food patches in animal enrichment and welfare regimes can benefit zoo-housed species, particularly okapi, by providing not only a valuable form of enrichment, but by also allowing animals to directly reveal their individual perceptions and exhibit preferences to their caretakers.

B. Introduction

In the wild, animals must assess and respond flexibly to their heterogeneous and constantly changing environment. Resources vary spatially and temporally. Foragers therefore

assess and adjust habitat use, foraging tactics, and time allocation appropriately. Similarly, risks, including competitors and predators, must be assessed and accommodated. The foraging ecology of animals in nature can and should be sophisticated. Foraging theory represents a well-developed body of conceptual and methodological tools for understanding how free-ranging animals respond to food availability, predation risk, their own motivational state, and other costs and benefits (Stephens, Brown & Ydenberg 2007).

For animals in zoos, in contrast, the environment is far less complex and often quite predictable. Regulated and predictable cleaning, feeding, and management routines coupled with reduced environmental complexity may limit the breadth or significance of “natural” behaviors (Carlstead 1996, McPhee 2003, Shepherdson 2010). Chronic suppression of behavioral sophistication and expression may result in suboptimal welfare (Morgan & Tromborg 2007), including the expression of abnormal or repetitive behaviors (Mason & Latham 2004, Mason & Veasey 2010, Shyne 2006, Swaisgood & Shepherdson 2005). Strategies to invite zoo animals to engage in a broader and more natural range of behaviors are therefore highly desired.

Providing zoo animals with environmental enrichment to increase behavioral diversity is now routinely employed to compensate for relatively homogenous zoo exhibits (Chamove 1989, Shepherdson 2010). Environmental enrichment typically involves adding auditory, visual, olfactory, and/or tactile stimuli to exhibits (Newberry 1995). Enrichment aims to achieve one or more of the following goals: reduce abnormal behavior, increase behavioral diversity, increase duration and/or frequency of target behaviors, increase enclosure utilization, or reduce physiological correlates of stress (Shepherdson 2010). Enrichment activities and devices that increase foraging complexity and foraging time are increasingly employed and they can be

particularly effective. Some species in the wild spend a significant proportion of their daily time budget on foraging-related activities (Herbers 1981), which is directly at odds with the highly predictable and pulsed manner in which captive environments traditionally provide food (Kistler et al. 2009, Newberry 1995).

Animals will generally forage in the most energy-efficient way possible (Charnov 1976). So, prolonged and extensive foraging related activities cannot be expected when resources are provided in a highly pulsed and predictable manner (Schneider, Nogge & Kolter 2014). To avoid this negative outcome, many captive facilities implement enrichment programs to extend foraging times. For example, food items may be dispersed throughout an exhibit rather than isolated to one area (Hare et al. 2003, Ryan, Proudfoot & Fraser 2012). Food may also be hidden in various locations (Carlstead, Seidensticker & Baldwin 1991, Ings, Waran & Young 1997), or distributed according to unpredictable feeding schedules (Kistler et al. 2009, Schneider, Nogge & Kolter 2014, Watters, Miller & Sullivan 2011). Foraging enrichments are typically considered successful if they increase foraging times compared to traditional methods. We suggest that placing foraging enrichment into a foraging ecology framework can provide additional insights that extend beyond increasing foraging times alone. Conjoining concepts from foraging theory with carefully designed feeding methodologies can provide a window into the perceptions and well-being of the target animals.

Under the guise of “zoo foraging ecology”, previous studies have discussed the potential benefits and conceptual framework of implementing foraging ecology techniques (specifically, placing food patches within exhibits) in zoos. Depleteable food patches spread throughout an exhibit can serve as an effective behavioral enrichment technique (Troxell-Smith et al. submitted). Food patches can also reveal exhibit and environmental preferences and aversions of

zoo animals (Howell-Stephens 2012, Mogerman 2011). Here, our objectives were to expand upon the existing zoo foraging ecology framework to explore two new applications of zoo foraging ecology: determining whether the enrichment benefits of food patches can effectively reduce performance of repetitive behavior in zoo animals, and evaluating to what extent individuals differ in environmental preferences within their exhibit.

We performed this study with two adult okapi (*Okapia johnstoni*) at the Brookfield Zoo, Brookfield, IL USA. A large proportion of the captive okapi population have been noted to perform repetitive behaviors (Bashaw et al. 2001, Bennett et al. 2015). We therefore predicted that the okapi would perform fewer repetitive behaviors and would forage more overall when provided with food patches compared to traditional feeding methods. We also predicted that provisioning of food patches would increase animal movement, foraging time and overall use of the exhibit space. Finally, we tested for whether the okapis demonstrate a landscape of comfort (van der Merwe & Brown 2008) within their exhibit. Namely, do the okapis consistently favor food patches in some areas of the exhibit relative to others, and do the okapi have similar perceptions of their exhibit landscape?

C. **Methods**

This study was approved by the UIC Office of Animal Care and Institutional Biosafety (OACIB; protocol # 13-152), and the Brookfield Zoo Biological Research Steering Committee (BRSC; protocol # 322).

1. **Subjects and housing**

This study was conducted on the two okapis (both adults) of the Brookfield Zoo, Brookfield, Illinois, USA. The okapis were chosen for this study due to their noted performance of repetitive behaviors, and because both animals were individually exposed to the same exhibit

space but at different times. We collected data on the two year old female between June-September 2013, and the 17 year old male between June-July 2014.

The study was conducted in the “Entry Yard”, the largest available outdoor exhibit space for the okapis (929 m²). As okapi are primarily solitary in the wild (Bodmer & Rabb 1992, Hart & Hart 1988), the Brookfield Zoo houses the animals individually, with one in the Entry yard, and the other in a smaller indoor/outdoor enclosure on the other side of the okapi building. In the evening, all animals are brought into an indoor barn, but continue to be housed individually. The female okapi was on exhibit in the Entry Yard during the summer of 2013, and the male during the summer of 2014.

The okapi were fed Mazuri Wild Herbivore diet® (PMI Nutrition International). On normal feeding days (traditional methods), each animal’s daily ration was divided into three meals: morning, mid-day, and evening. The feed was provided for the animals in a single feeding trough attached to the exhibit fence. The location of the trough was altered each day. Within this same trough, okapi were also provided with chopped vegetables and/or fruits during morning and evening feedings. Okapi also had *ad libitum* access to alfalfa hay throughout the day. During days with food patches, the entire pelletized diet ration was provided only in the food patches (as described below) in the morning. Hay was still provided, but fruits and vegetables were held until after the animals were brought into the holding barn for the night.

2. Food patches

To ensure familiarity with the food patches, each animal had approximately three weeks to acclimate to the food patches before data collection began. Each food patch consisted of a 19L plastic “carboy” bucket divided into two levels by a plastic plate. Each level was accessible to the okapi via an irregular hole (~15cm diameter; one on each level), and contained

50g of grain pellets (100g total/patch) and five, standard sized whiffle balls (Figure 6A). The whiffle balls provide a substrate that increases foraging difficulty and ensures diminishing returns. As the animal depleted the patch, its harvest rate declined as each piece of food became progressively harder to find (Brown 1988). Food patches were placed in the exhibit in the morning (0900hr), and were collected in the evening (1700hr) by the first author, allowing an approximately 8hr foraging period. Food remaining in each bucket at the end of the day was separated from the whiffle balls and weighed, providing the giving-up density (GUD). GUDs should be low at a patch if: 1) the food is highly desirable, 2) the animal is highly motivated to feed, 3) there are few alternative activities, and 4) the animal feels safe and secure at that location. This last feature of GUDs shall be crucial in evaluating each okapi's landscape of comfort.

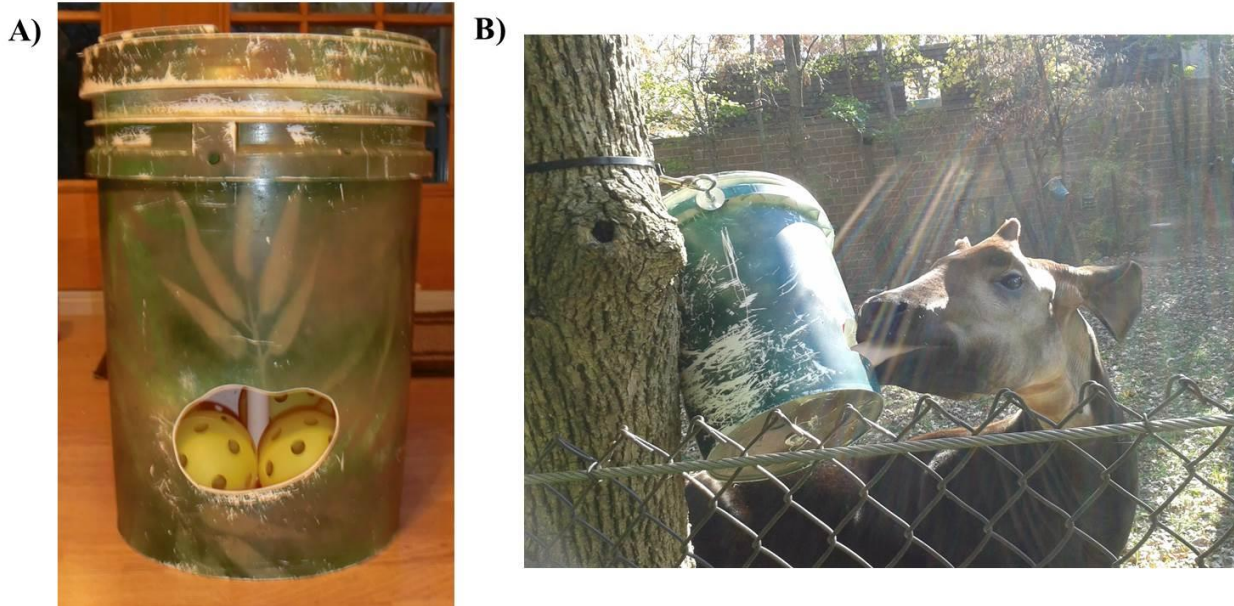


Figure 6. Photographs of okapi food patches. A) Close up of a food patch highlighting the bottom level containing five whiffle balls. B) The male okapi using his prehensile tongue to feed from a food patch in the exhibit. Food patches were attached to the tree approximately 2m off the ground via cable ties to simulate folivorous feeding.

3. Landscape of comfort

Under natural conditions, okapi move through a diverse landscape as individuals travel between shrub or trees in search of browse (Hart & Hart 1988). Predation risk will render portions of the landscape riskier than others (concept of landscape of fear; Laundré, Hernández & Altendorf 2001). Even within a relatively small space like the Entry Yard, the okapis may retain an instinctual sense of what constitutes safe and risky. This will manifest as a spatial landscape of comfort which can be revealed by significant spatial variation in giving-up densities. To mimic the okapis feeding from shrubs and trees, food patches were attached approximately 2m off the ground to tree trunks using cable ties (Figure 6B). On days with food

patches, we attached one food patch to each of the 24 trees of the exhibit space (Figure 7). The somewhat uniform spacing of the trees around the yard permits the GUDs to reveal an individual's landscape of comfort. We collected 10 days of food patch data from the female okapi in 2013 for a total of 240 GUD measures. We collected seven days of food patch data from the male okapi in 2014 for a total of 168 GUD measures.



Figure 7. Google Earth image of okapi yard at Brookfield Zoo. Each numbered circle represents the location of a tree with food patch.

4. Behavioral observations

Prior to data collection, behavioral observations were conducted on the okapi to generate a species-specific ethogram. A total of 23 behaviors were monitored (see Table 1), including repetitive behaviors unique to each individual. The female's predominant repetitive behaviors were "head-rolling" and "pacing", whereas the male primarily performed a "mouthing" behavior (for behavioral definitions, see Table 1). When official data collection began, at least two 30 minute observations were conducted each observation day during two time periods; one in the morning (0900-1230h) and one in the afternoon (1231-1700h). Instantaneous sampling (Altmann, 1974) of state behaviors and animal location within the exhibit were conducted at 1-min fixed intervals. A random number generator in Microsoft Excel was used to assign specific treatment days, and observation times within morning and afternoon periods. Data were recorded using the Animal Behavior Pro app for iPad (Newton-Fisher, 2012, University of Kent). Between June-September 2013, total observation time for the female okapi was 25 days; 10 days (49.5 hrs) with food patches and 15 days (24.6 hrs) without food patches. The male okapi received 14 days (totaling 39 hrs) of observation between June-July 2014; seven days (25.2 hrs) with food patches, and seven days (13.7 hrs) without food patches.

TABLE I
OKAPI ETHOGRAM OF OBSERVED BEHAVIORS AND CORRESPONDING
BEHAVIORAL CATEGORIES

Behavioral Category	Behavior	Definition
Active	Attentive	Sudden turning of head and ears toward a direction. No other movement.
	Running	Fast locomotion throughout exhibit.
	Sniffing	Nose pointed toward object accompanied by rapid head movement.
	Walking	Slow locomotion throughout exhibit.
Foraging	Browse (Enrichment)	Consumption of browse provided by keepers for enrichment.
	Browse (Fence)	Consumption of native plants/browse found in and near exhibit borders. Not provided by keepers.
	Chewing	Grinding of food (browse, grain, or hay) with teeth.
	Drinking	Consumption of water.
	Enrichment (Hay Ball)	Investigation and interaction with enrichment item holding hay. Present during both traditional and food patch feeding.
	Food Patch	Investigation and interaction with a food patch, including removal and consumption of pellets. Only used when food patches were available.
	Traditional Feeder	Investigation and interaction with traditional food trough, including consumption of food pellets. Only used when food was presented in traditional manner.
Maintenance	Defecation/urination	Waste excretion.
	Headshake	Quick head movement from side to side (usually to remove flies).
	Lying down	Animal has all parts of body (apart from head) in contact with the ground. No other movement occurs.
	Rubbing	Uses branch to scratch head/ears.
	Ruminate	Sudden chewing without recent prior consumption of food.
	Self-groom	Using tongue to lick body (ears, back, legs, etc).
Repetitive	Head Rolling	Moves head in circular pattern while facing vertical surface. Commonly accompanied by alternate stomping on front right and hind left leg. Only displayed by female okapi.
	Inactive	Upright on all four legs, but no other movement occurs. Ears are not fixed in a particular direction.
	Licking	Using tongue to touch objects in exhibit. Does not include self-grooming. Performed by both okapi.
	Mouthing	Rubs mouth on object (fence, enrichment, etc). Does not include self-grooming. Only displayed by male okapi.
	Pace	Repetitive walking pattern (not toward a destination).
	Not Visible	Animal is not visible to observer.

5. Data analysis

a. Time budget/behavioral enrichment

We were specifically interested in how effective food patches were as a tool to reduce repetitive behaviors and as a foraging enrichment. Hence we focused on the two pertinent behavioral categories of time spent Foraging and time spent on Repetitive behaviors. Time spent in each of these behavioral categories in the morning and in the afternoon was calculated for each animal as proportion of time the animal was visible to the observer. To test for the presence and absence of food patches on movement throughout the exhibit, we calculated the total number of times each animal moved from one exhibit section to another (hereafter “transitions”) as a proportion of the total number of observational scans for each day. All proportional data were then arc-sine square root transformed. A MANOVA was used to test for the effects of individual (female and male okapi) and the presence or absence of food patches on the proportion of time spent on the two behavioral categories and on transitions.

b. Landscape of comfort

To test for effects of patch location on GUDs for the okapi, GUD data were analyzed using an ANOVA under the general linear models of SYSTAT 13 (SPSS Inc., Chicago, IL, U.S.A.). Date was nested within individual for the analysis, with GUD as the dependent variable, and patch location and individual (male or female okapi) as independent variables. We used days of each experiment as replicates rather than repeated measures since the GUD measurement on one day does not dictate the GUD of the next. A Fisher’s Least Significant Difference (LSD) post-hoc test was used to identify differences between individual’s use of patch locations, and to group patch locations into comfort categories of high, intermediate, and low GUDs.

D. Results

1. Time budget/behavioral enrichment

The presence of the food patches significantly influenced the okapis' behaviors ($F_{(3,68)} = 6.28$, $P = 0.001$; Wilk's $\lambda = 0.78$). Univariate tests found that having the food patches significantly increased proportion of time spent Foraging ($F_{(1,70)} = 9.16$, $P = 0.003$), and increased the proportion of transitions within the exhibit ($F_{(1,70)} = 6.49$, $P < 0.05$) for both okapis. The presence of food patches did not significantly influence the proportion of time spent on Repetitive behaviors ($F_{(1,70)} = 0.56$, $P = 0.458$; Figure 8). Each okapi also significantly varied in their behavioral performance ($F_{(3,68)} = 3.87$, $P = 0.013$; Wilk's $\lambda = 0.85$). Univariate tests revealed that the female spent a significantly larger proportion of time on Repetitive behaviors than the male ($F_{(1,70)} = 9.80$, $P = 0.003$), however the two okapis did not differ in their proportion of time spent Foraging ($F_{(1,70)} = 1.71$, $P > 0.05$) or their exhibit transitions ($F_{(1,70)} = 1.29$, $P = 0.261$). Time of day (AM vs. PM) also significantly influenced the okapis' behaviors ($F_{(3,68)} = 3.85$, $P = 0.013$; Wilk's $\lambda = 0.86$). Univariate tests revealed that time of day influenced Foraging behavior ($F_{(1,70)} = 10.62$, $P = 0.002$), with more Foraging occurring in the morning vs. the evening. Time of day did not influence proportion of time spent Repetitive ($F_{(1,70)} = 0.04$, $P = 0.846$), or the proportion of exhibit transitions ($F_{(1,70)} = 0.27$, $P = 0.604$). There was no significant interaction effects between individual okapi and the presence or absence of food patches ($F_{(3,68)} = 1.22$, $P = 0.310$; Wilk's $\lambda = 0.95$), time of day and treatment ($F_{(3,68)} = 0.75$, $P = 0.524$; Wilk's $\lambda = 0.97$), or between individual and time of day ($F_{(3,68)} = 2.59$, $P = 0.060$; Wilk's $\lambda = 0.90$). The three-way interaction between individual, time of day, and treatment was also not significant ($F_{(3,68)} = 1.48$, $P = 0.228$; Wilk's $\lambda = 0.94$).

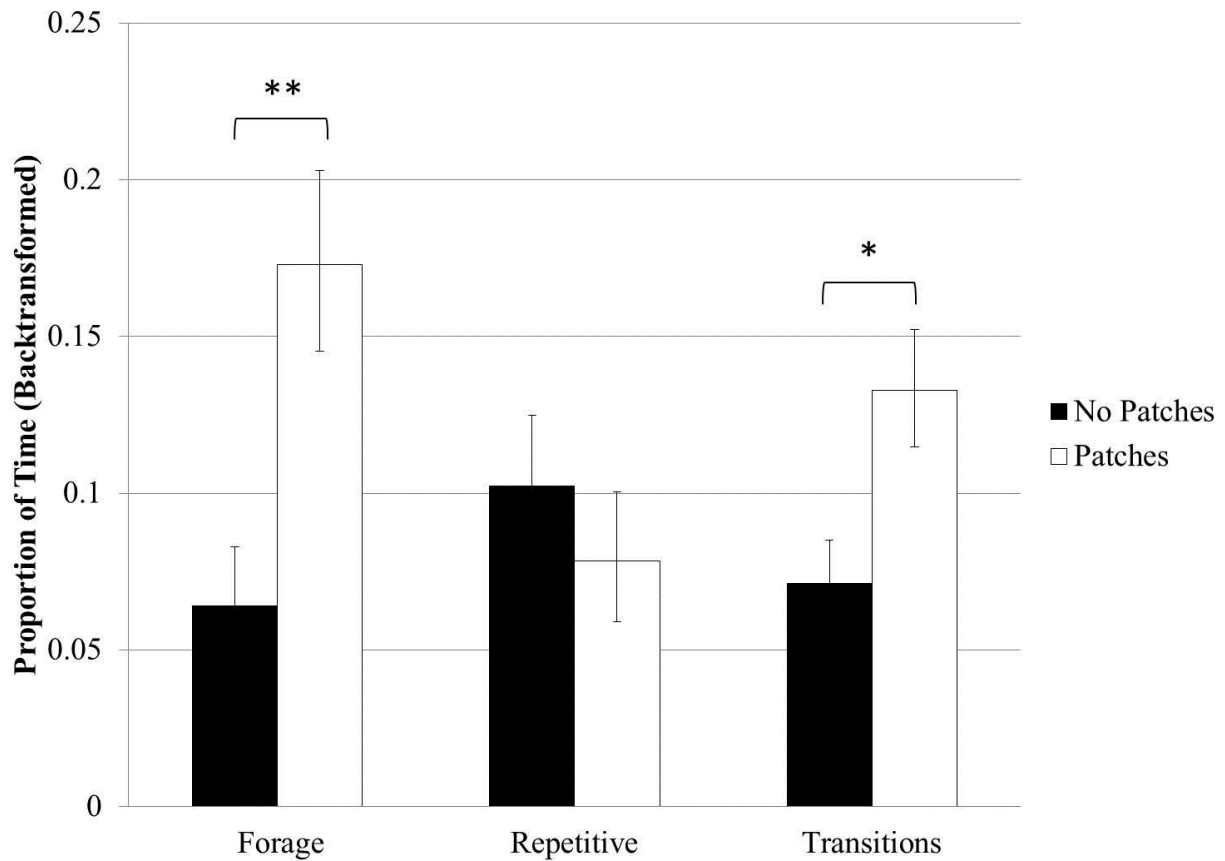


Figure 8. Pooled mean backtransformed proportion of time (\pm SEM) spent in the Foraging and Repetitive behavioral categories, and proportion of Exhibit Transitions in each treatment condition (with and without food patches). Patch treatment significantly increased proportion of time spent Foraging and proportion of exhibit transitions, but had no significant effect on proportion of time spent in Repetitive behaviors. Asterisks indicate level of significance (* = $P < 0.025$; ** = $P < 0.01$).

2. Landscape of comfort

Food patch location significantly influenced GUDs ($F_{(23,345)} = 5.95$; $P < 0.001$).

While the overall GUDs did not vary significantly between the male and female okapis ($F_{(1,345)} =$

0.28; $P = 0.074$), there was a significant interaction between individual and patch location on GUDs ($F_{(23,345)} = 4.89$; $P < 0.001$; Figs. 9A and 10A), suggesting that the male and female differed somewhat in their landscape of comfort. A Fisher's LSD revealed that the male and female okapi significantly varied in their use of nine out of 24 food patches (patches 1, 3, 5, 9, 10, 11, 17, 18 & 20). Overall, the female okapi had high GUDs (avg GUD > 80g remaining) in six of 24 food patches, intermediate GUDs (between 50-79g) in 13 patches, and low GUDs (< 49g) in five patches (Figure 9B). The male okapi had high GUDs (> 80g remaining) in seven of 24 food patches, intermediate GUDs (between 50-79g) in 14 patches, and low GUDs (< 49g) in four patches (Figure 10B). GUDs were similarly high (indicating discomfort) between both okapis in three spots (Patches 2, 7, and 22), whereas both okapis had low GUDs (indicating comfort) in three other spots (Patches 1, 12, and 15). There was only one location (patch 5) where the female had a GUD suggesting relative comfort, whereas the male's GUD suggested relative discomfort. If the high GUDs indicate a lack of comfort then the okapis seem less comfortable with approximately 25% of their exhibit space.

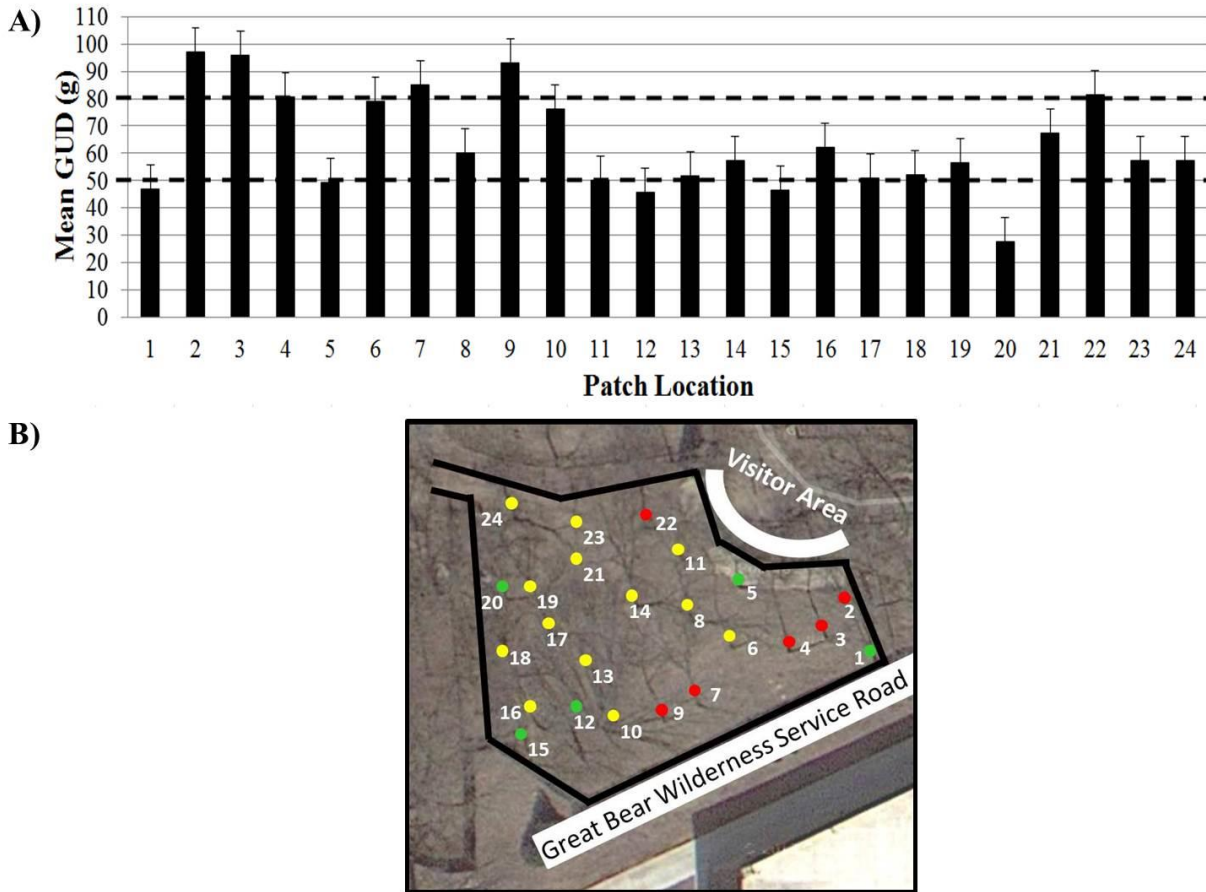


Figure 9. Pooled mean GUD (\pm SEM) and corresponding landscape of comfort for the female okapi. A) Patch location had a significant effect on GUDs ($P < 0.001$). Dashed lines indicate cut-offs for high (avg GUD $> 80g$), intermediate (avg GUD between $79-50g$), and low GUDs (avg GUD $< 49g$). GUDs in patches 2, 3, 4, 7, 9, and 22 were significantly higher than other locations, and GUDs in patches 1, 5, 12, 15, and 20 were significantly lower than other locations. B) Corresponding Google Earth map of the female okapi's "landscape of comfort" within the exhibit at the Brookfield Zoo. Each circle represents a foraging station, and circles are color-coded to represent pooled mean GUDs at patch location: red= high GUD (avg GUD $> 80g$); yellow= intermediate GUD (avg GUD between $79g - 50g$); green=low GUD (avg GUD $< 49g$).

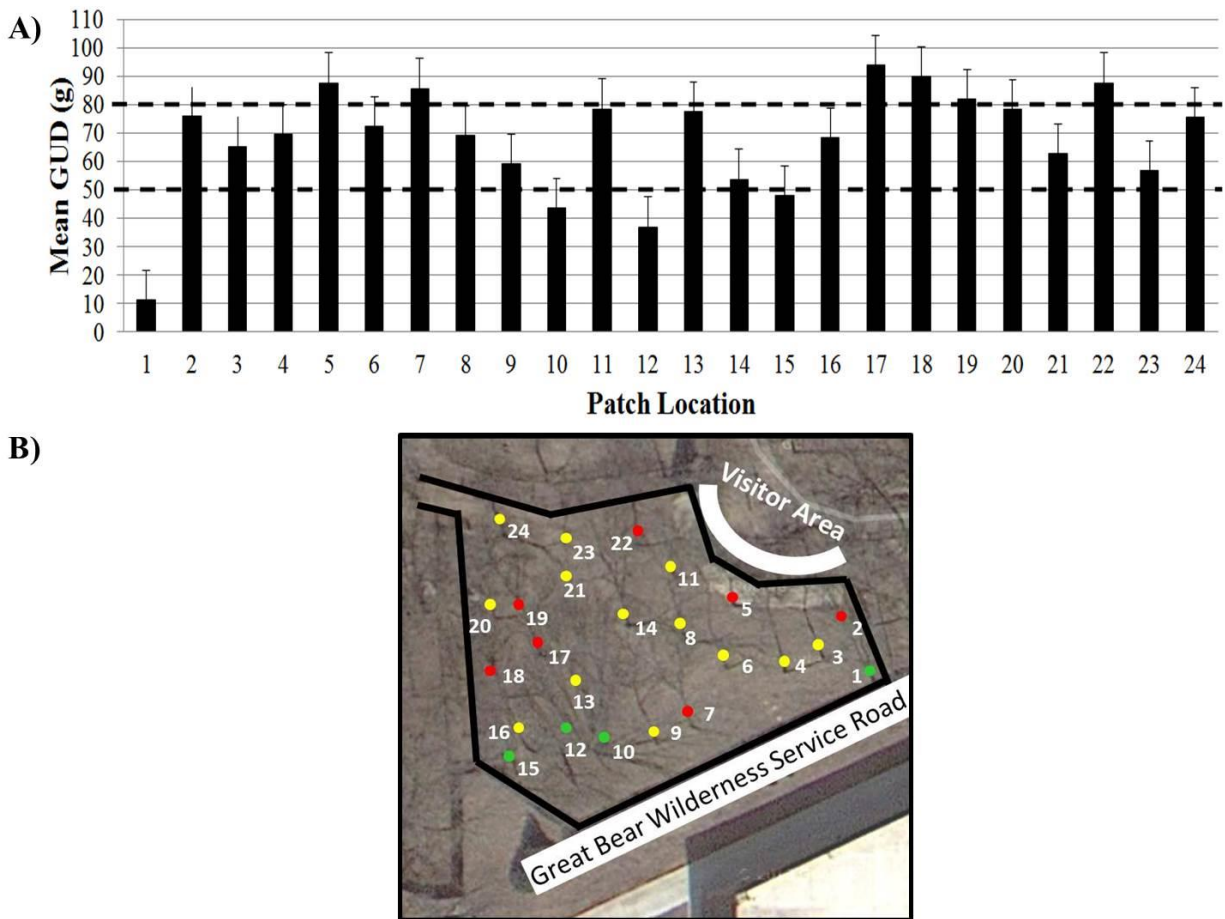


Figure 10. Pooled mean GUD (\pm SEM) and corresponding landscape of comfort for the male okapi. A) Patch location had a significant effect on GUDs ($P < 0.001$). Dashed lines indicate cut-offs for high (avg GUD $> 80g$), intermediate (avg GUD between $79-50g$), and low GUDs (avg GUD $< 49g$). GUDs in patches 2, 5, 7, 17, 18, 19, and 22 were significantly higher than other locations, and GUDs in patches 1, 10, 12, and 15 were significantly lower than other locations. B) Corresponding Google Earth map of the male okapi's "landscape of comfort" within the exhibit at the Brookfield Zoo. Each circle represents a foraging station, and circles are color-coded to represent pooled mean GUDs at patch location: red= high GUD (avg GUD $> 80g$); yellow= intermediate GUD (avg GUD between $79-50g$); green=low GUD (avg GUD $< 49g$).

E. **Discussion**

Using a combination of behavioral observations, food patches, and foraging ecology, we compared the behavioral and spatial preferences of two adult okapi. Behavioral results indicate that provisioning of food patches significantly increased the proportion of time both okapi spent foraging, decrease performance of repetitive behavior, particularly in the female okapi, and increased within-exhibit movement for the male okapi. In terms of the exhibit space, both animals exhibited largely similar landscapes of comfort but with some striking individual differences in spatial preferences.

As hypothesized, both okapi significantly increased the proportion of time spent foraging and the proportion of exhibit transitions when provided with food patches compared to normal feeding conditions. Taken together, these results suggest that providing okapi with food patches not only generates behavioral benefits, but may also more accurately replicate natural foraging behaviors and conditions. In the wild, okapi spend the majority of daylight hours moving within their home range to acquire their food (Hart & Hart 1988, Lindsey, Green & Bennett 1999). In captivity however, foraging options are typically far more limited, as food is provided on a highly regulated and predictable schedule (Morgan & Tromborg 2007, Newberry 1995), drastically reducing the control okapi have over their foraging patterns. It has been suggested that a lack of a sense of control, particularly over foraging (Shepherdson 2010), and the inability to perform natural foraging behaviors could lead to repetitive behaviors (Morgan & Tromborg 2007). In fact, previous studies have suggested that okapi that were fed more frequently throughout the day were less likely to exhibit repetitive licking behavior compared to individuals fed only once or twice a day (Bashaw et al. 2001). However, others suggest that increasing feeding frequency may also increase repetitive pacing in okapi (Bennett et al. 2015), possibly as

a result of anticipation of feeding events. As we found no interaction between patch treatment and time of day, we suggest that providing okapi with food patches is a possible solution to both problems, as food patches may provide animals with more control over their foraging habits throughout the day. Animals can choose when and where to eat, leave and return to multiple food patches throughout the day, and even avoid patches in uncomfortable areas, allowing a foraging experience similar to that in the wild. Moreover, by establishing several foraging locations throughout the exhibit, animals are encouraged to travel more to acquire their food as opposed to feeding from a single spot.

Food patches appeared to decrease time spent performing repetitive behaviors, particularly for the female okapi, though not significantly so. Similar to rates reported in other captive okapi (Bashaw et al. 2001, Bennett et al. 2015), the female in our study spent a significant proportion of her time (39.5%) engaged in repetitive behaviors prior when food patches were absent. In the absence of patches, the proportion of time spent in repetitive behaviors was the dominant behavioral category for the female (39.5% Repetitive, 27.4% Maintenance, 17.4% Active, and 15.5% Foraging). When the female had access to food patches however, the proportion of time she spent in Repetitive behaviors decreased from approximately 39.5% to 32.3%. This, combined with a doubling of Foraging time resulted in a shift in the female's predominant behavioral category to Foraging rather than Repetitive (32.8% Foraging, 32.4% Repetitive, 20.2% Maintenance, 14.3% Active). In general, the male okapi in our study performed far less repetitive behavior compared to the female (14.8% as opposed to 39.5% for the female prior to food patch implementation). Previous studies have suggested that males and females may have different factors motivating the performance of repetitive behavior (Bennett et al. 2015), and the drastic difference in performance of repetitive behaviors between our male and

female okapi appears consistent with these findings. However, when provided with food patches the male okapi also decreased the proportion of time spent in Repetitive behaviors (from 14.8% to 13.4%). These results suggest that provisioning animals, particularly okapi, with food patches may be an effective method to decrease problematic repetitive behavior.

Food patches also provided valuable information regarding individual exhibit perceptions. As predicted, food patches revealed detailed landscapes of comfort that were somewhat unique to each okapi. Namely, the food patches highlighted several areas of discomfort that could have important impacts on animal welfare. For example, the female okapi left high GUDs in six of the 24 food patches, suggesting that 25% of the exhibit was perceived as risky or uncomfortable from the female's perspective. Interestingly, the majority of the food patches with high GUDs were located directly adjacent to a service road bordering the exhibit. Given that wild okapi are native to remote, dense rainforest areas in the Democratic Republic of Congo (Hart 2013, Hart & Hart 1988), it is possible that the female okapi was highly sensitive to the general human activity and/or vehicle noise occurring along the road. These hypotheses could be tested further by measuring sound decibel output near vs. away from the service road, modulating traffic, or by experimentally increasing visual barriers (Troxell-Smith & Miller 2016) along the service road fence-line to create a more enclosed space. The exhibit preferences and aversions of the male okapi on the other hand, were often different from those of the female. Similarly, the male okapi also left high GUDs in seven out of 24 food patches, again suggesting that at least 25% of the okapi's exhibit was viewed as risky or uncomfortable from the male's perspective. Unlike the female, however, the majority of food patches with the highest GUDs were located either along the visitor area or toward the back of the exhibit. As previous studies have suggested, zoo visitors may represent a form of stress or risk to animals (Davey

2007, Stevens et al. 2013), it is possible that the male okapi was more sensitive to the proximity of human visitors compared to the female. This idea could be tested further by adding additional visual barriers or canopy cover along the exhibit barrier between the visitors and the okapi. Regardless of possible motivation, food patches did allow the okapi to reveal their individual preferences and aversions to their exhibit space. Such information can be used for exhibit design and improvements (i.e. adding additional visual barriers and/or canopy cover within the exhibit), and can ultimately have important welfare implications.

Overall, we suggest that implementation of foraging ecology and food patches within captive environments achieves important goals of effective behavioral enrichment (including reducing incidence of abnormal behavior, increasing targeted foraging behaviors, and increasing exhibit utilization; Shepherdson 2010) while simultaneously providing key insight into individual differences in preference, state, and welfare. The differences in individual behavioral performance and landscapes of comfort within the same exhibit reported in our study demonstrate the importance of considering individual behavioral needs and exhibit perceptions when making animal management and welfare decisions. Previous studies have investigated the effects of individual animal personality differences on breeding success (Carlstead et al. 1999, Fox & Millam 2014, Powell & Svoke 2008, Wielebnowski 1999), adrenal and hormonal cycling (Freeman, Weiss & Brown 2004, Wielebnowski et al. 2002), and general welfare (Schaefer & Steklis 2014), however few have investigated how individual personality characteristics may relate to animal exhibit perceptions and resulting behavioral performance. While we did not specifically address individual personality differences in our current study, we suggest that combining food patches with personality assessment could provide complementary information

regarding improvements to exhibit design by documenting and incorporating individual preferences.

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IV. ZOO FORAGING ECOLOGY- DEVELOPMENT AND ASSESSMENT OF A WELFARE TOOL FOR CAPTIVE ANIMALS

A. Abstract

Foraging ecology and food patch studies are commonly used to elucidate the environmental perceptions of wild, free ranging animals. Their application to captive animals, however, especially those in zoos, is still in its infancy. To illustrate some specific applications of zoo foraging ecology, we provide a study that evaluated (1) whether patch use and giving-up densities can reveal areas of preference within an exhibit for zoo species, (2) if food patches provide an effective form of behavioral enrichment, and (3) if visitor interest and behavior is affected by food patch presence. A combination of behavioral observations, and experimental food patches and giving-up densities (GUDs) were used to address these objectives in Parma wallabies and Patagonian cavies at the Lincoln Park Zoo, Chicago, Illinois USA. GUDs revealed distinct areas of preference and aversion within the exhibit for cavies, but not so for the wallabies. For both species, presence of food patches increased foraging behaviors, decreased inactive behaviors, and increased within-exhibit movement, demonstrating that food patches serve as an effective behavioral enrichment technique. The use of food patches also revealed striking differences between individuals, particularly for the pair of cavies. There were encouraging trends toward increased visitor number and stay-time when food patches were present in each exhibit, but the effect was not statistically significant. These results suggest that utilizing patch use, GUDs, and foraging theory in zoo populations can enhance animal welfare, and can inform improvements to exhibit design directly from the animal's perspective. We conclude with a broader discussion of zoo foraging ecology as an emerging field, with suggestions for future avenues of research.

B. Introduction

Zoological parks aim to improve the care and welfare of their animals. Welfare results from several inputs, including physiological, psychological, and physical conditions. Numerous methods have been developed to assess animal welfare, most of which focus on either the physiology, behavior, or general health of the focal animal(s) (Hill & Broom 2009, Melfi 2009, Whitham & Wielebnowski 2013). The effectiveness of any single method, however, may be limited, as each reflects only a portion of an animal's needs and perceptions (Swaigood 2007, Barber 2009). Additionally, animal care decisions may be constrained by interpreting animal experiences from a professional, yet inherently subjective human perspective, that may not accurately reflect the experiences of the animals themselves (Veasey et al. 1996, Rivas & Burghardt 2002). Therefore, new methodologies to assess and improve animal welfare are highly sought-after (Barber 2009, Whitham & Wielebnowski 2013), particularly those that allow the animals to reveal their perspectives (Melfi 2009).

To improve animal care and increase welfare, zoos increasingly incorporate aspects of a species' natural history and behavioral ecology (Forthman & Ogden 1992). As animals in the wild often invest considerable time and energy in acquiring resources (Herbers 1981), zoos have provided enrichment opportunities to mimic more natural foraging scenarios for their animals. For example, providing access to live fish significantly increased performance of natural hunting behaviors (Shepherdson et al. 1993, Mellen et al. 1998, Bashaw et al. 2003), and supplemental carcass feeding reduced stereotypic behaviors (Bond & Lindburg 1990, Mcphee 2002) for captive felids. When given the option, captive grizzly bears (*Ursus arctos horribilis*) spent more time actively manipulating objects to acquire food compared to when it was freely available (contrafreeloading; McGowan et al. 2010). Hiding food throughout the exhibit also increased

foraging time and decreased stereotypic behavior for chimpanzees (*Pan troglodytes*; Baker 1997), walrus (*Odobenus rosmarus*; Kastelein & Wiepkema 1989), and several species of bear (Carlstead et al. 1991). Furthermore, implementation of an random feeding schedule increased foraging behavior and activity for sun bears (*Helarctos malayanus*; Schneider et al. 2014), fennec foxes (*Vulpes zerda*; Watters et al. 2010), and chimpanzees (Bloomsmith & Lambeth 1995). These results demonstrate that providing captive animals with the opportunity to work for their food has positive implications for animal behavior.

Beyond the previously stated welfare implications, we suggest that foraging behaviors can reveal important information about how an animal perceives its environment. When provided with a number of experimental food patches that are equal in quality, an animal in a safe or comfortable spot or environment (ie area with little probability of injury or death) will exploit a food patch more thoroughly (ie leave less food behind) than an animal in a risky or uncomfortable environment (Whelan & Maina 2005). Measuring the amount of food left over by the forager in each food patch (the giving –up density: GUD; Brown 1988) provides a quantifiable metric of environmental perception, with extensive patch use (low GUD) indicating areas of comfort, whereas low patch use (high GUD) indicates that the forager feels uncomfortable (Brown 1988). In the wild, these procedures have been used to develop species-specific “landscapes of fear” (i.e. an environmental “map” of areas of preference and aversion; (Brown et al. 1999, Shrader et al. 2008, Laundré et al. 2010). We propose that similar methods can be used in captivity to develop an animal’s “landscape of comfort” within their exhibit. Such a map can provide valuable information to caretakers regarding how their animals perceive and use their exhibit space. For example, if GUDs reveal that individuals are too uncomfortable to forage extensively in several areas of their exhibit, the exhibit itself may be negatively impacting

the welfare of the animals housed within it. Furthermore, the food patches themselves may provide enrichment benefits to captive foragers by encouraging increased foraging and general animal activity.

Here, we investigate the utility of incorporating patch-use as a tool to investigate questions related to animal care in captive populations. We combined measures of patch-use with behavioral observations to determine: (1) whether patch use and giving-up densities can reveal landscapes of comfort for zoo species, (2) if food patches are an effective form of behavioral enrichment, and (3) is visitor interest and behavior influenced by food patch presence? Finally, we (4) aim to provide a more general discussion of zoo foraging ecology's potential as an emerging field.

This study was performed with two Patagonian caviies (*Dolichotis patagonum*), and two Parma wallabies (*Macropus parma*) at the Lincoln Park Zoo (LPZ) in Chicago, IL, USA. Based on previous patch use studies with these species in the wild, we predicted that caviies prefer foraging in open areas (away from exhibit borders and blocked sightlines; Sombra 2011), whereas wallabies prefer areas close to cover (near bushes or exhibit borders; While & McArthur 2006). In regard to behavioral enrichment, we predicted that presence of food patches would increase natural behaviors, particularly time spent foraging and movement throughout the exhibit, and decrease inactive behaviors for both species. Finally, as active animals are known to attract more zoo visitors than resting/inactive animals (Margulis et al 2003, Watters et al 2010), we predicted that presence of food patches within an exhibit would increase the number of zoo visitors and visitor stay-time.

C. **Methods**

Creation of a species-appropriate food patch is critical to achieving reliable GUD measurements (Bedoya-Perez et al 2013). Food patches typically consist of a measured amount of food mixed into an inedible substrate. The inedible substrate (topsoil) ensured diminishing returns: as an animal depleted the patch, its harvest rate declined as each piece of food became progressively harder to find (Brown 1988). If multiple food patches are accessible in an area with a uniform climate, differences in consumption of food between patches can be attributed to the spatial foraging preferences of the foragers (van der Merwe & Brown 2008), where animals will consume more from patches where they feel safe vs those where they feel uncomfortable (Whelan & Maina 2005). Resulting GUDs can be used to develop landscape of comfort within the exhibit space, and permit new and different insights into the preferences of captive foragers.

In our study, food patches consisted of 20 pieces (10g) of species-specific food randomly mixed into 2L of topsoil inside a black rubber foot tub for cavies (20.32cm diameter x 10.16cm H; Figure 11A), and 35 pieces (10g) of food randomly mixed into 1L of topsoil inside a plastic saucer for wallabies (40.1cm diameter x 9.6cm H Figure 11B). Final foraging patches varied between the species because the wallabies would not forage from the same tub as the cavies, necessitating the change to a plastic saucer to ensure proper foraging. This study was approved by the UIC Office of Animal Care and Institutional Biosafety (OACIB; protocol # 12-181), and the LPZ Research Committee (protocol # 2013-025).



Figure 11. Pictures of species-specific food patches. A) Cavy food patches consisted of 20 pieces of food mixed into 2L of topsoil inside a black rubber foot tub. B) Wallaby food patches consisted of 35 pieces of food mixed into 1L of topsoil inside a plastic saucer.

We let each species acclimate for approximately one month to the food patches before formal data collection began. At that time, food patches were placed in the exhibit in the morning (08:30h) by keepers, and were collected in the evening (16:30h), allowing approximately an 8hr foraging period. Exact daily foraging periods varied slightly according to keeper availability for placement and removal of patches. All food remaining in each patch was separated from the topsoil via a sieve and individual pieces were counted, providing the GUD.

1. **Subjects and housing conditions**

Two exhibits were used for this study, one with two adult male *Parma* wallabies (Wallaby A and Wallaby B), and the other housing two adult Patagonian caviés (one male, one female). *Parma* wallabies and Patagonian caviés were chosen for this study because background information regarding how wild individuals use depletable food patches was already available, and both species inhabit similar indoor, small exhibits with conspicuous heterogeneity

in topography. The cavies and wallabies occupied adjacent, indoor exhibits in the Small Mammal-Reptile House at the LPZ. The wallaby exhibit was approximately 24.2 m², and the cavy exhibit was approximately 12.5 m². In the non-food patch condition, the wallabies were fed the same total ration of Mazuri Kangaroo/Wallaby diet® (PMI Nutrition International) provided in the patches, however the ration was presented in two dishes only in the morning, plus chopped vegetables and/or fruits in the evening. Similarly, the cavies were provided their Mazuri Rodent diet® (PMI Nutrition International) in a single dish, plus chopped vegetables and/or fruits in the morning. When measuring GUDs and feeding behaviors from the depletable food patches, the pelletized diet ration for both species was provided only in the food patches (see below) in the morning. The cavies continued to receive their raw produce in the morning with the food patches, and the wallabies continued to receive their raw produce in the evening after food patches were removed.

2. **Environmental preferences**

To investigate environmental preferences, the animals' locations within each exhibit were recorded during each observation period when patches were present and absent. The cavy yard was divided into 8 quadrats (hereafter "quads A-H") of approximately equal area (Figure 12A). Due to multiple levels within the exhibit, the wallaby exhibit had 10 quads (Figure 12B). When patches were deployed, every quad also contained a single numbered food patch at its center. When patches were collected, the GUD for each quad was recorded to determine foraging location preferences within the exhibit. The wallabies had 11 total days with food patches, and the cavies had 10 total days with food patches. Patch design for cavies required iterative design modifications that permitted 5 days of GUD data for analysis, while providing 10 days of behavioral data with patches for behavioral analyses.

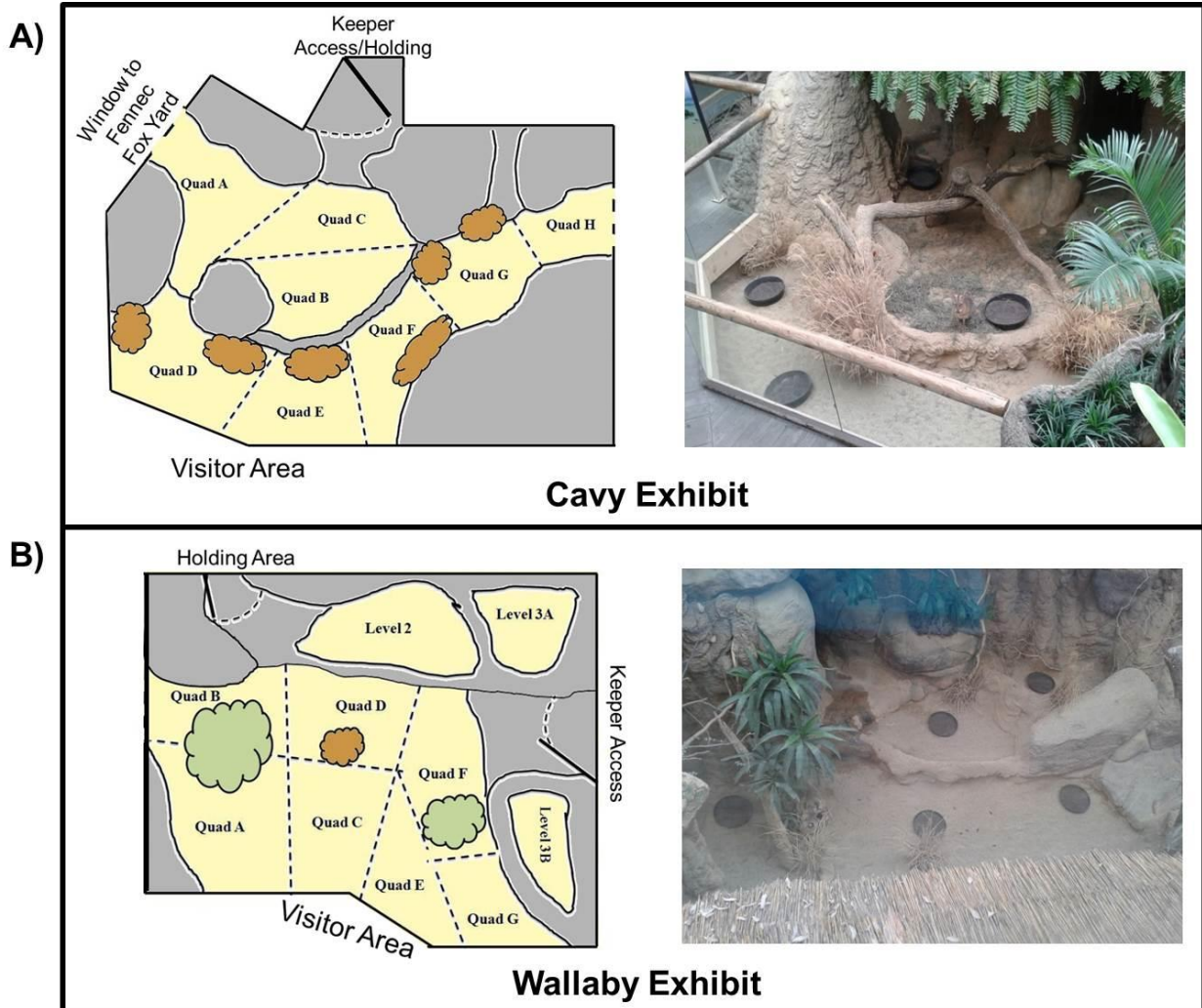


Figure 12. Schematics and corresponding photographs of the cavy and wallaby exhibits at the Lincoln Park Zoo. Dark brown areas indicate bushes or dry foliage and green areas indicate live foliage within the exhibits. Gray areas indicate rock formations and exhibit walls and borders that were inaccessible to the animals. In the food patch condition, patches were placed in the approximate center of each section. A) The cavy exhibit depicting the eight environmental preference sections (tan), and photograph depicting food patch placement within the exhibit. Quads A, B, and C are approximately 0.2m higher than the rest of the sections. B) Schematic of the wallaby exhibit with the 10 environmental preference sections (tan) delineated, and photograph depicting food patch placement within the exhibit. Level 2 was approximately 0.5m higher than Quads A-G, and Levels 3A and 3B were approximately 1m higher than Level 1.

3. **Behavioral enrichment**

Behavioral observations were conducted on the cavies and wallabies prior to data collection to generate species-specific ethograms. The resulting cavy ethogram contained 20 behaviors, whereas the wallaby ethogram contained 18 behaviors (see Tables II and III). In both ethograms, all behaviors were collapsed into six main behavioral categories (Active, Inactive, Foraging, Social, Maintenance, and Other/Out of Sight). Once official data collection began, behavioral observations consisted of 15 minute periods using scan sampling at 1 minute intervals (Altmann 1974). The location of each individual at each scan was also recorded. Four 15 minute observation bouts were conducted per individual in the morning (08:30-12:30h), and again in the afternoon (12:31-16:30h), generating eight observation periods (two hours of observational data) for each individual animal per day. A random number generator was used to assign observation times within morning and afternoon time periods, and to determine daily assignment of patch treatment. All observations were recorded using the Animal Behavior Pro app for iPad (Newton-Fisher 2012, University of Kent).

TABLE II
CAVY ETHOGRAM OF OBSERVED BEHAVIORS AND CORRESPONDING
BEHAVIORAL CATEGORIES.

Behavioral Category	Behavior	Description
Active	Dig	Use of forepaws to move sand.
	Running	Quick locomotion throughout exhibit.
	Sniffing	Movement of nose near an object or another individual.
	Vigilant	Attentive sitting or standing in an upright rigid posture with a stretched-out head.
	Walking	Slow locomotion throughout exhibit.
Forage	Chewing	Consumption of hay. Used during both food patch and traditional food presentations.
	Food patch	Investigation and interaction with a food patch, including removal and consumption of pellets. Only used when food patches were available.
	Normal food	Investigation and interaction with traditional food tray, including consumption of food pellets. Only used when food was presented in traditional manner.
	Produce	Consumption of fruits and vegetables. Used during both food patch and traditional food presentations.
Inactive	Inactive	Upright posture, but no other movement occurs. Ears are not fixed in a particular direction.
	Resting	Lying down on ground or rock with all parts of body (apart from head) in contact with the ground. No other movement occurs.
	Sitting	Rear end is in contact with the ground with no other movement.
Maintenance	Defecation/urination	Waste excretion.
	Dust bathing	Animal repeatedly rolls on back in sand.
	Self-grooming	Care of own body. May include licking, scratching, etc.
Social	Affiliative Contact	Physical contact is made with a conspecific, but recipient does not move away.
	Courtship	Male rapidly moves throughout exhibit in circles or “figure eights” around female.
	Displacement	Quick approach toward conspecific, and receiver moves away quickly. No physical contact is made.
	Scent marking	Rubbing of rear on ground or object such as tree limb, rock, etc.
Not Visible		Animal is not visible to the observer.

TABLE III
WALLABY ETHOGRAM OF OBSERVED BEHAVIORS AND CORRESPONDING
BEHAVIORAL CATEGORIES

Behavioral Category	Behavior	Description
Active	Alert	Sudden orientation of head and ears in a direction with cessation of other movement.
	Hopping	Locomotion using hind limbs only.
	Licking	Touching of tongue to an object. Does not include interaction with a conspecific.
	Sniffing	Movement of nose near an object or another individual.
	Walking	Locomotion using all four limbs.
Forage	Chewing	Consumption of hay. Used during both food patch and traditional food presentations.
	Food patch	Investigation and interaction with a food patch, including removal and consumption of pellets. Only used when food patches were available.
	Normal Food	Investigation and interaction with traditional food tray, including consumption of food pellets. Only used when food was presented in traditional manner.
Inactive	Inactive	Upright posture, but no other movement occurs. Ears are not fixed in a particular direction.
	Resting	Lying down with no other movement.
	Sitting	Resting on rear with no other movement.
Maintenance	Defecation/urination	Waste excretion.
	Drinking	Consumption of water.
	Ruminating	Forward and backward movement of neck followed by chewing.
	Self-grooming	Interaction with own body. May include licking, scratching, etc.
Social	Displacement	Quick approach toward conspecific (usually hopping), and receiver moves away quickly. No physical contact is made.
	Ear Twitch	Head is orientated toward a conspecific while ears rapidly shake/twitch.
	Not Visible	Animal is not visible to the observer.

Experimental observations occurred for 8 weeks (March 10, 2014 - May 14, 2014) generating approximately 40 hours of behavioral data for caviés, and 39 hours for wallabies. Caviés had 10 observation days (totaling 20.6 hrs) with the food patches, and 11 days (19.8 hrs) with traditional food presentation methods. Wallabies had 11 days (21.8 hrs) with food patches and 10 days (17.9 hrs) with traditional methods. Differences were due to weather and maintenance-related building closures on two separate observation days.

4. **Visitor effects**

Following documentation of animal behavior at each 1 min scan, the number of zoo visitors at the exhibit were estimated and noted in the following increments: no visitors, 1-5, 6-10, 11-15, 16-20, 21-25, 25+ (Margulis et al 2003). Visitor interest in the exhibit was also documented by recording length of stay. If the majority of visitors (approximately 75% of group) present at the time of the scan spent < 20 sec actively looking at the animals or into the exhibit, that observation received a rank of “low”; 21-40 sec ranked “medium”, and 41-60+ sec ranked “high” (Margulis et al 2003).

5. **Data analysis**

a. **Environmental preferences**

To test for effects of patch location on GUD for each species, GUD data were analyzed using randomized block ANOVAs under the general linear models of SYSTAT 13 (SYSTAT SOFTWARE Inc., San Jose, CA U.S.A.). We conducted separate analyses for each exhibit space (or species). GUD was the dependent variable, and patch location and date of treatment served as independent variables. We used days of each experiment as replicates rather than repeated measures since the GUD measurement on one day is independent of the GUD of the next. We also satisfy conditions of sphericity (von Ende 1993). A Fisher’s Least Significant

Difference (LSD) post-hoc test was used to evaluate which feeding locations differed significantly.

To evaluate whether the patch treatment influenced how the animals used their exhibit space, we performed goodness of fit test comparing the number of instances each animal was observed in each exhibit quad when patches were present or absent. Associations between exhibit location preferences and GUDs were also tested using a Spearman rank-order correlation. Finally, we used descriptive statistics to compare proportions of time spent in preferred quads vs unpreferred quads with and without patches.

b. **Behavioral enrichment**

As we were specifically interested in how effective food patches were as an enrichment option, we focused on the two salient behavioral categories- foraging and inactive. Time spent foraging and inactive (in minutes) was calculated for each animal on each day of observation as a proportion of time the animal was visible to the observer. Similarly, to test for patch treatment effects on movement throughout the exhibit, we calculated the total number of times each animal moved from one quad to another (hereafter called “transitions”) as a proportion of the total number of observational scans for each day. We then used a MANOVA to test whether individual animal ID and patch treatment (with or without patches) influenced the proportion of time each animal spent in the two behavioral categories and on proportion of transitions. Proportion of time spent foraging and inactive, and proportion of transitions were the dependent variables, and individual animal and patch treatment were the independent variables. We did not attempt to test for differences in effects between the two exhibits, as we had just four individuals (of only two species) that were nested within exhibit spaces, different patch designs, and the potential for between individual interactions.

c. **Visitor effects**

To determine whether patch treatment influenced visitor interest and stay time at each exhibit, we first converted our descriptive data into coded, qualitative scores for both visitor number and stay time at the exhibit (0 visitors = 0, 1-5 visitors = 1, 6-10 visitors = 2, and 0-20 sec = 1, 21-40 sec = 2, etc.). Each scan's qualitative visitor scores were then averaged within day to obtain a daily visitation and duration rate. We then used two-sample *t* tests to compare the daily visitor number and stay-time rates to detect possible effects of patch treatment on visitor behavior.

D. **Results**

1. **Environmental preferences**

For caviies, the location of food patches had a significant effect on GUDs ($F_{(7,28)} = 3.59$; $P < 0.01$; Figure. 13A). Fisher's LSD revealed that GUDs in Quad D were significantly higher than any other section, and GUDs in Quad B were significantly lower than most other sections (Figure. 13B). Date had no significant effect on GUDs ($F_{(4,28)} = 1.90$; $P > 0.05$). While the wallabies showed strong trends toward low GUDs in Quad B and high GUDs in Levels 3A and 3B, the effect of patch location on GUDs was not significant ($F_{(9,90)} = 1.35$; $P = 0.07$; Figure. 14A & 14B). Date also had no significant effect on GUDs ($F_{(10,90)} = 1.57$; $P > 0.05$) for wallabies.

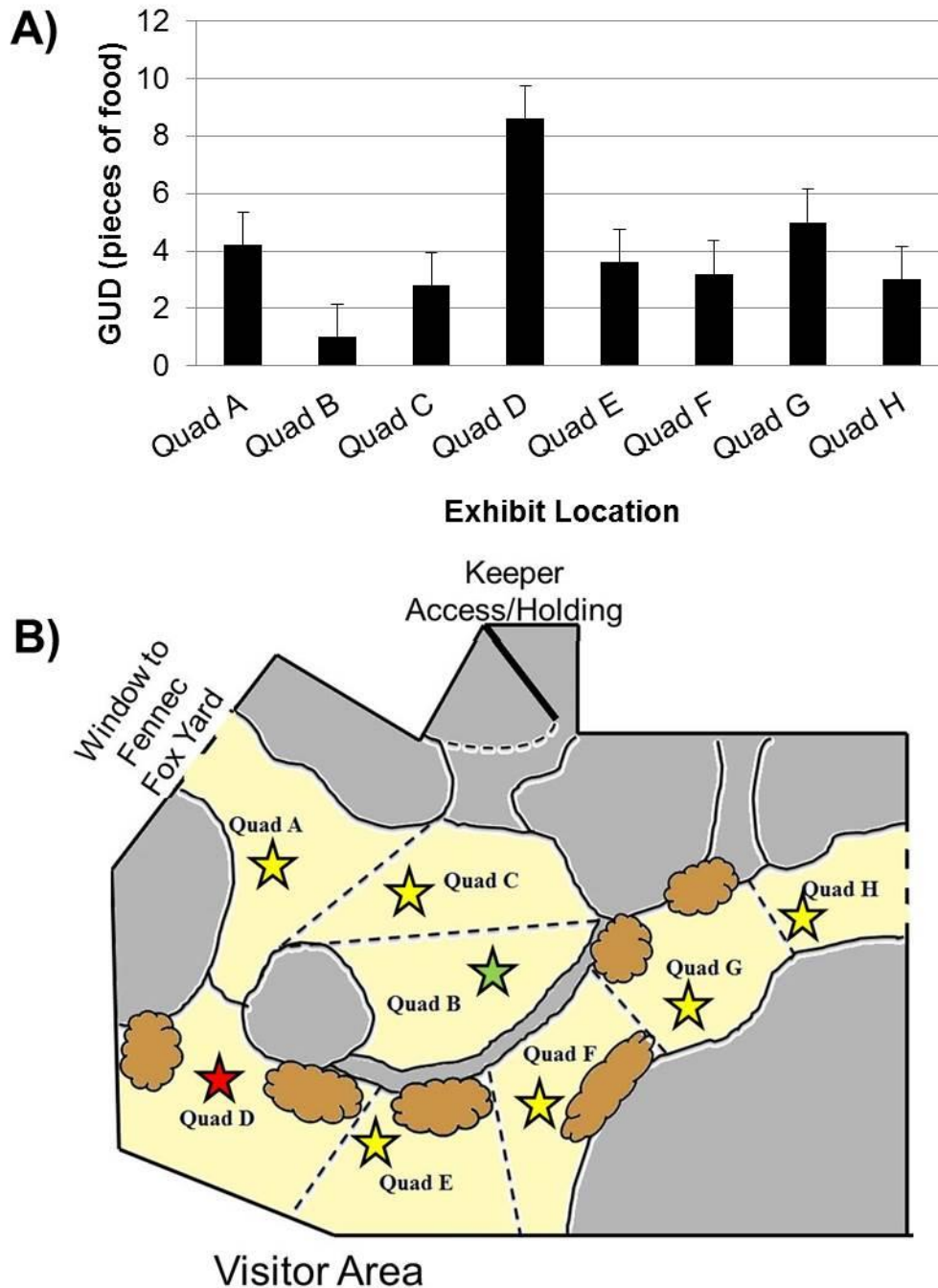


Figure 13. Mean GUD (\pm SEM) and corresponding exhibit graphic map of the cavy's landscape of comfort for the eight patch locations in the cavy exhibit. A) Patch location had a significant effect on GUDs ($P < 0.01$). GUDs in Quad D were significantly higher than other locations, and GUDs in Quad B were significantly lower than most of the other locations. B) The red star indicates an area of discomfort (highest GUDs), yellow stars indicate areas of intermediate comfort (intermediate GUDs), and the green star indicates the area of highest comfort (lowest GUDs).

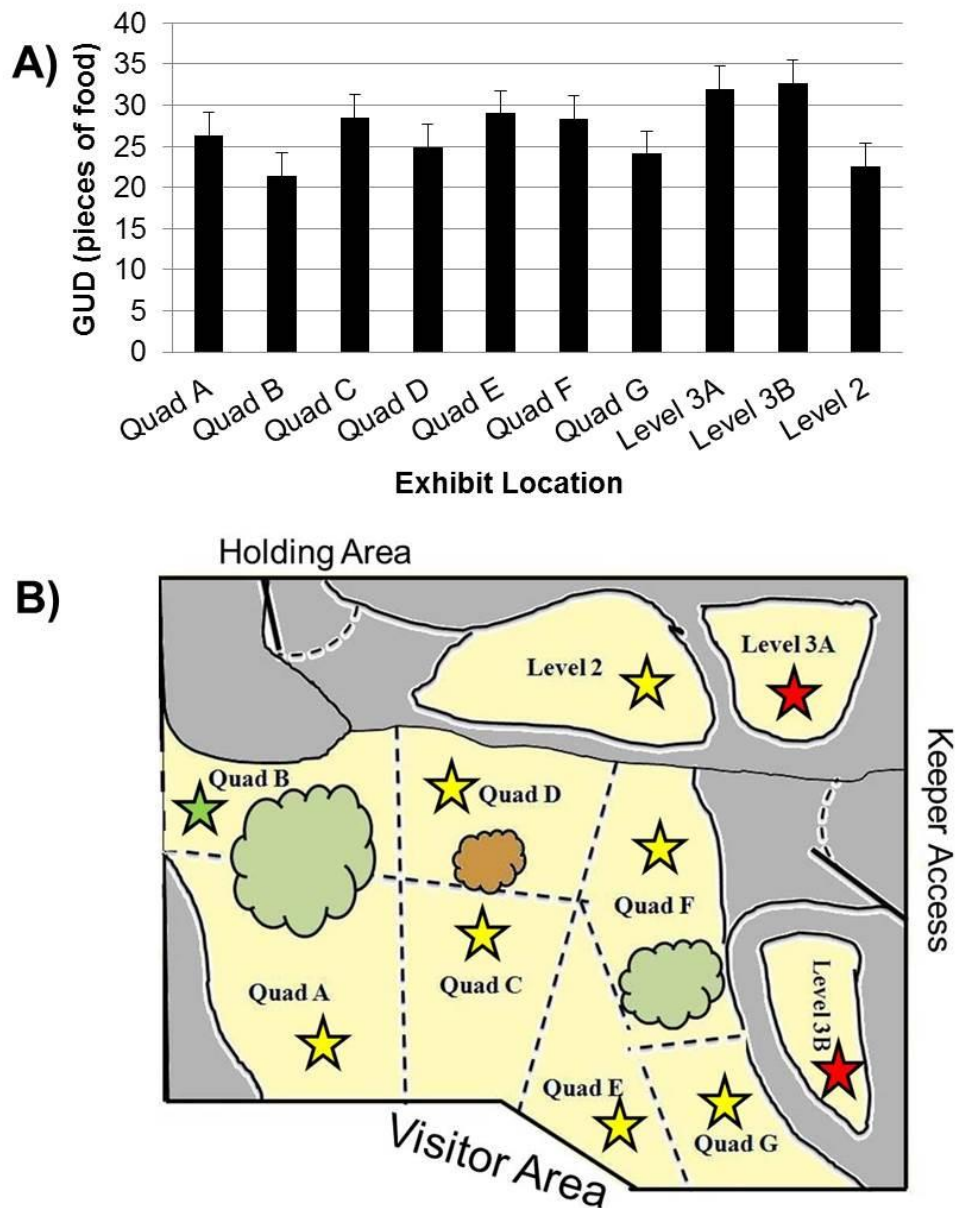


Figure 14. Mean GUD (\pm SEM) and corresponding exhibit graphic map of the wallabies' landscape of comfort for the 10 patch locations in the cavy exhibit. A) While trends did exist, the effect of patch location on GUDs was not statistically significant ($P = 0.07$). B) The red stars indicate areas of discomfort (highest GUDs), yellow stars indicate intermediate comfort (intermediate GUDs), and the green star indicates the area of highest comfort (lowest GUDs).

Goodness of fit tests revealed that food patch treatment significantly influenced where the cavies spent their time in their exhibit ($\chi^2 = 51.22$, $df = 7$, $P < 0.001$). Analysis of residuals indicates that quads previously underutilized without patches became more utilized when patches were present, indicating an increase in exhibit space use. For cavies, GUDs were lowest in quads where they spent most of their time, however, the association was not significant (Spearman rank correlation: $r = -0.37$; $N=16$; $P > 0.1$). When patches were available, the cavies spent most of their time in Quad B (60.1%), followed by Quad C (13.8%) and Quad A (12.2%). The cavies spent less than 5% of their time in the five remaining quads: Quad D (3.3%), Quads E, F, G (2.8%), and Quad H (2.2%), respectively. When patches were absent, cavies again spent the majority of their time in Quad B (68.2%), followed by Quad C (13.8%), and Quad A (9.0%), with less than 5% of time spent in Quads G (2.6%), Quad E (2.3%), Quad F (2.0%), Quad H (1.3%), and Quad D (0.8%). Cavies left the lowest GUDs in Quad B, followed by Quad C, which correspond to the locations where they spent most of their time. However, the next lowest GUDs occurred in Quad H, followed by Quads F, E, A, G, and D (Figure 13A).

Goodness of fit tests revealed that food patch treatment significantly influenced where the wallabies spent their time in their exhibit ($\chi^2 = 116.22$, $df = 9$, $P < 0.001$). As with the cavies, analysis of residuals with the wallabies indicates that quads previously underutilized without patches became more utilized when patches were present, again suggesting an increase in exhibit space use. For wallabies, there was a significant association between quads where GUDs were lowest and where they spent most of their time (Spearman rank correlation: $r = -0.65$; $N=20$; $P < 0.025$). When patches were present, the wallabies spent most of their time in Quad A (41.2%), followed by Quad G (33.7%) and Level 2 (6.0%). The wallabies spent less than 5% of their time in the seven remaining quads: Quad E (4.7%), Quad B (4.0%), Quad D (3.5%), Quad F (3.0%),

Quad C (2.2%), Level 3B (1.0%) and Level 3A (0.7%) respectively. When no patches were present, wallabies again spent the majority of their time in Quad A (39.3%), followed by Quad G (38.2%). The wallabies spent less than 5% of their time in the seven remaining quads: Quad E (4.5%), Quad B (4.4%), Level 3A (3.8%), Quad D (2.9%), Level 2 (2.8%), Quad F (1.3%) and Level 3B (1.0%). The wallabies left the lowest GUDs in Quad B, followed by Level 2, which correspond to the locations where they spent most of their time. The next lowest GUDs occurred in Quad G, followed by Quads D, A, F, C, E, Level 3A And Level 3B (Figure 14A).

2. **Behavioral enrichment**

The presence or absence of food patches had significant effects on proportion of time spent on particular behaviors ($F_{(3,74)} = 13.13$, $P < 0.001$; Wilk's $\lambda = 0.65$). Univariate tests showed that the presence of patches significantly increased proportion of time spent foraging ($F_{(1,76)} = 21.31$, $P < 0.001$), significantly decreased proportion of time spent inactive ($F_{(1,76)} = 5.55$, $P < 0.05$), and significantly increased proportion of transitions within the exhibit ($F_{(1,76)} = 11.31$, $P < 0.01$; Figure. 15A) for all individuals. Individuals also significantly varied in behaviors ($F_{(6,150)} = 51.69$, $P < 0.001$; Wilk's $\lambda = 0.11$). Univariate testing found that the female cavy spent significantly more time foraging than any other individual, followed by the male cavy, then by the wallabies ($F_{(3,76)} = 137.51$, $P < 0.001$). Proportion of time each individual spent inactive also differed, with male Wallaby A spending more time Inactive compared to all other individuals, followed by male Wallaby B, the male cavy, and finally the female cavy ($F_{(3,76)} = 37.25$, $P < 0.001$). There was no individual effect on proportion of exhibit transitions ($F_{(3,76)} = 1.99$, $P > 0.05$; Figure. 15B). There was also no significant interaction between each individual and patch treatment on any of the studied behaviors ($F_{(9,180)} = 0.78$, $P > 0.05$; Wilk's $\lambda = 0.91$).

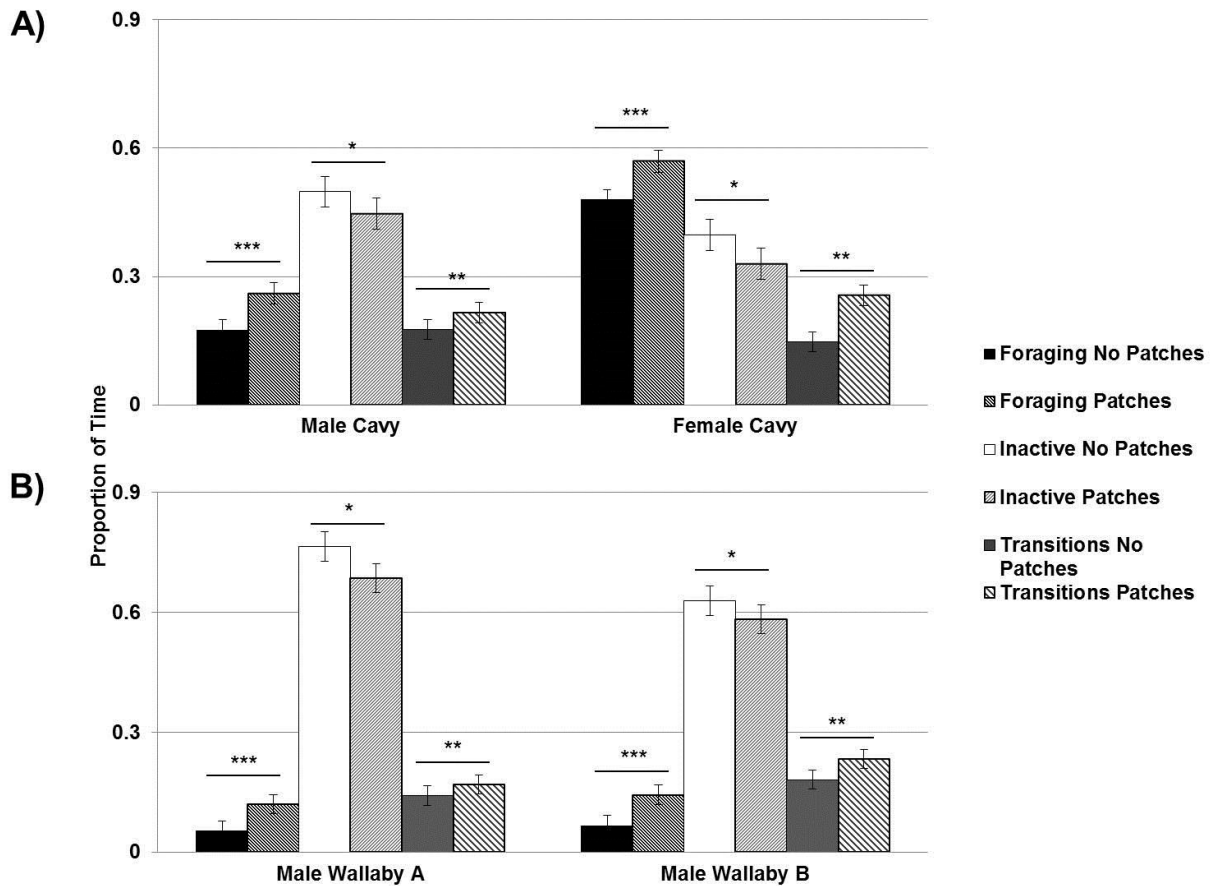


Figure 15. Mean proportion of time (\pm SEM) spent foraging, inactive, and the proportion of exhibit transitions in each treatment condition (with and without food patches) for A) the cavies, and B) the wallabies. Solid bars represent means in the “no patch” condition, whereas hashed bars represent means in the “food patch” condition. Asterisks indicate level of significance (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

3. Visitor effects

Despite trends toward higher visitor number and stay-time during days with food patches compared to days without, for both species there were no significant effects of patch treatment at the cavy exhibit, on either visitor number (one-tailed t test: $t_{19} = 0.33$, $P > 0.05$) or stay time (one-tailed t test: $t_{19} = -0.08$, $P > 0.05$). Similarly, at the wallaby exhibit patch

treatment had no significant effect on visitor number (one-tailed t test: $t_{19} = 0.14$, $P > 0.05$) or stay time (one-tailed t test: $t_{19} = 0.78$, $P > 0.05$).

E. **Discussion**

Captive animal welfare is currently assessed using a variety of methods, but typically require either human interpretations of animal perception, or may only reveal a portion of an animal's welfare. Quantification of foraging effort via patch use and GUDs, in contrast, has the potential to reflect the psychological states of the forager as it assesses and responds to its environment. This information can ultimately assist caretakers in making caretaking decisions based directly on the animal's revealed preferences and perceptions. Integrating principles of foraging ecology, enrichment use, and behavioral observations can produce a comprehensive view of how animals perceive their environment directly from *their* perspective, while contributing important behavioral benefits for captive animals. We therefore envision zoo foraging ecology (employing food patches that allow quantification of GUDs) as a practical, inexpensive, and useful method with which to address a variety of animal welfare-related questions.

1. **Zoo foraging ecology- theory and practice**

Optimal foraging theory explores how animals balance the costs and benefits of foraging (MacArthur & Pianka 1966). Foraging choices depend on how foragers perceive both the reward from the resource, and the costs of obtaining it (Charnov 1976). Brown (1988) identifies costs as metabolic expenditure (C), foregoing alternative fitness-related opportunities (missed opportunities; MOC), and exposure to forms of predation risk or discomfort (P). Foraging costs differ in captivity from those experienced in the wild. For instance, in the wild, missed opportunity costs (MOC) typically include courtship, mating, provisioning young,

maintenance, and seeking shelter (Brown 1988). In captivity, however, most of these activities are either controlled (for example, mating) or provided (for example, shelter). In the wild, energetic costs of foraging (C) include search, attack, and handling prey. Diets of zoo animals, on the other hand, are strictly regulated by nutritionists and veterinarians. They may differ greatly from natural foods (Morgan & Tromborg, 2007), and are typically provided on a predictable schedule in a predetermined location(s) (Watters 2009). Each of these aspects can reduce both effort and energetic expenditure of foraging. Perceived or realized predation risk (P) in captivity also differs from what an animal experiences in nature. Predation risk accounts for detection, response, and avoidance of predators (Brown 1988). Zoos minimize exposure to actual predation risk, rendering predation risk negligible. However, *perceived* predation risk may still be experienced by captive animals. For instance, exhibits of prey and predator species may be close together, allowing for olfactory, auditory, and sometimes visual exchange between animals. Predator/prey odors are also sometimes incorporated into enrichment regimes (see Wells, 2009 for a review). Even visitors represent a form of stress, and perhaps risk, to zoo animals (Davey 2007, Morgan & Tromborg 2007). Therefore, as both the costs of C and MOC are held relatively constant in captive situations (Mogerman 2011), differences in patch use within exhibits can be attributed to the risk perceived by the inhabitants (P).

Despite wide-spread application to wild animal systems and obvious similarities to existing exhibit assessment and zoo foraging enrichment techniques (such as scatter feeding), food patch-GUD methodology has only recently been implemented in zoos. For example, Mogerman (2011) placed several foraging trays throughout species' exhibits at the Brookfield Zoo, in Brookfield, IL, and discovered that rock hyrax (*Procavia capensis*), and American bison (*Bison bison*) perceive areas near exhibit borders as risky (leave higher GUDs) compared to open

areas throughout their exhibits. Similarly, Grant's zebra (*Equus quagga boehmi*) left higher GUDs in areas with blocked sightlines, demonstrating that zoo animals' landscapes of comfort can be reliably established for animals in captivity. Further, Howell-Stephens (2012) compared fecal glucocorticoid metabolites with GUDs to generate "well-being" state categories for zoo-housed Southern Three-banded armadillos (*Tolypeutes matacus*). Interestingly, animals with high stress levels also tended to have high GUDs (and vice versa), demonstrating that changes in GUDs have the potential to reflect (or provide an index of) the physiological state of an animal (Howell-Stephens 2012).

2. **Cavy and wallaby case study**

We expanded upon previous zoo foraging ecology research to explore three specific applications: 1) whether we could determine landscapes of comfort using patch use and giving-up densities, (2) if food patches could increase animal foraging time and activity, thus providing an effective form of behavioral enrichment, and (3) whether food patch presence impacted visitor interest. Despite only having four individuals of two species participating in the study, food patches revealed fine-scale exhibit preferences for foraging, increased foraging and active behaviors while decreasing inactive behaviors of animals, and demonstrated a potential to increase visitor interest.

Regardless of the relative safety of living in an indoor zoo environment, the results indicate that the cavies perceive areas of preference (comfort) and aversion (vulnerability) within their exhibit. Unlike most rodent species, wild cavies forage preferentially in open areas away from bushes and blocked sight lines (Sombra 2011). We therefore predicted that captive cavies would likewise prefer exhibit areas with more open visual fields, and would avoid areas with impeded views. While other aspects of the exhibit also varied among foraging locations, such as

proximity to visitors, we found that the exhibit space most avoided by the cavies (Quad D) had several large visual obstructions (tall grasses, large boulders, etc), which likely limited the cavies' sightlines. The most preferred foraging area, in contrast (Quad B), had arguably the best field of vision. These results indicate that even though these cavies were bred and born in captivity, they retain environmental preferences similar to their wild counterparts.

Foraging location preferences for the Parma wallabies also followed wild foraging studies. Wild Parma wallabies live in forested areas with dense ground cover (Read & Fox 1991), and previous patch-use studies with other wild wallaby species found a preference for foraging near areas of cover (hedges and forest edges) compared to open areas (While & McArthur 2006). We therefore anticipated that our wallabies would also preferentially forage in areas that provided cover and near exhibit borders. We found a nearly significant trend toward greater GUDs/greater discomfort ($P = 0.07$) in the tallest exhibit levels (Levels 3A and 3B), and lowest GUDs/greater comfort in Quad B. Quad B contained a large live plant and exhibit borders that may have created a sense of cover and safety for the wallabies. In contrast, Levels 3A and 3B, where wallabies left higher GUDs, were substantially higher (approximately 1m above the ground quads) and more exposed than the other exhibit areas, which may have resulted in the wallabies feeling vulnerable.

A common goal of behavioral enrichment can be to increase species-specific behaviors, making the captive environment more biologically relevant to the housed animals (Mellen & MacPhee 2001, Swaisgood & Shepherdson 2005). Despite individual variation in the proportion of time spent foraging, inactive, and proportion of exhibit transitions, all participants of our study responded to food patches as predicted- patches significantly increased proportion of time spent foraging, decreased proportion of time spent inactive, and increased movement throughout the

exhibit for both species. Consequently, creating biologically realistic foraging opportunities via food patches and GUDs increased the time spent foraging and the overall use of the exhibit spaces. Natural foraging opportunities are less predictable, requiring greater searching and handling of food items than in captivity (Newberry 1995). Therefore, providing captive animals with opportunities to work for food improves behavioral and physiological indicators of welfare (Morgan & Tromborg, 2007). Food patches also require animals to move from patch to patch to search for and acquire their food, thus increasing within-exhibit movement, thus providing opportunities for choice and flexibility in foraging decisions. Animals are able to forage according to their own schedules, may choose to leave and return to a patch at any time throughout the day, and can avoid foraging in areas of perceived discomfort. As a result, provisioning exhibits with food patches may more accurately replicate natural foraging conditions.

Visitor number and stay-time at each exhibit increased slightly when animals were provisioned with patches compared to traditional methods, suggesting that food patches can alter animal behavior in a manner that may influence the interest of human visitors. It is possible that an increase in sample size (i.e. number of days with each treatment) could reduce some of the variance in daily scores, allowing for more definitive detection of effects. Studies of more active or charismatic species may also result in a greater visitor effect. Regardless, food patches may influence the behavior of not only the animals, but also the human visitors that come to the zoo.

3. **Future applications of zoo foraging ecology**

Our case study demonstrated that GUDs can be used to determine foraging location preferences and exhibit perceptions, are a useful behavioral enrichment tool for animals in captivity, and have the potential to increase visitor interest and experience. Further testing and

validation of zoo foraging ecology is required to realize the full potential of food patches as a tool to assess animal welfare in captivity, and provides many opportunities for future research. For example, longitudinal patch use studies can examine temporal changes in an animal's welfare state via changes in their foraging habits (ie seasonal differences, changes in physical and psychological health, pairing of new individuals in a single exhibit space, etc.). Food patches can be utilized to help alleviate stereotypic behaviors by providing increased opportunities for animals to perform productive foraging and active behaviors (Troxell-Smith et al, unpublished data). Similar to a goal of scatter-feeding (Ryan et al. 2012), provisioning of food patches may also help alleviate aggressive interactions in situations where species' dominance hierarchies and access to resources are a concern, as the dominant animal cannot monopolize all food patches at the same time. Further, researchers can investigate visitor effects on animal preferences and perceptions without having to invest significant amounts of time in behavioral observations by comparing animal foraging location preferences via food patches on days with and without large numbers of visitors. Similarly, for immersive free-range exhibits, food patches can be used as a preference indicator to determine "landscapes of comfort" for animals in constant proximity to humans, and inform further management decisions regarding placement of visitor footpaths, providing areas of cover for the animals, etc. Individual differences in environmental preferences via patch use can be noted and accounted for to customize exhibit design toward the preferences of the animals living in the exhibit, and can aid in tracking long-term health and wellness by revealing changes in food consumption to caretakers. For facilities engaged in wildlife rescue programs, the use of the same food patch methodology in the wild and in captivity can allow researchers to compare the preferences and foraging abilities of rescued animals to their wild

counterparts, providing a quantifiable evaluation of adequate and appropriate responses to environmental stimuli for rescued individuals.

4. **Conclusions and prospectus**

We present an opening application that illustrates some of the potential of zoo foraging ecology with the goal of inspiring additional validation and application of patch-use techniques in captive environments. In particular, we encourage continued studies to more directly link food patches and GUDs to more traditional approaches for measuring animal welfare. Here, we offer several suggestions and guidelines for incorporating foraging ecology into captive animal care and enrichment practices.

First, establishing a positive relationship with animal care staff is critical. Concerns regarding safety, nutritional needs, and feasibility need to be thoroughly discussed and addressed before studies are implemented. Animal care staff are the best and most effective resource regarding the personalities and behavior of their own animals, and thus their input regarding patch design and project implementation is critical to advance the project quickly and effectively. Continued flexibility and open communication between managers, care staff, and researchers throughout the project is essential for project success.

Secondly, creating an appropriate food patch may take significant persistence, creativity, and determination. While the species' natural history should be taken into account, (eg arboreal species will forage more thoroughly in patches above rather than on the ground), finding effective substrates and foraging containers may require trial and error. Initial reluctance to forage in patches may either indicate the patch is ineffective and must be altered, or may simply result from neophobia, in which case animals may simply need more time to adjust to the food

patches. Preliminary trials should always be conducted to ensure animals are foraging appropriately from the patches.

Finally, once an effective patch is created for the target species, we encourage using patches in a wide variety of conditions. As mentioned above, there are many possible applications. When used alone, long-term food patch studies can quickly reveal changes to animal psychological and physiological health, and changes in environmental preferences with minimal time investment. When used in conjunction with behavioral observations, time investment may be greater, the combination allows for more flexibility in the types of questions asked and information gained.

With further validation, foraging ecology, patch use, and GUDs can provide a simple, affordable, and practical tool to assess aspects of animal welfare that can be utilized by anyone working with captive animals. Caretakers in zoos, wildlife rehabilitation/conservation centers, laboratories, and even pet owners can all actively engage in ecological and behavioral research with their animals. Widespread implementation of foraging ecology principles to aid captive animal care can greatly contribute to the fields of animal welfare and behavior by providing caretakers with a quantifiable metric to objectively answer a wide variety of welfare related questions.

F. **Acknowledgments**

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V. UTILIZING NATURAL HISTORY INFORMATION FOR ZOO ANIMAL MANAGEMENT: A CASE STUDY WITH OKAPI (*OKAPIA JOHNSTONI*)

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A. Abstract

Until recently, the impact that presence of conspecifics may have on stereotypic behavior in naturally solitary species exhibited in zoological institutions has largely been ignored. This study examined the effect of adding a visual barrier between animal holding areas at the Brookfield Zoo in Brookfield, IL USA, on stereotypic head-rolling behavior in an adult female okapi (*Okapia johnstoni*). Instantaneous sampling was used to document the proportion of time the female okapi spent head-rolling prior to and after visual barriers were constructed. Behavioral surveys were also distributed to animal care staff to document behavioral change following the implementation of visual barriers. Results from both behavioral observations and animal care surveys suggest obstructing the view of neighboring conspecifics significantly reduced head-rolling behavior and had a positive impact for this okapi. Information gained from this case study will hopefully stimulate discussion around how zoos manage solitary species, and increase research efforts to better understand the effect of the presence of conspecifics on stereotypic behavior for solitary species. Moreover, we hope these results will contribute to both husbandry recommendations and best practice guidelines for zoo-housed okapi.

B. **Background**

A common goal shared by zoos is to improve the welfare and management of the animals under their care. Incorporating characteristics of species' natural history into management practices is often essential to achieving this goal (Foster & Vincent, 2004). Providing animals with species-appropriate social groups and housing is an important first step, and can have a dramatic impact on animal well-being (Morgan & Tromborg, 2007; Swaisgood & Schulte, 2010). For example, housing naturally solitary felids in small groups in zoos can result in chronic stress (Mellen et al., 1998). Similarly, reducing zoo-housed duiker groups to a more natural population size dramatically reduced stress-related jaw abscesses (Barnes et al. 2002). Studies in primates suggest that natural group compositions can lead to more diverse and natural behaviors (Bloomsmit et al., 1994), and improved reproductive success (Cox, 1997; Kuhar et al., 2003; Leong et al., 2004; Maple & Hoff, 1982). Additionally, natural group structure with higher levels of affiliative behavior can reduce stereotypic regurgitation and reingestion (R/R) in bonobos (Miller & Tobey, 2012). It therefore appears that a naturalistic social environment is vitally important to several aspects of zoo animal health and welfare.

Stereotypic behaviors such as pacing or R/R can be defined as invariant and repetitive behaviors that seem to have no immediate function (Mason, 1991), and are typically considered an indicator of negative welfare (Mason & Veasey, 2010). Many authors provide suggestions on how to reduce stereotypic behavior, with increasing enrichment opportunities as one of the most common strategies (Mason et al., 2007; Shyne, 2006; Swaisgood & Shepherdson, 2005). While adding enrichment opportunities for animals can indeed reduce the performance of stereotypic behavior, such solutions treat the symptom (the behavior) and may fail to address the motivation or cause behind the behavior itself. Some research has examined possible motivations behind

stereotypic behavior in zoo animals (Miller et al., 2008), but few studies have investigated the motivation behind stereotypic behavior in okapi, specifically.

Limited data suggest that performance of stereotypic behaviors in okapi may be related to environmental variables, such as exhibit size and complexity, and the length of hay provided during feeding (Fripp et al., 2013; Gilbert & Turner, 2003). Social density was found to impact stereotypic behavior, with small indoor enclosure size and night access to conspecifics predicting oral and pacing behavior in okapi and giraffe (Bashaw et al., 2001). In the Bashaw et al. (2001) study, okapi represented in the survey were mostly housed in groups, and incidence of stereotypic behavior was combined and analyzed with giraffe stereotypic behavior. While the species are related phylogenetically, the social structure between giraffe and okapi differs considerably. Unlike giraffe, which can typically be found in loose, gregarious herds (Estes, 1991; van der Jeugd & Prins, 2000), okapi are typically solitary in the wild (Bodmer & Rabb, 1992; Hart & Hart, 1988; Hart, 2013). Therefore, considering how okapi are housed in zoos (solitary vs. in groups; with or without visual access to conspecifics) may play a more important role in understanding the motivation and frequency of stereotypic behavior in okapi than previously investigated factors. The goal of our case study was to investigate whether blocking visual access to conspecifics by constructing visual barriers could reduce stereotypic head-rolling behavior in an adult female okapi (*Okapia johnstoni*).

C. **Methods**

1. **Subject**

This study was conducted between June and September 2014 at the Brookfield Zoo, Brookfield IL. The subject of this study was an adult female okapi, born at the Brookfield Zoo in April 2011. An adult male okapi (born in March 1996), and an adolescent male okapi

(born in June 2013) were housed in the same barn; however, behavioral observations were only conducted on the female okapi as this was the only individual to perform stereotypic behavior (as described below). All okapi were housed and fed in individual stalls in the barn, and had no direct physical access to one another throughout the observation period.

2. **Exhibits and holding areas**

Animals were on exhibit daily between approximately 0900-1600h. The female okapi had free access to an indoor and outdoor exhibit yard connected by a holding area. The public had visual access to all three of the female's exhibit areas. While in the holding area, the female okapi could choose to have visual access to both the adult male and adolescent male okapi prior to going on exhibit in the morning, and again after the males were shifted into holding for the evening. The female had no visual access to the males while they were on exhibit, but did have visual access if males were kept indoors due to inclement weather. A variety of enrichment items, including brushes, browse and pellet feeders, and a variety of salt and seed licks, were available in each animal's exhibit and holding area, and were rotated daily. Enrichment items that were provided to each animal were consistent throughout the study.

3. **Data collection and analysis**

a. **Behavioral observations**

Eight 30 minute observations were conducted daily for at least three days a week (Monday-Friday) during two time periods; four observations in the morning (0900-1230h) and four in the afternoon (1230-1600h). Instantaneous sampling of state behaviors were conducted at one minute intervals (Altmann, 1974). A random number generator was used to assign specific observation times and dates. Data were recorded using the Animal Behavior Pro app for iPad (Newton-Fisher, 2012, University of Kent). Several behaviors were monitored as

part of a larger project, but for purposes of the present study, only results on stereotypic head-rolling behavior will be discussed. Head-rolling was defined as a clockwise circular rotation of the head along a vertical surface (fence line, shift door, etc), accompanied by alternating stomping of the right front and back left hoof. Inter-observer reliability was achieved using one full day of live observations between two main observers ($r > 0.80$).

Baseline observations were collected from 9th June to 18th July 2014. During the baseline condition, no manipulations to the enclosure were conducted. Visual barriers were then constructed out of plywood and attached to stall walls in the female's holding area via zip ties. The barriers completely blocked the female's view of the males in holding, but did not prevent the female from observing keepers or the public. Behavioral observations with visual barrier were collected from 21st July to 26th September 2014. A total of 132 hours of data were collected for this study, with approximately 62 hours of baseline data, and 70 hours of data with the visual barrier.

Time spent head-rolling was calculated for each day of observation as a proportion of time visible. Proportions were then plotted against sequential observation day, and the celeration line approach was used to examine if a significant change had occurred (Krischef, 1991). The celeration line approach has been used extensively in the fields of physical therapy, behavioral therapy and psychiatry to evaluate the efficacy of treatment in single-subject designs (Krischef, 1991; Nourbakhsh & Ottenbacher, 1994; Wolery & Harris, 1982). As our case study also focused on determining effect of treatment on the behavior of a single subject, we chose to apply this approach to our data to determine treatment effects. Following the procedure outlined in Krischef (1991), the celeration trend line was first constructed from mean data in the baseline phase. Specifically, the baseline period is sectioned into two equal parts based on total number of

baseline observations, and a mean is calculated for each portion of the baseline. The celeration line is then created from the two mean baseline values and extended to the treatment phase to evaluate the effect of the treatment. The proportion of data points above and below the line was compared across phases, and a binomial test was used to determine whether the change in the proportion of data points below the celeration line was statistically significant. A significance level of $P < 0.05$ was set to determine effect of treatment.

b. **Survey data**

A survey was distributed to all okapi animal care staff (including both full-time and rotational staff; total of 8 individuals) that worked with the female okapi both prior to and following the implementation of the visual barriers. The survey asked staff to rate whether they had observed any changes in the female's behavior/welfare following the addition of the visual barriers. There were 17 items on the survey, and the survey used a Likert type scale (-3 to +3). A score of -3 indicated a large reduction in the particular behavior or item (i.e. less/lower) following the construction of the visual barriers, 0 indicated no change and a +3 indicated a large increase in the behavior or item (i.e. more/higher) after visual barriers. A "Don't Know" category was also available. "Don't Know" responses were treated as a null response and removed from subsequent data analysis.

Survey results were tallied, and descriptive statistics (mean \pm SD) were calculated for each survey item. Intra-class correlations (ICCs) were then performed to determine inter-rater reliability and agreement of survey responses.

D. **Results**

The average proportion of visible scans in which the female was engaged in head-rolling behavior in the baseline phase was 9.27 ± 7.33 min, as opposed to 5.88 ± 3.83 min in the treatment

phase. Daily proportion of time engaged in head-rolling behavior is displayed in Figure 16. Once the visual barrier treatment was implemented, all data points during the 19 day treatment phase fell below the celeration line. Binomial test results indicate that implementation of visual barriers significantly decreased the proportion of time the female okapi spent head-rolling ($P < 0.001$).

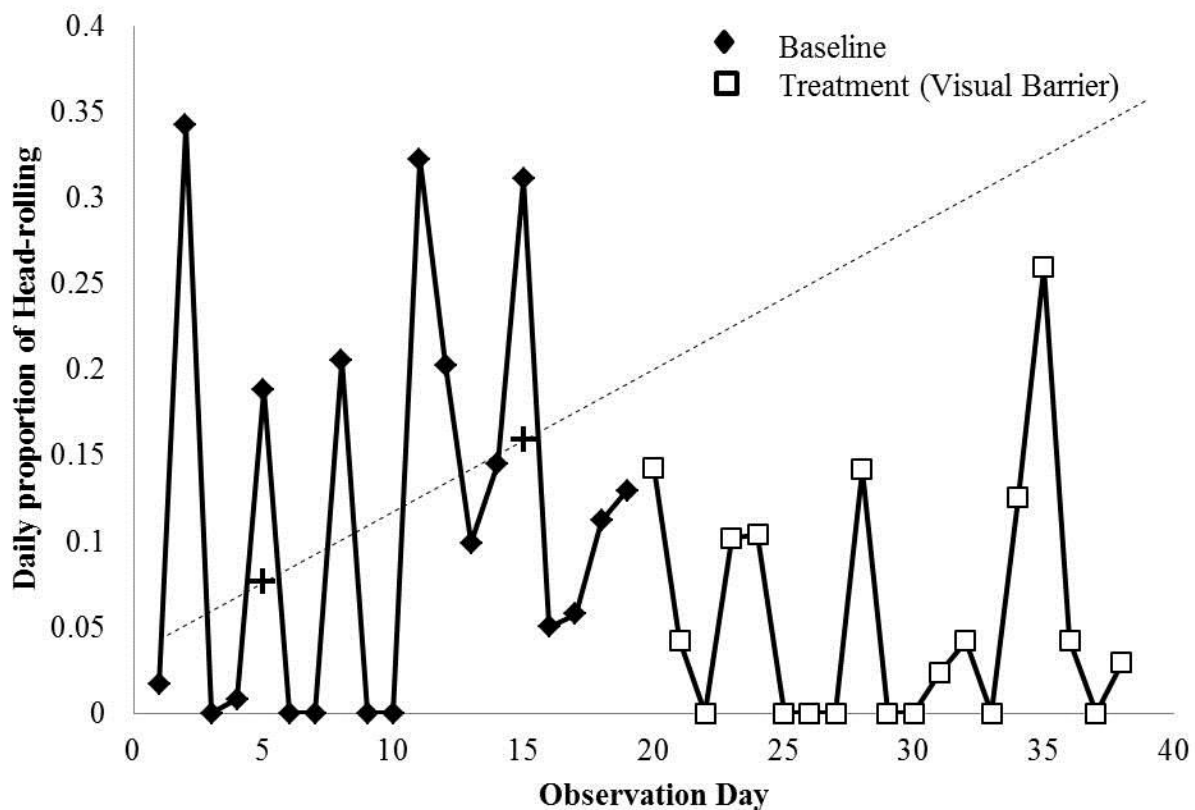


Figure 16. Scatterplot and corresponding celeration line of proportion of time spent Head-rolling prior to (baseline) and after implementation of the visual barrier (treatment). Dashed line indicates the celeration line constructed from two mean data points (indicated by +) in the baseline phase.

Eight okapi keepers and curators completed the survey. Of those eight animal care staff, average years of experience with okapi as a species was 10.75 ± 9.81 yrs, and average experience with the female okapi in particular was 2.38 ± 1.51 yrs. Results of the ICC indicated that there was a high level of agreement in survey responses; the average measure ICC was 0.928 with a 95% confidence interval from 0.825 to 0.981 ($F_{(8,56)} = 13.86$, $P < 0.001$). The three highest scoring survey items (indicating greatest increases in item/behavior after implementation of visual barriers) included overall welfare (1.63 ± 1.60), interest in human interaction (1.00 ± 1.31), and interest in environment (0.86 ± 1.46) (Figure 17). The three lowest scoring survey items (indicating greatest decreases after adding visual barriers) were pacing (-1.38 ± 1.19), anxiety (-1.88 ± 0.99), and head-rolling (-1.88 ± 0.99).

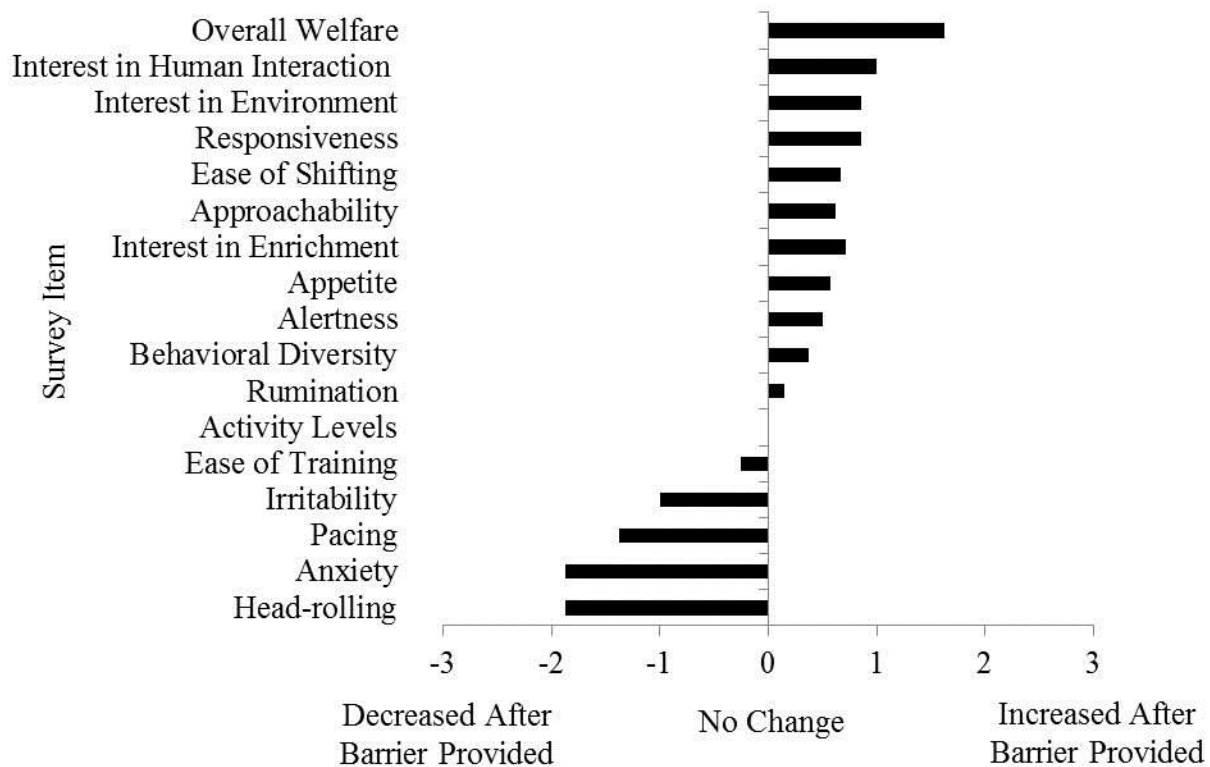


Figure 17. Mean score results from animal care staff surveys ($n=8$) addressing changes in the female okapi's behavior after implementation of visual barriers. -3 indicates a large reduction in the survey item (i.e. less/lower) after implementation of the visual barrier, 0 indicates no change, and +3 indicates a large increase in the survey item (i.e. more/higher) after installation of visual barriers.

E. Discussion

The behavioral results of this study suggest that the female okapi head-rolled significantly less often when her visual access to conspecifics was impeded ($11.53\% \pm 11.78\%$) compared to baseline ($5.56\% \pm 7.19\%$). The location of visual barriers did not obstruct the female's view of visitors, nor did it obstruct her view of keepers, lending further support to the notion that blocking visual access to conspecifics was the main variable driving the decrease in

her stereotypic behavior. While it did not exceed the celeration line, a spike in head-rolling did occur during the visual barrier condition. After reviewing keeper records, the temporary increase in head-rolling could have been in response to extensive carpentry activity that occurred near the okapi exhibit on that particular observation day. Even with the disturbance, the visual barriers appear to have a very positive effect on stereotypic behavior in this female.

The behavioral results coincided with animal care staff perceptions regarding changes in the female okapi's behavior. Following the implementation of visual barriers, keepers perceived the greatest decreases in all undesirable survey items, namely irritability, pacing, anxiety, and head-rolling. Such responses could indicate that, in addition to improving head-rolling, the visual barriers may have decreased other negative behaviors that were observed by the care staff, but not addressed in the behavioral portion of this study. There was a perceived decrease in ease of training, however this was due to a single staff member providing a score of -1 whereas all others reported no change (0) or "Don't Know". Animal care staff also perceived changes in several positive behavioral items, with interest in environment, interest in human interaction, and overall welfare all showing the greatest increases after visual barriers. Taken together, these results suggest that animal care staff perceived an overall positive effect of the visual barriers on the female okapi's behavior.

Wild okapi are typically only found together when mating, or when females are with young (Hart & Hart, 1988; Hart, 2013). The elusive nature of okapi, combined with the densely forested areas of the Democratic Republic of Congo in which okapi are found (Bodmer & Rabb, 1992; Hart & Hart, 1988; Hart, 2013), may reduce their opportunities to come into visual contact with conspecifics on a frequent basis. Thus, housing okapi in a solitary manner in zoos could provide a social environment similar to that experienced the wild. However, if singly-housed

individuals retain the ability to see conspecifics in other exhibit areas while direct interaction is prevented, the situation could become frustrating since natural social behavior cannot be expressed. Over time, such situations could lead to the expression of stereotypic behavior (Mason et al., 2007). Therefore, providing visual barriers to block access to conspecifics, in addition to housing okapi individually, may more thoroughly represent the social situation experienced by okapi in their native habitat. While our results are based on the responses of a single individual, we nevertheless suggest that visual barriers may be an important management tool to help ameliorate stereotypic behavior for solitary species, particularly okapi.

In summary, by incorporating natural history information into animal care and management decisions, we were able to positively influence the welfare of an adult female okapi by significantly reducing the proportion of time she spent performing stereotypic head-rolling behavior. Despite extensive research into the importance of social interactions for zoo-housed gregarious species, little research has investigated the effects of social isolation in solitary species. Multi-institutional studies may be particularly effective in improving our understanding of the influence of conspecifics and social setting on stereotypic behaviors for solitary animals. Such studies can simultaneously facilitate data collection on larger sample sizes and allow for investigation of additional environmental variables that may influence stereotypic behaviors.

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**VI. RELATIONSHIPS BETWEEN ANIMAL PERSONALITY, RISK-TAKING, AND
FORAGING DECISIONS IN THE COMMON BRUSHTAIL POSSUM
(*TRICHOSURUS VULPECULA*)**

A. Abstract

Acquiring nutrients in a landscape of fear poses a challenge for foraging animals. While studies have investigated this tradeoff at a population level, few have investigated the role of personality. We explored the connections between personality and foraging decisions regarding food and fear in a generalist herbivore, the brushtail possum (*Trichosurus vulpecula*). After quantifying behavioral traits in a series of tests, we studied how previously trapped individuals approached the choice between nutritional quality and safety in a free-ranging foraging scenario. We created several foraging stations throughout our field site to investigate the dual costs of predation risk and nutritional quality. We varied the concentration of nitrogen in manually prepared foods, and provided each diet (low, medium and high nitrogen) in either a safe or risky (high nitrogen only) location within the foraging station. We then used a combination of Giving-Up Density experiments and video behavioral analysis to determine the foraging choices of individual possums. At a population level, possums significantly preferred safe, high-nitrogen food patches over patches of lesser quality. However, possums were still willing to forage in risky patches to attain high-quality food. At the individual level, the personality trait “*activity*” appeared to influence foraging decisions of possums: more active individuals spent more time at risky feeders, and spent more time searching for food at the feeder, compared to less active individuals. Interestingly, we found no effect of *boldness* on risk-taking behavior, suggesting that future studies should not limit their assessment of personality on foraging to the effects of *boldness* alone.

B. **Introduction**

Foraging animals must constantly balance finding food with the threat of predation risk (Lima and Dill 1990). Traversing this landscape of fear (Brown et al. 1999, Laundré et al. 2001) can become even more complicated for herbivores. Not only must herbivores balance overall food consumption with predation risk, but they must also take into consideration food quality. In general, animals should prefer foods with a higher nutritional content over less nutritious foods. However, if highly nutritious foods are presented in a risky situation, animals may sacrifice nutritional value for the sake of safety (Hernández and Laundré 2005). Previous studies have demonstrated that individual state also impacts foraging choices. For example, hungry individuals tend to take more risks to attain food compared to sated or overweight individuals (Brown 1992, Olsson et al. 2002, Berger-Tal et al. 2009). In addition, animals with higher ectoparasite loads have been found to spend less time foraging in than individuals with lower loads (Webster et al. 2007, Raveh et al. 2011), demonstrating that individual state directly impacts how animals balance foraging choices. Aside from individual state, the decision on whether to sacrifice nutrition in the face of risk should also be influenced by individual personalities.

Consistent intra-individual variation in behavior within a species, or animal “personality” (Sih et al. 2004, Réale et al. 2007), has recently received increasing attention regarding its influence on the behavioral choices of individual animals. Animal personality traits (including boldness, exploration, activity, and aggressiveness; Réale et al., 2007) are known to have large ecological implications for survival (Bremner-Harrison et al. 2004) and reproductive success (Both et al. 2005), and thus are inherently linked to individual fitness (Réale et al. 2007, Smith and Blumstein 2007). As foraging is an essential component of individual fitness, personality

should influence the foraging decisions of individual animals. Several studies have demonstrated that bold individuals are more willing to venture into risky areas when foraging compared to shy individuals (Michelena et al. 2009, Bergvall et al. 2011, Dammhahn and Almeling 2012). However, the direct effect that individual personality may have on navigating tradeoffs of nutrition and predation risk has received limited investigation.

We explored how individual personality may influence tradeoff decisions in nutritional quality and predation risk in brushtail possums (*Trichosurus vulpecula*). Brushtail possums offer an ideal study species, as previous research has demonstrated how possums respond to variation in nutritional quality (Freeland and Winter 1975) and predation risk (Pickett et al. 2005, Mella et al. 2014). Moreover, recent studies have also established and validated ecologically relevant personality traits in the species. Mella et al. (2015) discovered three consistent personality traits (docility, activity/exploration, and boldness) in brushtail possums after briefly housing wild individuals in captivity. It has been suggested, however, that prolonged captivity alters the behavior of wild animals (Butler et al. 2006), and could result in habituation to testing procedures (Dingemanse et al. 2012). In effort to mitigate these potential issues, Mella et al. (in press) recently outlined procedures for several field-based behavioral tests designed to consistently evaluate the same personality traits for possums (docility, activity/exploration, and boldness) while eliminating the need to house individuals in captivity for extended periods of time. These behavioral tests produced highly repeatable results for the three personality traits of interest, and thus provide a valuable standardized protocol for future personality testing in wild brushtail possums.

In this study, we used a combination of behavioral/personality testing and artificial foraging stations to determine how individual brushtail possums navigate the tradeoff between

nutritional quality and predation risk. We predicted that, at a population level, brushtail possums should bias their foraging toward safe, highly nutritious patches as opposed to risky high nutrition patches, but the preferences for risky patches would increase as the nutritional quality of safe patches declines. We also predict that individual personality traits will influence the value that the forager places on nutritional quality relative to predation risk. In particular, we anticipated that individuals with bold personality traits will spend a higher proportion of time foraging in risky food patches, whereas shy individuals will focus their foraging toward the safer patches. Further, we predict that shy individuals will display more vigilance behavior, particularly at risky food patches, compared to bold individuals.

C. **Methods**

1. **Study species and field site**

The brushtail possum is an arboreal, nocturnal generalist marsupial herbivore with an extensive geographic range throughout Australia (Kerle and How 2008). The majority of their diet consists of a variety of *Eucalyptus* leaves, but they are also known to encounter and consume less toxic food, such as grasses and flowers, on the ground (Freeland and Winter 1975, Pietrzykowski et al. 2003). However, previous studies also suggest that ground foraging is perceived as more risky than above-ground foraging for this arboreal species (Pickett et al. 2005, Mella et al. 2014, 2015).

The field sites were located in the Ku-Ring-Gai Chase National Park, NSW, Australia (33°41'16.24''S, 151°09'11.90''E). The national park consists of primarily eucalypt woodland, and is inhabited by several possum predators, like the invasive red fox (*Vulpes vulpes*), domestic dog (*Canis lupus familiaris*), and powerful owl (*Ninox strenua*) (Mella et al. 2015).

2. Personality assessment

We trapped possums in the park over three separate sessions between May and October 2015, each session lasting either two or four weeks: Session 1 (4 weeks) occurred between 5/12/2015- 6/5/2015, Session 2 (4 weeks) occurred between 7/14/2015-8/7/2015; and Session 3 (2 weeks) occurred between 10/20/2015- 10/30/2015. Live traps (66 cm L \times 24 cm W \times 24 cm H; model 205; Tomahawk, USA) were used to capture possums throughout the study site. Traps were opened for four consecutive nights per week. A total of 33 traps were placed along either side of two main walking trails in the park (Sphinx Walking Trail and Warrimoo Walking Trial) in the park, and were placed approximately 70m apart, and 70m into the bush.

Traps were covered with hessian sack and a plastic bag to protect captured possums from the elements. Traps were baited each evening at approximately 16:00. Bait consisted of half of a peanut butter sandwich attached to the back of each trap by a cable-tie. Half of a red apple was also placed in each trap to provide a water source for trapped animals. All traps were checked once per night, beginning at 00:00 the next morning, allowing at least an eight-hour trapping window.

As described in Mella et al. (in press), captured possums underwent four different personality tests at time of capture to measure qualities related to exploration, boldness and docility: the “Emergence test”, “Handling Bag test”, “Open-Field”, and “Release test”. All personality assays were conducted in the field immediately following the discovery of an animal in the trap. Where applicable, data for possums trapped on the University of Sydney campus (hereafter USyd campus) as part of a previous study (Sunderasan 2014) were also included. These animals were subjected to the same personality assessment protocols (see below).

“Emergence Test”: A total of 23 possums (13 females and 10 males) from the national park were tested. Upon finding a captured possum, the individual was encouraged from the trap into a hessian handling sack. The *latency to move* from the trap into the handling bag was recorded in seconds. We feel this provides a measure of *boldness*.

“Handling Bag Test”: A total of 47 possums (24 females and 23 males) from both the national park ($n = 23$) and USyd campus ($n = 24$) campus underwent this test. To measure *docility*, possums in the handling sack were suspended for 60 sec, and *time spent immobile* was quantified for each possum. Previous studies defined animals that were less mobile during human handling as docile (Martin and Réale 2008a).

“Open-Field Test”: A total of 47 possums (24 females and 23 males) from both the national park ($n = 23$) and USyd campus ($n = 24$) campus underwent this test. To measure *activity/exploration*, possums were transferred from the handling sack to a modified open field apparatus. The apparatus consisted of a plywood bookshelf (IKEA, Besta Bookcase; 60 x 40 x 128cm) with four shelves, each shelf containing a red light. Plastic mesh (1cm^2) covered the front of each level preventing escape and allowing the filming of the animal within the apparatus. Two rectangular holes were cut into each shelf to allow possums to pass from level to level. Each level also contained a small food container with a single piece of apple in the corner. Possums were released into the bookcase via a circular hole (10cm diameter) in the right side of the lowest shelf level, and behavior was filmed in the maze for 5 minutes. Videos were analyzed using *JWatcher* (Blumstein and Daniel 2007), where we recorded several behavioral variables related to boldness, docility and activity/exploration (see Table IV for complete ethogram).

TABLE IV
ETHOGRAM OF QUANTIFIED BEHAVIORS DURING OPEN-FIELD TEST VIDEOS

Behavior	Description
Not Moving	not performing any behaviors
Head Moving	moving the head only
Semi Moving	moving the front two feet only
Fully moving	moving all four feet
Number of level changes	number of times animal moves from one level to another
Unpreferred level time	total time spent in the three least preferred levels by the individual
Sniffing	movement of nose toward object
Eating	chewing
Grooming	licking and scratching
Pushing	pushing against the mesh doors of the open field test
Vigilance	not moving, on four feet with ears up and eyes focused on something outside of the open field test

Following completion of the Open-Field test, each possum was placed back into the handling sack. Possum weight and sex were recorded, along with its microchip ID if the animal was already PIT tagged. If the possum did not already have a PIT tag, a unique tag was injected subcutaneously under the skin between the shoulder blades. Animals trapped in the national park were also given a unique fur-clip pattern to aid in camera-trap identification for later foraging trials.

“Release Test”: A total of 23 possums (13 females and 10 males) from the national park underwent this test. This test was the final personality assessment, and was quantified as another measure of *boldness*. Each possum was returned to its site of capture, and the handling sack was placed gently on the ground. The sack was removed to only expose the head of the possum,

which faced away from the handler. *Latency to escape* was quantified as the time it took for the possum to move from the bag to a distance of 1m, measured in seconds.

Following procedures outlined in Mella et al. (2015) and Mella et al. (in press), we tested whether each observed behavior represented a personality trait using a log-likelihood ratio test in SAS (PROC MIXED, SAS Institute Inc. 2003) by comparing two different linear mixed-effect models for each behavior. Each model contained an identical fixed effect structure (i.e. test-number, body weight and sex), and were tested with and without individual possum identity as a random effect (after Dingemanse and Dochtermann, 2013). Akaike information criterion corrected (AICc) values were compared as an additional model assessment, where a difference of at least 2 units indicates improved explanatory power (Burnham and Anderson 2003). Repeatability (r ; i.e. the proportion of behavioral variation displayed over multiple tests that is due to inter-individual rather than intra-individual differences; Réale et al. 2007) for all traits was calculated as described in Dingemanse and Dochtermann (2013).

3. Free-ranging foraging trials

To determine if personality traits influence foraging decisions in our possums, we created a total of 13 foraging stations or “Blocks” in the national park, following methods described in Mella et al. (2015): eight blocks were located along the Sphinx trail, and five blocks were located along the Warrimoo trail. Each block was spaced at least 400 m apart from a neighboring block. All blocks contained four feeders: three “safe” feeders which were situated near large trees and approximately 1.5 m high above-ground, and one on-ground “risky” feeder that was located at least 2m from the nearest tree, requiring possums to travel along the ground to access the patch (Mella et al. 2015; Figure 18). Each feeder also had an infra-red motion

sensitive digital camera (Scout-Guard SG550v), allowing for the identification of individual possums (via their fur-clips) and to record behavior at the feeder.



Figure 18. Picture of a typical foraging block. Yellow circles indicate the above-ground, “safe” feeders (located approximately 1.5m off the ground and next to a tree to facilitate easy entry and escape). The red circle indicates the on-ground “risky” feeder (located at least 2m from the nearest tree, requiring possums to travel along the ground to access the feeder).

To evaluate the impact of food quality on foraging decisions, we created food pellets that varied in the amount of nitrogen. We prepared three different diets by first grinding commercial

rabbit pellets into powder. We then added one of four concentrations of ground oaten hay stalks (0, 40, 60, 80 %), 6% water and 20% raw sugar to each rabbit pellet mixture before repelletting (approximate size: 8 mm diameter \times 2 cm L) in a stock feed pelleting machine (Buskirk Engineering, model PM605). Average nitrogen content for the rabbit pellets prior to mixing was 2.5%. Average nitrogen content for the oaten hay was 0.9%. Final nitrogen concentrations of our prepared diets were: Diet A- 0.7%N, Diet B- 1.4%N, Diet C- 2.0%N. Each diet was prepared separately to prevent cross-contamination. The range of nitrogen present in our diets was similar to that found in *Eucalyptus* leaves, the main food source of wild brushtail possums (Freeland and Winter 1975, Dearing and Cork 1999). Each feeder consisted of a foraging container (12cm H x 27cm L x 33 cm W) with 20 pellets of diet mixed randomly into 1.2kg of sawdust. Sawdust generated diminishing returns from foraging in the patch (i.e. each piece of food is harder to find than the last; Brown, 1988). One pellet was placed on top of the sawdust, and served as an indicator of patch visits. If the pellet remained, the feeder was considered not visited unless video showed otherwise. Each day, remaining pellets of food in the patch were counted (providing the Giving-Up Density/GUD; Brown, 1988), and patches were restocked for the next night's test.

The experiment ran for a total of nine nights over a three week period. Some sites were not visited every night, and patches were closed on some nights due to rain. Diet location for safe feeders was randomly assigned within the block, and remained in the same location throughout the experiment. Following McArthur et al. (2012) and Nersesian, Banks, & McArthur (2011), the diet in each safe feeder within the block was randomly assigned (hereafter Diets A, B, and C), but risky feeders always contained the high-nitrogen diet (hereafter Diet CGround). In nature, possums encounter foods that contain less toxic compounds (and thus high-quality) on the

ground, such as grasses and flowers, as opposed to their main staple of eucalypt leaves that they consume in the trees (Freeland and Winter 1975).

Following Mella et al. (2015), we tested whether patch Treatment (A, B, C, or CGround) effected GUDs using the mixed model procedure in SAS (PROC MIXED). Treatment, Experimental Day, and the interaction of Treatment X Experimental Day were included as fixed effects. Site (i.e. Sphinx or Warrimoo trail), Feeder Number within Block (i.e. 1-4), and Block Location within Site were included in the model as random factors.

Food patch videos were analyzed using *JWatcher* to identify individual possums, and to quantify the amount of time each individual spent in several foraging and vigilance related-behaviors (Table V). Behaviors were then combined to generate behavioral categories of interest, including *Searching*, *Foraging*, and *Vigilance*. We quantified total time spent *Searching* and *Foraging* at the feeder, and the proportion of time spent *Vigilant* (Mella et al. 2015).

TABLE V
ETHOGRAM OF QUANTIFIED VIDEO BEHAVIORS AND BEHAVIORAL CATEGORIES
FOR FEEDER VIDEOS

Category	Behavior	Description
Vigilant	vigilant	2 feet, ears up, immobile
	alert	on 4 feet, ears up, immobile
Foraging	feeding	head out of the feeder eating/chewing (not looking around)
	aware	on 4 feet, ears up but chewing
Search	search	head in the feeder searching/eating (head in box)
Other	grooming	licking and scratching
	interact	any interaction with other possum (e.g. fighting, sniffing)
	marking	rubbing chest on feeder
	tail wagging	staring and simultaneously wagging the tail
	investigation	sniffing the feeder
	climbing	climbing up/down the pole
	escape	jumping abruptly off feeder
	out of view	possum behaviour not visible

4. The effect of personality on foraging choices

As an initial exploratory analysis, we examined associations between personality metrics and the *time spent at each feeder* (A, B, C, and CGround), and time spent in *Foraging*, *Searching*, and *Vigilance* behavioral categories using Spearman rank-order correlations. While we acknowledge that conducting multiple tests on the same individuals generates issues with the experiment-wise error rate, this exploratory analysis will be supplemented in the future with more extensive and appropriate analyses.

D. Results

1. Assessment of personality traits

A total of 47 (24 female 23 male) possums were captured between the national park and USyd campus. Of the 23 possums (13 females and 10 males) captured at the national park, 12 possums (6 females and 6 males) were trapped at least twice, which allowed for testing of personality metrics. 12 additional possums (2 females, 10 males) were trapped at least twice on the USyd campus, generating a pool of 24 possums to test for individual-variation in personality metrics.

Possum identity had a significant effect on five behaviors assessed for personality (Figure 19): *Time spent immobile* during the Handling Bag test (i.e. *docility*; $n = 24$); *not moving*, *head moving*, and *unpreferred level time* during the Open Field test (*activity/exploration*; $n = 24$); and *latency to escape* during the Release test (*boldness*; $n = 12$; Table VI). However, *test-number* also explained a significant amount of variation in *time spent immobile* and *not moving*, suggesting that these behaviors were also plastic over time (i.e. possums became more or less immobile (depending on the animal) as the number of repeat tests increased). All other behaviors were discarded, as they did not prove to be consistent within individuals.

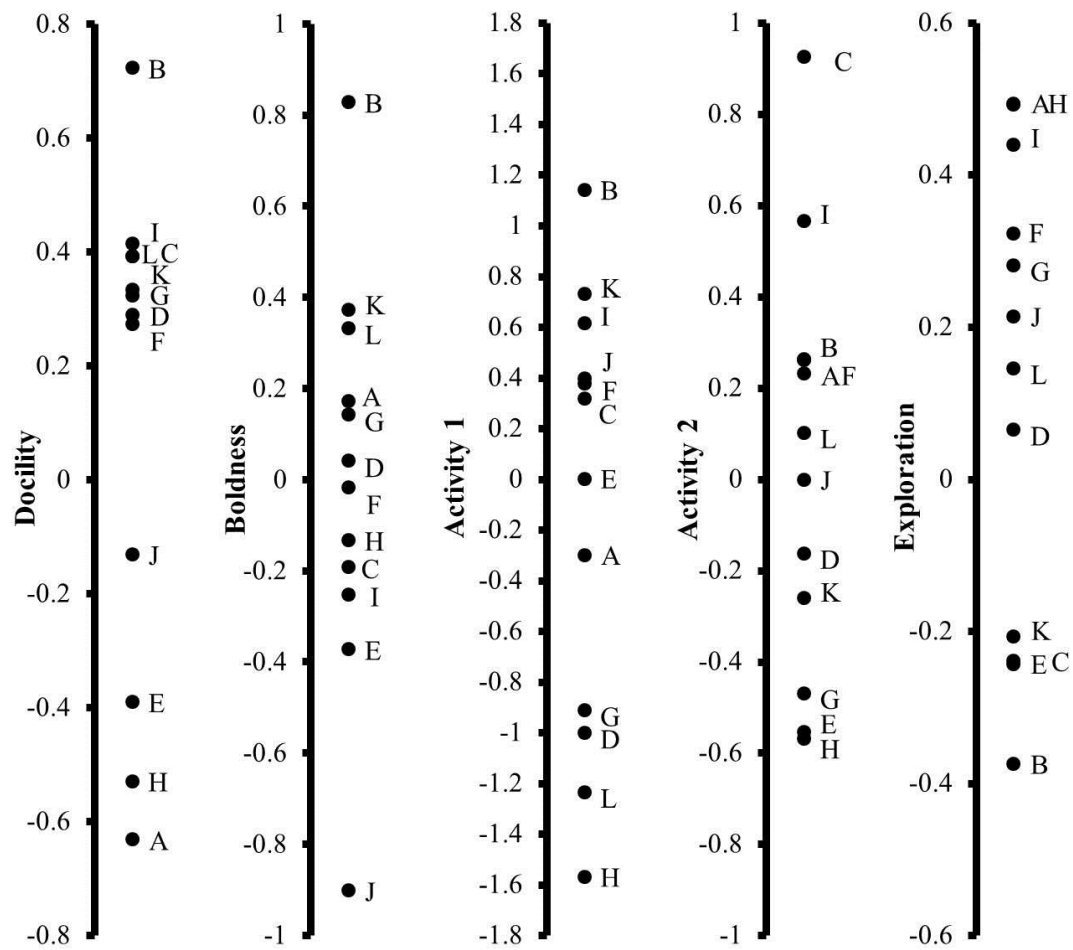


Figure 19. Best linear unbiased predictor (BLUP) values from the univariate models of docility, boldness, and activity/exploration demonstrating the relative value of each personality trait for each possum. Each individual is represented by a different letter ($n = 12$).

TABLE VI
BEHAVIORS REPRESENTING PERSONALITY TRAITS FOR BRUSHTAIL POSSUMS

Test	Behavior/ Trait	ID	Model	K	-2LL	Test	LRT	df	P	AICc	ΔAIC_c	r
Handling -bag test (n = 24)	Time Spent Immobile	no	1	3	298.82					309.46		
	<i>Docility</i>	yes	2	4	284.14	1 vs. 2	14.68	1	< 0.001	288.27	21.19	0.40
	Not Moving	no	1	3	241.38					247.77		
Open- field test (n = 24)	<i>Activity</i>	yes	2	4	228.88	1 vs. 2	12.5	1	< 0.001	233.07	14.7	0.46
	Head Moving	no	1	3	194.6					200.94		
	<i>Activity</i>	yes	2	4	185.56	1 vs. 2	9.04	1	0.002	189.73	11.21	0.43
	Unpreferred level time	no	1	3	218.59					224.95		
	<i>Exploration</i>	yes	2	4	212.22	1 vs. 2	6.37	1	0.011	216.6	8.55	0.31
Release test (n=12)	Latency to escape	no	1	3	227.50					233.85		
	<i>Boldness</i>	yes	2	4	220.59	1 vs. 2	6.91	1	0.009	224.76	9.09	0.36

Significant differences are based on log likelihood ratio tests (LRT) and are indicated in bold. ID = indicates whether individual identity was included in the model; K = number of parameters tested in the model; -2LL = -2 log-likelihood; Test = indicates which models were compared; df = degrees of freedom; P = probability; AICc = Akaike information criterion corrected; ΔAIC_c = difference between AICc values; r = repeatability value for that behavior.

2. Population-level GUDS

At the population level, *Treatment* had a significant effect on GUDs ($F_{3,189} = 14.21$, $P < 0.0001$; Figure 20). GUDs were highest in Treatment A (safe, low nutrition; mean GUD (pieces of food) \pm SEM; 16.48 ± 1.56), followed by B (safe, medium nutrition; 11.48 ± 1.50), CGround (risky, high nutrition; 10.06 ± 1.49), and lowest in Treatment C (safe, high nutrition; 8.85 ± 1.50). Neither *Experimental Day* or the interaction of *Treatment X Experimental Day* ($F_{8,189} = 1.87$, $P > 0.05$ and $F_{24,189} = 1.28$, $P > 0.05$ respectively) had significant effects on GUDs.

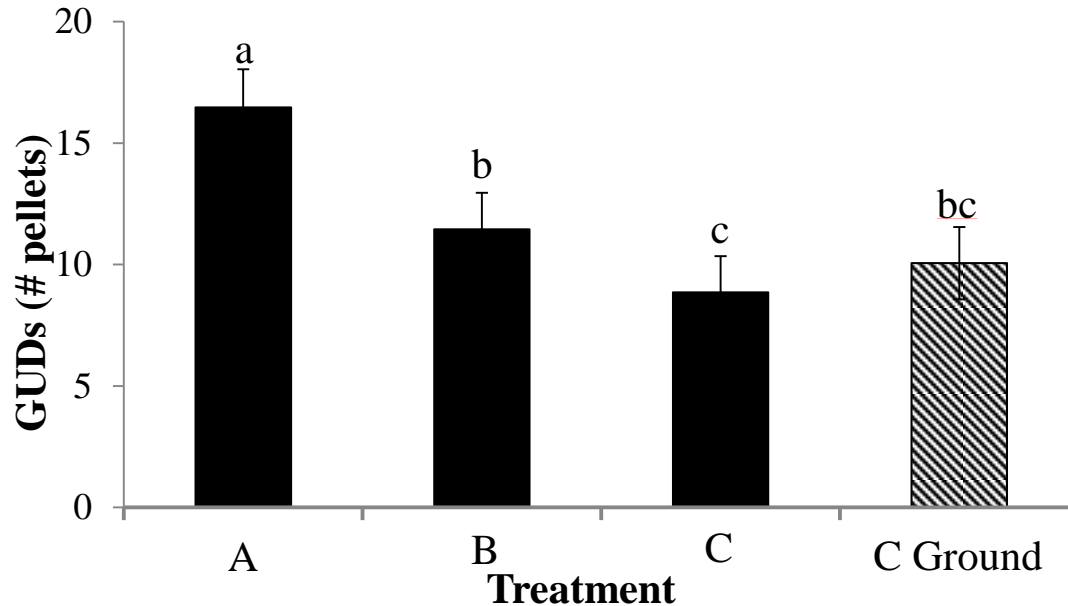


Figure 20. Population-level mean GUDs (\pm SEM) for the brushtail possums. Patch *Treatment* had a significant effect on GUDs ($P < 0.0001$). Significant differences between GUD Treatments are indicated by different letters. GUDs in Treatment A were significantly higher than all other treatments ($P < 0.0001$). GUDs in Treatment B were significantly higher than Treatment C ($P < 0.05$), but did not significantly differ from Treatment CGround ($P > 0.05$). GUDs did not significantly differ between Treatment C and CGround ($P > 0.05$).

3. The effect of personality on foraging choice

A total of 10 identifiable possums (7 female, 3 male) visited the foraging stations. Six of those possums (5 female 1 male) had previously quantified personality traits (i.e. trapped more than twice). Only two possums lacking identification visited the foraging stations, indicating that we had successfully trapped and tested the majority of the free-ranging possum population around our field sites in the park.

In general, possums spent the smallest proportion of time at Diet A (average 15.9%), followed by Diet CGround (average 26.0%), and Diet B (average 26.2%). Possums spent the most time at the safe, high nutrition feeder (Diet C; average 31.8%). Possums did demonstrate substantial individual variation, however, indicating that individuals differed in how they chose to balance the tradeoff between nutrition and fear. Possums #01153368, #01153997, and #01155555 all spent the majority of their time at Diet C (45.7%, 48.1%, and 48.9% respectively; Figure 21). Possums #01156174 and #01156174 spent the majority of their time at Diet B (39.0% and 57.2% respectively), whereas possum #01156308 spent the majority of its time (45.6%) at patch CGround.

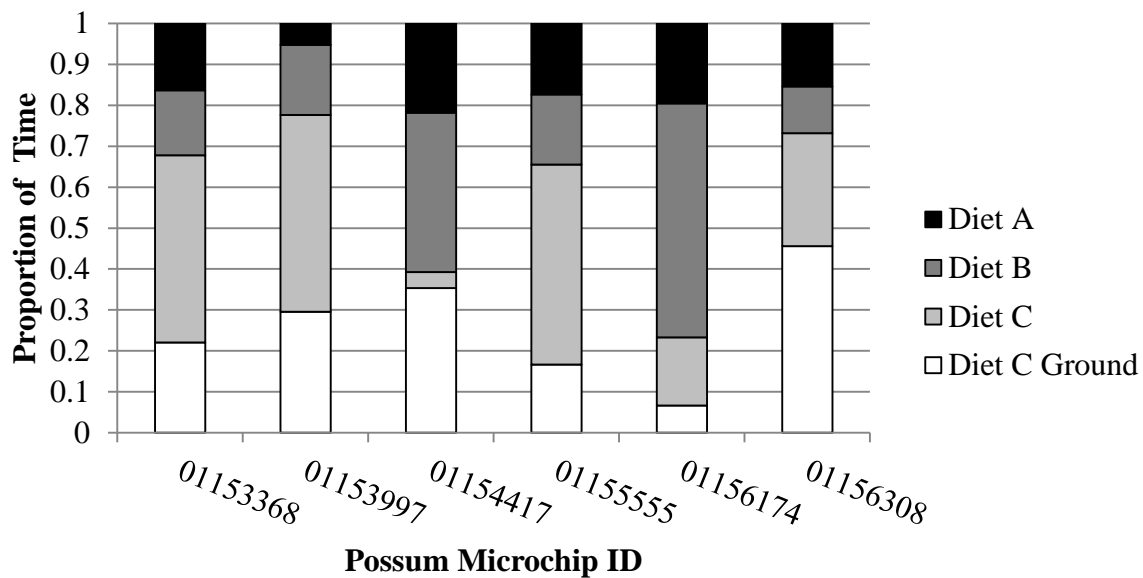


Figure 21. Portion of time spent at each treatment for possums with established personality metrics.

Overall, the dominant behavioral categories displayed at feeders were *Foraging* (average 23.9%), *Searching* for food (average 29.9%), and *Vigilance* (average 30.2%). Possums spent an average of 15.3% of their time in *Other* behaviors, which we did not consider in the following analyses.

Spearman rank correlation tests revealed that *Activity 1 (not moving)* had significant negative associations with time spent in Diet DG and time spent *Searching*, with active (least time *not moving*) individuals spending the most time in feeder DG ($r = -0.94$; $n = 4$; $P < 0.025$), and the most time *Searching* ($r = -1.0$; $n = 4$; $P < 0.005$; Table VII). *Activity 1* also had a positive association with time spent *Foraging* (i.e. most active individuals spent least time foraging; Spearman rank correlation: $r = 0.94$; $n = 4$; $P < 0.025$). *Activity 2 (head moving)* had a significant positive association with time spent in Diet DG (i.e. most active individuals spent the most time in Diet DG; $r = 0.89$; $n = 4$; $P < 0.025$). There was no significant association between the personality traits of *Docility*, *Boldness* or *Exploration* on time spent in any of the feeders, nor in any of the behaviors at the feeder (*Foraging*, *Searching*, and *Vigilance*).

TABLE VII
SPEARMAN'S CORRELATION COEFFICIENTS OF PERSONALITY COMPONENTS
COMPARED TO FORAGING LOCATIONS AND BEHAVIOR

	Diet A	Diet C	Diet D	Diet DG	Search	Foraging	Vigilant
Docility	-0.314	-0.257	0.543	0.029	-0.143	0.314	-0.6
Boldness	0.143	0.086	-0.657	-0.029	0.086	0.029	-0.429
Activity 1	0.2	0.6	0.314	-0.943**	-1***	0.943**	-0.029
Activity 2	-0.714	-0.429	0.314	0.886**	-0.029	0.086	0.2
Exploration	0.086	-0.657	0.257	-0.029	0.257	-0.2	0.486

Bold indicates significant results: ** = $P < 0.025$; *** = $P < 0.005$

E. Discussion

We found consistent differences between individuals in five behavioral traits representing three different personality metrics: boldness, docility, and activity/exploration. Each of these behavioral traits was highly repeatable ($r = 0.31 - 0.46$) over several tests, indicating that the variation in the traits can be explained by consistent differences between individuals within the population, rather than behavioral inconsistency within individuals (Réale et al. 2007). Therefore, we interpret these results as genuine personality metrics, rather than a reflection of an animal's temporary state. However, we did find evidence of within-individual plasticity (i.e. significant effect of *test number*) for *time spent immobile* in the Handling Bag test and *not moving* in the Open-Field test. Previous studies suggest that frequent exposure to the same test over a short period of time can lead to variable individual responses as a result of habituation (Mella et al. in press, Martin and Réale 2008b). The Handling Bag test was performed every time a possum was trapped, which in some cases meant it was performed multiple times within the same week. The Open Field test, on the other hand, was only performed if there was a minimum of three nights between tests (i.e. once per trapping session) per animal. However, habituation is still possible as animals continued to be exposed to both tests. Despite evidence of plasticity, individuals maintained their relative rank compared to other possums, indicating that these measures still represent reliable personality traits.

As predicted, possums at a population level strongly preferred foraging from safe feeders with high nitrogen (Diet C; lowest GUDs) over safe feeders with medium (Diet B) and low nitrogen (Diet A; highest GUDs). Despite the risk associated with foraging on the ground (Mella et al. 2015), GUDs were lower in risky feeders with high nitrogen (Diet DG) compared to safe feeders with medium nitrogen (Diet B). Such results demonstrate that at the population level,

possums value nitrogen-rich food to a point where they're willing to forego safety to obtain it. These results are consistent with previous studies in brushtail possums (Nersesian et al. 2011, Mella et al. 2015), oribi antelope (*Ourebia ourebi*; Stears & Shrader 2015), and red foxes (*Vulpes vulpes*; Berger-Tal et al. 2009), which demonstrate that animals were willing to venture into more risky situations to attain high quality food. Alternatively, despite setting up our risky feeders in a manner similar to previous studies (Mella et al. 2014, 2015), and the increased risk from fox predation associated with ground-foraging (Pickett et al. 2005), perhaps the level of perceived risk experienced by the possums on the ground was actually lower than hypothesized.

Individual possums did appear to vary in their foraging preferences, however. Similar to our population level results, five of the six identified possums biased their foraging toward safe feeders: three possums preferred the high-quality, safe feeder (Diet C), whereas two possums preferred to forage at the medium-quality, safe feeder (Diet B). This is perhaps the result of appetitive differences- for these possums, Diet B may have simply tasted better. Alternatively, it is possible that these possums arrived to the foraging blocks after other individuals, resulting in the more preferred food (Diet C) having been already depleted. Further analyses will reveal whether the differences in diet preference are reflective of differences in timing of nightly approach to the blocks or of foraging sequence within the blocks. A single possum spent the majority of its time foraging at Diet C Ground, indicating a willingness to take risks while foraging.

Results from our preliminary analyses suggest that the personality trait of *Activity* shaped the foraging responses and behaviors of individual possums. More *Active* animals may be more motivated to expend energy to venture into different situations and search for food as opposed to inactive individuals. Contrary previous studies on brushtail possums (Mella et al. 2015), and our

initial hypotheses, our preliminary analyses did not reveal a significant association between *boldness* and foraging choices or behavior. In particular, the boldest possum that visited the foraging blocks (#01156174) actually preferred to forage at the safe, Diet B feeder, whereas the possum (#01156308) that spent the majority of its time foraging at the risky feeder (Diet CGround), was only ranked second most bold.

While further validation is certainly required, our results are nevertheless interesting. Overwhelmingly, studies have exclusively focused on the effect of *boldness* on foraging habits (Michelena et al. 2009, Kurvers et al. 2010, Couchoux and Cresswell 2011, Dammhahn and Almeling 2012, Patrick and Weimerskirch 2014), without considering the possible implications other personality traits may have on foraging decisions. As our preliminary results indicate that there may be other aspects of individual personality that influence foraging decisions, we suggest that future studies investigating personality and foraging decisions consider the possible influence of other personality metrics, not just *boldness*.

F. Conclusions

Animal personality studies have consistently revealed that there are multiple ways that individuals within a population approach ecological tradeoffs (Réale et al. 2007). Here, we not only demonstrate that at a population level, brushtail possums are willing to take risks to acquire highly valued food, but we also demonstrate that multiple personality traits may influence how individuals approach foraging decisions relating to food quality and fear. However, further analysis is needed to determine whether our established personality traits reliably impact the foraging decisions of brushtail possums. Differences in personality beyond the evaluation of simply bold vs. shy should be considered when investigating how individual personality can impact the foraging success of animals.

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APPENDIX A



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VITA

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2010 - 2011: Sr. Research Technician- University of Chicago

2008- 2010: Research Technician – Michigan State University

2007-2008: Animal Behavior Research Intern- Disney's Animal Kingdom, Orlando, FL

2006-2007: Primate Training and Enrichment Intern- Potter Park Zoo, Lansing, MI

2006-2006: Primate Behavior and Research Intern- Toledo Zoo, Toledo OH

HONORS: 2016- UIC Biology Department Research Excellence Award- \$100

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2013- UIC Undergraduate Mentoring Award for Graduate Students- Honorable Mention

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2013- UIC Biology Department Teaching Excellence Award- \$200

2013- NSF Graduate Research Fellowship (GRFP)- \$100,000

2012- UIC LAS Alumni Association Board of Directors Merit Award- \$2000

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2016- (with Mr. Daniel Hughes) Holly Area Schools Education Foundation Mini-Grant- \$600

2015- UIC Elmer Hadley Graduate Research Grant - \$2879

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2014- Animal Behavior Society David Tuber Applied Animal Behavior Award-\$1100

2013- Sigma Xi Grant-in-Aid of Research- \$810

2013- UIC Elmer Hadley Graduate Research Grant- \$2500

2012- Sigma Xi Grant-in-Aid of Research- \$850

PUBLICATIONS:

Sandra M. Troxell-Smith, Jason V. Watters, Christopher J. Whelan, Joel S. Brown. "Foraging Ecology and Welfare Assessment of Two Okapi (*Okapia johnstoni*) at the Brookfield Zoo." (*Under review- Zoo Biology*)

Sandra M. Troxell-Smith, Christopher J. Whelan, Seth B. Magle, Joel S. Brown. "Zoo Foraging Ecology- Development and Assessment of a Welfare Tool for Captive Animals". (*Under review-Animal Welfare*)

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Jamie A. Johansen, Sandra M. Troxell-Smith, Zhigang Yu, Kaiguo Mo, D. Ashley Monks, Andrew P. Lieberman, S. Marc Breedlove, Cynthia L. Jordan. (2011). "Prenatal flutamide enhances survival in a myogenic mouse model of Spinal Bulbar Muscular Atrophy". *Neurodegenerative Diseases* 8: 25-34.

ORAL

PRESENTATIONS: S.M. Troxell-Smith, Lance Miller. "Reduction in stereotypic head-rolling behavior in a female Okapi: application of natural history information". The International Giraffid Conference 2016, Brookfield Zoo, Brookfield, IL.

S.M. Troxell-Smith. "Relationships between animal personality, risk-taking, and foraging decisions in the common brushtail possum (*Trichosurus vulpecula*)". Ecology and Evolution Seminar Series 2016, University of Illinois at Chicago, Chicago, IL.

S.M. Troxell-Smith. "Zoo Foraging Ecology: Assessing Animal Welfare Through Foraging Activity". Small Mammal and Reptile House Department Meeting 2016, Lincoln Park Zoo, Chicago, IL.

S.M. Troxell-Smith. "So what *was* with all those buckets? Zoo Foraging Ecology and the Effect of Management Decisions on Okapi Behavior". Mammals West Team Meeting 2014, Brookfield Zoo, Brookfield, IL.

S.M. Troxell-Smith. "Welfare Assessment Through Foraging: An Okapi (*Okapia johnstoni*) Case Study". Ecology and Evolution Seminar Series 2014, University of Illinois at Chicago, Chicago, IL.

S.M. Troxell-Smith, M. Tutka, J. Albergo, J. Brown. "Legacies of Foraging and Predation Past: What Does Life in the Lab Select For?" The 11th International Mammalogical Congress 2013, Queens University, Belfast, UK.

POSTER

PRESENTATIONS: S.M. Troxell-Smith, J.V. Watters, C.J. Whelan, J.S. Brown. "Zoo Foraging Ecology: Animals Reveal Their Preferences." Association of Zoos and Aquariums Annual Conference 2014, Orlando, FL.

S.M. Troxell-Smith, J.V. Watters, C.J. Whelan, J.S. Brown. "Zoo Foraging Ecology: Okapi Reveal Their Preferences." Animal Behavior Society 51st Annual Conference 2014, Princeton, NJ.

S.M. Troxell-Smith, J.V. Watters, J.S. Brown. "Ask the Animals: Foraging Decisions Reveal Animal Preferences." Chicago Zoological Society's 2nd International Symposium on Zoo Animal Welfare 2013, Brookfield, IL.

J. Albergo, S. Troxell-Smith, J. S. Brown. "Effects of patch characteristics on the foraging patterns of *Mus musculus*." University of Illinois at Chicago Student Research Forum 2013, Chicago, IL.

M.J. Tutka, S.M. Troxell-Smith, C.J. Whelan, J.S. Brown. "Perceived Predation: a study of fear in three strains of *Mus musculus*." University of Illinois at Chicago Student Research Forum 2013, Chicago, IL.

J.A. Broder, S.M. Troxell, T.L. Vratnina, M. Sanderson. "Past, Present, and Future: The challenge of training a group of mandrills." Animal Behavior Management Alliance 2010, Pittsburg, PA.

S.M. Troxell, J.A. Johansen, M.Q. Kemp, A.P. Lieberman, S. M. Breedlove, C. L. Jordan. "Androgen receptor antagonist increases lifespan and motor function in mouse models of SBMA." Society for Neuroscience 2009, Chicago, IL.

J.A. Johansen, S.M. Troxell, S.M. Breedlove, C.L. Jordan. "Flutamide is an effective therapeutic in transgenic female mice with Spinal Bulbar Muscular Atrophy." Society for Behavioral Neuroendocrinology 2008, Groningen Netherlands.

S.M. Troxell, J.A. Johansen, C.L. Jordan, S.M. Breedlove. "Use of Flutamide as a Potential Therapy in a Mouse Model of Kennedy's Disease." University Undergraduate Research and Arts Forum 2007, Michigan State University, East Lansing, MI.

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