

UNDERSTANDING BEHAVIOR AND NEST BOX USAGE IN THREE SPECIES OF
OWL MONKEYS: AZARA'S OWL MONKEY (*AOTUS AZARAI*), SPIX'S OWL
MONKEY (*A. VOCIFERANS*) AND NANCY MA'S OWL
MONKEY (*A. NANCYMAAE*)

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	x
ABSTRACT.....	xi
CHAPTER	
INTRODUCTION	1
Sleeping Sites in Primates	2
Sleeping Site Selection	3
Protection from Predators	3
Protection from Weather and Thermoregulation.....	3
Owl Monkey Sleeping Sites	5
Dense Foliage Nest Usage in Owl Monkeys	5
Tree Hole Nest Usage in Owl Monkeys.....	6
Owl Monkeys (<i>Aotus</i> spp.)	7
Phenotypic Variation	8
Taxonomy and Distribution.....	8
Reproduction	10
Social Structures	11
Feeding Behavior.....	12
Activity and Resting	12
Travel, Home Range and Density	13
Current Study.....	13
Hypothesis	14
Predictions	14
METHODS	14

Population.....	14
Setting.....	16
Lighting/Temperature.....	18
Feeding	18
Caging.....	20
Nest Boxes.....	20
Data Collection.....	20
Statistical Analysis	21
RESULTS	22
Focal Animal Sampling Results	22
Behavioral Budget.....	22
Play Behavior	23
Social Behavior	23
Cathemeral versus Nocturnal	24
Nesting Sites.....	24
Tree Hole Type Sleeping Site.....	24
Dense Foliage Type Sleeping Site.....	25
Alternative Nesting Site	25
Group Size	26
Scan Sampling Behavior Results.....	27
Nesting Behavior	27
Nesting Site	27
Alternative Sleeping Sites	28
Site Fidelity	29
DISCUSSION	29
Dense Foliage Type Sites	30
Tree Hole Type Sites	31
Open Sites.....	32
Available Space	32
Owl Monkey Nest Use in Captivity	33
Nesting Sites for Other Species in Captivity	34
Site Fidelity	35
Nest Site Behavior: Sleep.....	36

Other Nest Site Behavior	37
Marking and Tail Twining.....	37
Huddling	37
Behavioral Budget	38
Cathemeral and Nocturnal Circadian Rhythms	38
Play Behavior	39
Food Behaviors.....	40
Nursing	41
Strengths and Limitations	42
CONCLUSIONS.....	43
APPENDIX A: REGARDiNG IACUC APPROVAL.....	45
REFERENCES	47

LIST OF TABLES

Table	Page
1. <i>Aotus</i> spp. individual information on date of birth, sex, weight and group composition.	15
2. Ethogram of behaviors for recording both scan and focal animal sampling.	19
3. Average percentage (+SD) Focal Time in Nest Box by Type.	25
4. Frequency, chi-square and p-values for all groups by species for the top 6 locations used for resting during the morning scan sampling data collection.....	28

LIST OF FIGURES

Figure	Page
1: Phylogenetic reconstruction of tree hole or nest building in primates. (Original in Kappeler 1998).....	1
2. Examples of tree hole cavities used for sleeping by <i>Aotus</i> spp. (Original from Aquino and Encarnación 1986).	4
3. Examples of dense foliage used by <i>Aotus</i> spp. (Original in Aquino and Encarnación 1986).	6
4. <i>Aotus azarai</i> at UT MD Anderson Cancer Center, 2012.	9
5. <i>Aotus nancymae</i> , at UT MD Anderson Cancer Center 2012.	9
6. <i>Aotus vociferans</i> at UT MD Anderson Cancer Center, 2012.	10
7. Cladogram for <i>Aotus</i> spp. (Adapted from Ford 1994)	10
8. Continuous waterfall in the middle of a colony room	17
9. One <i>Aotus</i> cage with all substrates, nest boxes and surfaces labeled.	17
10. A family group of <i>A. vociferans</i> with face markings clear.	18
11. Percentage of focal time spent in each behavioral category for each species of owl monkeys.	23
12. Percentage of focal observation time in each site for resting behavior, all three <i>Aotus</i> species.	26

ABSTRACT

UNDERSTANDING BEHAVIOR AND NEST BOX USAGE IN THREE SPECIES OF OWL MONKEYS: AZARA’S OWL MONKEY (*AOTUS AZARAI*), SPIX’S OWL MONKEY (*A. VOCIFERANS*) AND NANCY MA’S OWL MONKEY (*A. NANCYMAAE*)

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The use of tree holes and nest building behavior is uncommon among most primate species. In research facilities that house species requiring nesting sites, such as those that use tree holes in the wild, providing nest boxes is crucial to their psychological well-being. The goal of this study was to determine nest box preferences among three species of captive owl monkeys: Azara’s owl monkey (*Aotus azarai*), Nancy Ma’s owl monkey (*A. nancymae*) and Spix’s owl monkey (*A. vociferans*). The subjects were housed at The University of Texas M.D. Anderson: Keeling Center for Comparative Medicine and Research Core and Animal Resources, Bastrop, TX. Five family groups

from each species, ranging from 2-5 individuals, were observed for location and behavior from 21 June 2012 to 23 August 2012. The facility provided four different types of nest boxes: [1] a mesh box, [2] a covered box of the same size, [3] a horizontal bucket, and [4] an opaque white box. As a nocturnal species, the owl monkeys are maintained on a partial reverse light cycle and the rooms are equipped with louvers, allowing the owl monkeys to experience a “dusk” period in the lighting. While *A. azarai* spend more of their sleeping time in dense foliage in the wild compared to *A. nancymae* and *A. vociferans*, in the current study, although they were provided with nest boxes meant to mimic this type of nesting site, *A. azarai* did not spend more time (13.6+19.1%) in the nest boxes compared to the other two species (*A. vociferans* 18.4+18.8% and *A. nancymae* 8.1+18.0%). All three species employed a variety of sleeping sites including the nest boxes as well as other cage substrates such as the floor and perches. One factor contributing to sleep site selection was the size of the group and the size of the nest box. *A. azarai* are generally considered more cathemeral than the other species. In the current study, the activity level of *A. azarai* was much higher (77.3 + 15% resting) compared to *A. nancymae* (93.3 + 11) or *A. vociferans* (97.9+3) during the dusk period. Dusk is usually a peak time for all owl monkey species to forage and to travel; however, in captivity the pattern may be different.

INTRODUCTION

Nesting behavior is uncommon among most primate species (Kappeler 1998). Selective pressures related to life histories, ecological niches, behavioral repertoire and social organization in response to predation, available resources, competition, thermoregulation and parasites contribute to the employment of nesting behavior during

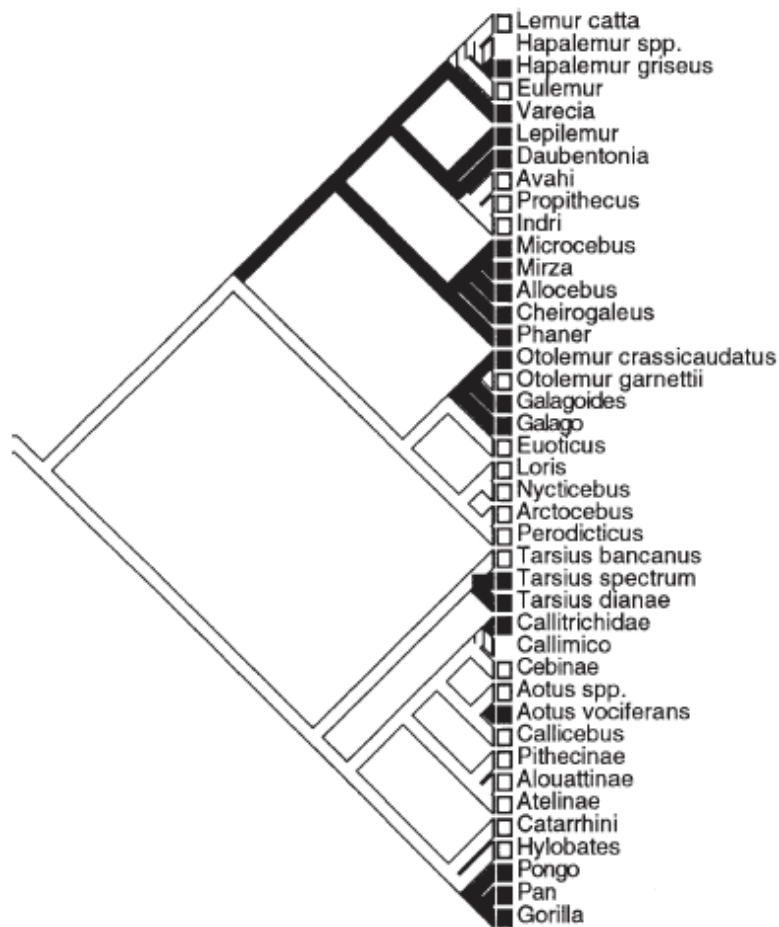


Figure 1: Phylogenetic reconstruction of tree hole or nest building in primates. (Original in Kappeler 1998).

periods of rest (Anderson 1998; Kappeler 1998). Most primate species do not hide or manipulate materials into forming nests (Kappeler 1998). However, great apes, callitrichids (marmosets and tamarins), *Aotus* spp. and many strepsirrhines use tree holes or build nests in which to sleep (Figure 1) (Kappeler 1998).

Sleeping Sites in Primates

Some lemur species use both tree holes and dense foliage as sleeping sites. The weasel sportive lemurs (*Lepilemur mustelinus*) and Milne Edwards' sportive lemur (*Lepilemur edwardsi*) use tree holes far more often than dense vegetation (Rasoloharijaona et al. 2003; Rasoloharijaona et al. 2008). In these species, the availability of sleeping sites for protection and predator avoidance may be an important resource worthy of defense (Rasoloharijaona et al. 2003; Rasoloharijaona et al. 2008). Similarly, mouse lemurs (*Microcebus* spp.) have been observed using tree holes and building leaf nests (Radespiel et al. 1998; Perret 1998; Thorén et al. 2010). Nest building is a rare behavior in primates though it has been documented in all of the great apes and some strepsirrhines (Anderson 1998; Kappeler 1998). Gorillas (*Gorilla* spp.), chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*) and orangutans (*Pongo* spp.) build nests in the canopy every night by bending branches into a large platform (Goodall 1963; Anderson 1998; Kappeler 1998; Russon et al. 2007). Of the New World primates, callitrichids and owl monkeys (*Aotus* spp.) are considered cavity-dwelling species (Anderson 1998; Kappeler 1998). Although no nest building behavior has been documented in a New World primates, many callitrichids and owl monkeys use specific sleeping sites such as tree holes and dense foliage for sleeping sites (Kappeler 1998). Tamarins (*Saguinas mystax* and *Saguinas fuscicollis*) use a variety of sleeping sites

including tree holes, dense foliage and open branches (Heymann 1995). Owl monkeys have been well documented in their use of these three types of sleeping sites in the wild (Wright 1978; Aquino and Encarnación 1986; Garcia and Braza 1993; Kappeler 1998).

Sleeping Site Selection

A sleeping site is a location chosen by animals in order to engage in sleep or rest behavior for an extended period of time (Anderson 1998). Due to the difficulty in determining physiological sleep of individuals at a sleep site, the term is interchangeable with rest site and nest site. Several factors contribute to the decision of sleeping sites, such as thermoregulation, social structures, parasite risk, predator avoidance, foraging ranges, time of retiring and body size (Kappeler 1998; Anderson 2000).

Protection from Predators

Predator avoidance is an important strategy for most primate species. Many species choose locations well above the ground, sometimes on the terminal ends of branches in order to detect any movement in the tree from predators (Anderson 1998; Anderson 2000). Tree hole use may also be effective in avoiding predators and concealing young (Kappeler 1998). Marmosets have been shown to change behavior near sleeping sites in order to reduce predation (Franklin et al. 2007). Many species that employ tree holes or dense foliage use these sites as visual barriers from predators (Anderson 1998).

Protection from Weather and Thermoregulation

Hygiene and comfort may be strong factors in selecting a sleeping site and contribute to using nest sites that also provide protection from the weather and

thermoregulation, especially in smaller primates (Anderson 1998; Anderson 2000).

Thermoregulation is one factor in nest site selection for a variety of species. For example, tamarins (*Saguinus mystax* and *S. fuscicollis*) use *Jessenia* palms and tree holes for protection from the rain and cold (Heymann 1995). Garcia and Braza (1993) suggested that *A. azarai* choose the open nest sites for warming in the sun. Likewise, Aquino and Encarnación (1986) proposed that the thermoregulatory benefits of avoiding direct rainfall and sharing nest site with family unit for influences nest site selection for *A. vociferans* and *A. nancymae*. However, thermoregulation benefits of either sunning behavior on branches and open sites or within tree hole cavities have not been documented in owl monkeys, although sleeping position may have an impact on cooling or warming in the sunlight for *A. azarai* (Garcia and Braza 1993). Owl monkeys also use dense foliage and tree hole in order to avoid direct rainfall or wind (Aquino and Encarnación 1986).

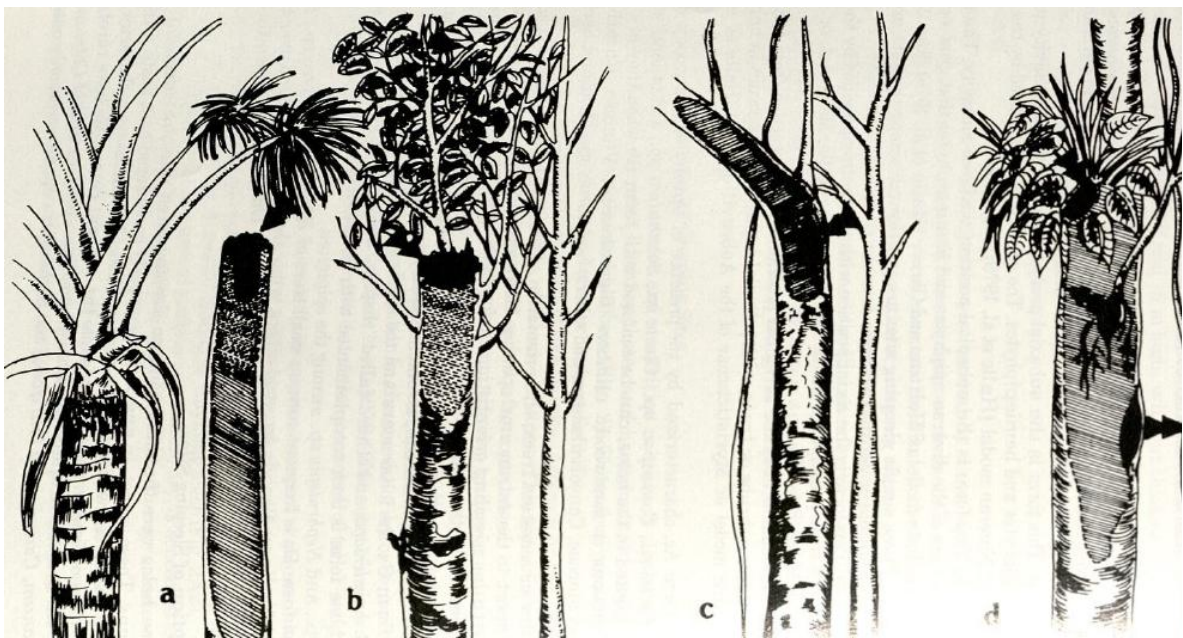


Figure 2. Examples of tree hole cavities used for sleeping by *Aotus* spp. (Original from Aquino and Encarnación 1986).

Huddling behavior is seen in many tree hole dwelling primate species (Heymann 1995; Perret 1998; Kappeler 1998; Radespiel et al. 1998; Rasoloharijaona et al. 2008). Huddling behavior at nesting sites might provide important thermoregulation effects for tamarins (Heymann 1995). Similar huddling behavior within tree holes of grey mouse lemurs (*Microcebus murinus*) has energetic and thermoregulatory advantages such as maintaining a steady temperature needed for torpor, a type of hibernation (Perret 1998; Radespiel et al. 1998). Social factors such as group cohesion and hierarchy may also contribute to the selection of sharing of sleeping sites and huddling behavior in addition to thermoregulatory benefits (Anderson 2000).

Owl Monkey Sleeping Sites

Most *Aotus* species use tree holes and dense foliage in a variety of sleeping sites. Indeed, owl monkeys rarely use open branches to sleep during the day (Garcia and Braza 1993). Aquino and Encarnación (1986) documented 4 types of sleeping sites, with 11 variations among *A. nanymaae* and *A. vociferans*.

Four of these includes tree holes (Figure 2), cavities of branches (Figure 2), dense foliage of epiphytes and hemiepiphytes (Figure 3), and dense foliage among thickets with many access points (Figure 3) (Aquino and Encarnación 1986). Tree holes are cavities within branches or trunks of usually old or senescent trees (Aquino and Encarnación 1986; Puertas et al. 1995).

Dense Foliage Nest Usage in Owl Monkeys

Dense foliage includes masses of different types of plant growth including lianas and vines, shrubs, thickets, hemiparasites, epiphytes and hemiepiphytes (which are plants

that grow on or take nutrients from another plant). Dense foliage such as thickets of genipapo (*Genipa Americana*) and bakupari (*Rheedia* spp.) have been used by *A. azarai* for sleeping sites (Garcia and Braza 1993). *A. vociferans* and *A. nancymae* also used a variety of epiphytes, hemiepiphytes and hemiparasites (Bromeliaceae, Araceae, Loranthaceae, Guttiferae, Moraceae, Gesneriaceae, ferns and others) that are found covering cavities, invading tree holes, and part of dense thickets and shrubs (Aquino and Encarnación 1986). *A. nancymae* were observed in all 4 types (tree holes in trunks, tree holes in branches, dense foliage in epiphytes and hemiepiphytes and dense foliage in thickets) (Figures 2 and 3) of sleeping sites, while *A. vociferans* were observed mainly in tree holes and occasionally in dense foliage of hemiepiphytes (Aquino and Encarnación 1986; Puertas et al. 1995).

Tree Hole Nest Usage in Owl Monkeys

Tree holes used by owl monkeys are found in a variety of tree species, including ollita (*Eschweilera*), acacia (*Macrobolium acaciaefolium*), huacapurana (*Campsiandra lauriflora*), mulateiro (*Calycophyllum spruceanum*) (Aquino and Encarnación 1986;



Figure 3. Examples of dense foliage used by *Aotus* spp. (Original in Aquino and Encarnación 1986).

Puertas et al. 1995). Tree holes are the most common nesting sites for *A. nancymae* and *A. vociferans*, though not for *A. azarai* (Aquino and Encarnación 1986; Garcia and Braza 1993). *A. azarai* use branches and lianas as platforms at an average height of 10.8m with lianas around for coverage (Garcia and Braza 1993). *A. nancymae* and *A. vociferans* choose tree holes with circular openings between 14 and 30cm in diameter and depths of 0.6 to 13.4m at various canopy levels (7.1 to 37.0 m) (Aquino and Encarnación 1986).

Predators of owl monkeys include the hawk-eagle (*Spizaetus*), solitary eagles (*Harpyhalietus*), ocelots (*Felis pardalis*), and tayra (*Eira barbara*) (Garcia and Braza 1993). These predators likely contribute to the selection of certain sleeping sites for owl monkeys (Garcia and Braza 1993), helping with visual concealment of the individuals from interested predators, which has been a factor noted in sleeping site selection in primate species such as callitrichids, strepsirrhines and other primates (Kappeler 1998). Although there has not been a study specifically on the effect of tree hole use and predation in owl monkeys, it is reasonable to expect that predators would have more difficulty locating and extracting the owl monkeys well concealed in a tree hole during their vulnerable sleep periods (Anderson 1998). Site fidelity has not been demonstrated by owl monkeys, many species do not return to the same nesting site more than one night in a row, though there has not been a study on whether they return to the sites on other occasions (Aquino and Encarnación 1986; Fernandez-Duque 2011).

Owl Monkeys (*Aotus* spp.)

Owl monkeys (*Aotus* spp.) are the only nocturnal anthropoid primate and are one of the few primates to exhibit social monogamy (Wright 1994; Erkert 2010). Like most New World primates, owl monkeys are arboreal primates endemic to Central and

South America and inhabit dry deciduous to tropical rainforests (Aquino and Encarnación 1988; Fernandez-Duque et al. 2008; Erkert 2010; Fernandez-Duque 2011). The three owl monkeys of focus are Spix's owl monkey, *A. vociferans*, Nancy Ma's owl monkey *A. nancymae* and Azara's owl monkey *A. azarai* (due to availability, see Methods).

Phenotypic Variation

Owl monkeys weigh between 700 and 1200g, measure 250 to 475mm in body length and 220 to 420mm in tail length with short hair ranging from black on the sides to bright orange on the neck and dorsal region, depending on the species (Erkert 2010). *A. azarai* (Figure 4) males weigh 1256 g, females weigh 1246 g and are generally heavier and larger than *A. nancymae* and *A. vociferans* (Fernandez-Duque 2011). *A. nancymae* (Figure 5) males weigh, on average, 794 g and females weigh 780 g (Fernandez-Duque 2011). *A. vociferans* (Figure 6) males weigh, on average, 697.5 g, but there is no evidence from the wild on female weights (Fernandez-Duque 2011). Newborn owl monkeys weigh between 80 and 105g (Dixson 1994; Dixson and Fleming 1981).

Taxonomy and Distribution

Initially, research focused on only one species, the three-striped night monkey, *A. trivirgatus* (Ford 1994). However, over time there became a clear delineation between two groups of owl monkeys and genetic evidence of more species: the grey-necked group north of the Amazon and the red-necked group south of the Amazon (Ford 1994; Erkert 2010). The species north of the Amazon (the grey-necked group) are *A. lemurinus*, *A. brumbacki*, *A. trivirgatus* in the east and *A. vociferans* in the west (Ford 1994; Erkert 2010). The five species of the red-necked group are *A. nancymae*, *A. miconax*, *A. nicriceps*, *A. infulatus* and *A. azarai* at the southern extreme (Ford 1994; Erkert 2010) *A.*

vociferans ranges are in the tropical regions of South America including Ecuador, Bolivia, Brazil and Peru (Fernandez-Duque et al. 2008; Erkert 2010). *A. nancymae* have a smaller range in western Brazil, eastern Peru and southern Columbia (Ford 1994; Erkert 2010). *A. azarai* have the most southern range from southern Bolivia to northern Argentina (Fernandez-Duque et al. 2001; Fernandez-Duque and Erkert 2006).

Based on pelage, cranial and genetic data, the three species in the current study split three different times (Ford 1994). The earliest branch of *Aotus* spp. split with *A. vociferans* and *A. trivirgatus*; *A. nancymae* branches off then finally *A. azarai* (Figure 7). There is evidence that some owl monkey species, including these three, are capable of interbreeding and producing hybrid offspring, which would indicate that they are very biologically closely related (Williams, pers. Comm.; Ford 1994).



Figure 4. *Aotus azarai* at UT MD Anderson Cancer Center, 2012.



Figure 5. *Aotus nancymae*, at UT MD Anderson Cancer Center 2012.



Figure 6. *Aotus vociferans* at UT MD Anderson Cancer Center, 2012.

Reproduction

Owl monkeys are socially monogamous and lack sexual dimorphism (Wright 1994). The females do not show overt sexual swellings or colorations and the male external genitalia are not proportionally large or easily visible (Dixson 1994; Wright 1994). Likely due to the social monogamy, mating behavior is infrequent and only observed rarely in captivity (Dixson 1994). Similar to most primates, owl monkeys give

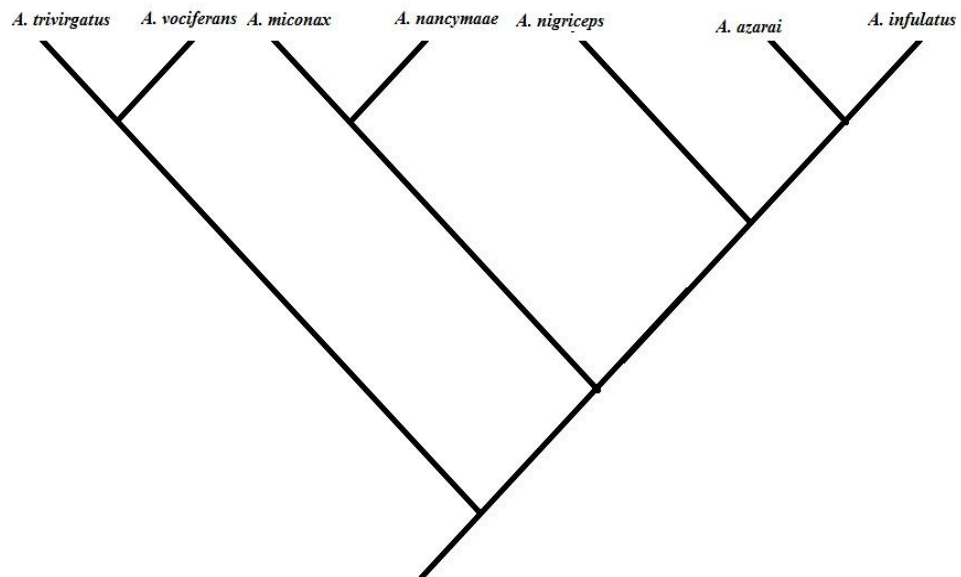


Figure 7. Cladogram for *Aotus* spp. (Adapted from Ford 1994)

birth to one infant at a time and gestation is approximately 133 days (Gozalo and Montoya 1990; Dixson 1994). Females start reproduction between 40 and 60 months of age and show an interbirth interval of 166 to 419 days (Fernandez-Duque 2011). Infant owl monkeys are born furred with their eyes open and for the first week of life, they are carried by the mother (Rotundo et al. 2005). Infants are carried and cared for by their fathers by the third week and only returned to the mother for nursing (Rotundo et al. 2005). Infant owl monkeys begin venturing off the parents between 22 and 46 days and eating solid food between 35 and 60 days (Dixson 1994). By 5 months of age, the parents carry the infant only 6% of the time (Rotundo et al. 2005).

Social Structures

Owl monkeys live in a family group of male/female pairs and their offspring with up to 5 individuals (Wright 1994; Erkert 2010; Fernandez-Duque 2012). Evidence for owl monkey grooming in the wild is limited but is thought to be much less frequent than grooming in titi monkeys (*Callicebus*), which are also socially monogamous New World monkeys (Wright 1994). Garcia and Braza (1993) observed allogrooming and playing among wild *A. azarai* during the time after waking but before leaving the nest site. Fights between subadults and parents occasionally occur in captivity at 18 to 24 months of age, and is likely due to natural emigration from the group in the wild (Gozalo and Montoya 1990; Dixson 1994). Agonistic behaviors between groups of owl monkeys are not uncommon at borders between species' territories and has occasionally been observed near sleeping sites (Aquino and Encarnación 1986; Wright 1994).

Feeding Behavior

Owl monkeys consume a variety of foods including fruit, flowers, young leaves and insects (Wright 1994). *Aotus* spp. spend up to 82% of their active period for feeding and foraging (average 53%) (Wright 1978). The most active feeding times are at dawn (04:00 to 05:00) and dusk (19:30 to 20:30) (Wright 1978). Owl monkeys tended to concentrate on nectar, figs and insects during seasons of reduced fruits (Wright 1994). Advantages to nocturnal lifestyle include reduced competition for food and the increased presence of larger nocturnal insects (Wright 1994). One interesting food behavior involves the sharing of food between family members, which can be an important social interaction (Wolovich et al. 2006). Among owl monkeys, males often share their food with their female partner while she is lactating, as well as with any offspring, strengthening social bonds (Wolovich et al. 2007).

Activity and Resting

Owl monkeys spend 22% of their time resting (range 9-45%) during the night (Wright 1978). *Aotus* spp. show peaks of activity at dawn and dusk (Fernandez-Duque 2011). *A. azarai* are more cathemeral than other owl monkeys and are active during the day time as well as night (Fernandez-Duque et al. 2001; Fernandez-Duque 2011). *A. azarai* are active for approximately 4.5 hours in both day and night times (Fernandez-Duque et al. 2001; Fernandez-Duque 2003; Fernandez-Duque and Erkert 2006). *A. vociferans* and *A. nancymae* are both strictly nocturnal, mainly active at night, especially with a brighter moon (Aquino and Encarnación 1986; Puertas et al. 1995; Fernandez-Duque et al. 2008).

Travel, Home Range and Density

Owl monkeys spend 21% of their time travelling during the night (Wright 1978). Their average territory size is 9.2 ha (Wright 1994). *A. azarai* have territories of 4 to 12 ha, *A. vociferans* have territories an average of 6.3 ha and there is no current territory size information for *A. nancymae* (Fernandez-Duque 2011). Owl monkeys actively defend their home ranges from other groups (Erkert 2010). The large ranges in densities of different species of owl monkeys is dependent on hunting pressure and intensive deforestation in certain areas (Aquino and Encarnación 1994). Densities of owl monkeys ranged 3.3 to 13.5 groups per km² and 8.8 to 46.3 individuals per km² (Aquino and Encarnación 1988; Erkert 2010).

Current Study

The goal of this study is to understand nesting behavior and general behavior in three captive owl monkey species: Azara's owl monkey (*Aotus azarai*), Spix's owl monkey (*A. vociferans*) and Nancy Ma's owl monkey (*A. nancymae*). In the wild, these three species differ in their use of nests and tree holes, body sizes, circadian rhythms and home ranges (Garcia and Braza 1993; Puertas et al. 1995; Fernandez-Duque 2003; Erkert 2010; Fernandez-Duque 2011). Differences observed in the wild may translate to differences in the preference and use of certain nesting boxes in captivity. Nesting behavior can be tested by comparing the use of some nest boxes and the exclusion of others during periods of rest. Understanding differences in behavior during different time periods provides information for the construction and design of nest boxes and caging material specified to each species, in order to improve the psychological well-being of the owl monkeys.

Hypothesis

I hypothesized that there would be significant differences in the use of nest boxes and activity levels between *A. azarai*, *A. vociferans* and *A. nancymae*.

Predictions

Based on observations of wild owl monkeys, I predicted that *A. azarai* would use the more open, less dense nesting sites while *A. nancymae* and *A. vociferans* would use the tree hole type nest sites. *A. azarai* would be more cathemeral in captivity than both *A. nancymae* and *A. vociferans*.

METHODS

I observed groups of owl monkeys at University of Texas MD Anderson Keeling Cancer Center and Medical Research in Bastrop, Texas. *Aotus* spp. were housed in compliance with *The Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academy Press, Washington) and in guidelines from the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Population

The study was conducted from 21 June 2012 to 23 August 2012. Five groups of 2 to 5 individuals per group from each species of *Aotus* were observed. At the start of the study there were 45 total *Aotus* spp. individuals. At the beginning of the observation period (21 June 2012), there were 17 individuals of *A. nancymae*, 14 of *A. azarai* and 14 of *A. vociferans*. All of the groups were family combinations except one that contained an established, unrelated pair of males of *A. vociferans* (Table 1).

Table 1. *Aotus* spp. individual information on date of birth, sex, weight and group composition.

Location (Room.Cage)	Name	Species	Sex	Weight (g)	DOB
3400.68	Rafiki	<i>A. azarai</i>	M	1085	8/20/2001
	St. Pauli	<i>A. azarai</i>	F	1124	9/10/2001
	Timon	<i>A. azarai</i>	M	1042	7/26/2010
	Nala	<i>A. azarai</i>	F	172	7/19/2012
3400.67	Heineken	<i>A. azarai</i>	M	1285	8/20/2001
	Dos Equis	<i>A. azarai</i>	F	785	6/6/2011
3400.66	Funny Face	<i>A. azarai</i>	M	1056	9/10/2001
	Earth	<i>A. azarai</i>	F	1114	8/20/2001
	Harli	<i>A. azarai</i>	F	1083	6/21/2010
	Joker	<i>A. azarai</i>	M	964	8/23/2011
3400.65	Fach	<i>A. azarai</i>	M	989	9/10/2001
	Ophelia	<i>A. azarai</i>	F	1430	8/20/2001
3400.64	Hubcap	<i>A. azarai</i>	M	1095	9/10/2001
	Annie Difazio	<i>A. azarai</i>	F	1050	2/16/2004
	Tire Iron	<i>A. azarai</i>	M	837	3/22/2011
3400.51	Xander	<i>A. nancyma</i>	M	857	12/31/2001
	Amanda	<i>A. nancyma</i>	F	1201	3/28/2003
	Katydid	<i>A. nancyma</i>	F	1052	1/24/2010
	Mantis	<i>A. nancyma</i>	M	881	4/18/2011
	Lovebug	<i>A. nancyma</i>	M	553	2/19/2012
3400.50	Vivitar	<i>A. nancyma</i>	M	840	9/10/2001
	Gibson	<i>A. nancyma</i>	F	972	9/10/2001
	Cordoba	<i>A. nancyma</i>	M	644	9/30/2011
3400.48	Thorgrim	<i>A. nancyma</i>	M	949	6/7/2002
	Truffle*	<i>A. nancyma</i>	F	591	4/27/2003
	Art	<i>A. nancyma</i>	M	958	8/3/2009
	Leonardo	<i>A. nancyma</i>	M	857	3/18/2011
3400.47	Oliver	<i>A. nancyma</i>	M	1041	9/8/2007
	Emma	<i>A. nancyma</i>	F	1171	9/29/2007
340.46	Hansolo	<i>A. nancyma</i>	M	1242	3/4/2006
	Princess Leia	<i>A. nancyma</i>	F	730	6/2/2006
	Cadeus	<i>A. nancyma</i>	M	811	6/15/2011
3400.01	Roulette	<i>A. vociferans</i>	F	882	9/10/2001
	Grease	<i>A. vociferans</i>	M	811	9/10/2001

Table 1. *Aotus* spp. individual information on date of birth, sex, weight and group composition.

Location (Room.Cage)	Name	Species	Sex	Weight (g)	DOB
	Black Jack	<i>A. vociferans</i>	M	826	9/1/2010
3400.02	Zane	<i>A. vociferans</i>	M	771	3/5/2005
	Paisley	<i>A. vociferans</i>	F	979	3/23/2006
	Denim	<i>A. vociferans</i>	M	892	7/9/2010
	Pleather	<i>A. vociferans</i>	M	430	4/19/2012
3400.04	Chocula	<i>A. vociferans</i>	F	789	8/20/2001
	Peeper	<i>A. vociferans</i>	M	812	8/20/2001
	Cheerios	<i>A. vociferans</i>	F	773	7/27/2010
3400.05	Rupert Everett	<i>A. vociferans</i>	M	974	8/27/2003
	Rhonda	<i>A. vociferans</i>	F	806	12/21/2010
3400.07	Bamboo	<i>A. vociferans</i>	M	969	11/20/2004
	Alex	<i>A. vociferans</i>	M	890	8/30/2006

*Euthanized 7/17/2012.

Detailed characteristics of each study subject are included in Table 2. However, several events impacted the sample size of each species. On 16 July 2012 one juvenile *A. vociferans* male was removed (decreasing the number of *A. vociferans* to 13) due to naturally occurring aggression between him and his father. On 17 July 2012 one adult female *A. nancymae* was euthanized due to illness (decreasing the number of *A. nancymae* to 16). On 19 July 2012, one *A. azarai* gave birth (increasing the number *A. azarai* to 15).

Setting

UT MD Anderson houses all 340 *Aotus* spp. individuals inside a large building with two large colony rooms. In each colony room, there are rows of cages that run along each side of a room-length continuous waterfall. There is a waterfall running down the middle of the room where the colony is housed that provides a visual barrier, as well as

olfactory and auditory reduction for anxiety between groups (Figure 8). On 21 August 2012 the waterfall was shut off for maintenance but a sound recording loud enough for the entire colony was provided.



Figure 8. Continuous waterfall in the middle of a colony room. The main lights are on and the sky lights are open.



T: Top of Cage
CF: Cage Front
CFP: Cage Front Panel
UP: Upper Perch
MP: Middle Perch
LP: Lower Perch
L: Ladder
F: Floor
M: Mesh Nest Box
B: Bucket Nest Site
O: Opaque Nest Box
C: Covered Nest Box
FT: Food Trough

Figure 9. One *Aotus* cage with all substrates, nest boxes and surfaces labeled.



Figure 10. A family group of *A. vociferans* with face markings clear.

Lighting/Temperature

The temperature is maintained at 27°C throughout day and night. The lights automatically turn off at 12:00-noon with a partial sun roof open until approximately 15:00. The partial sun roof light is considered a “dusk” period and is substantially darker than the daylight (from 0:00 to 12:00) though not as dark as the night period (15:00 to 0:00). The night time has red light available for staff and study throughout the main area.

Feeding

The monkeys are fed a twice daily with a mixture of produce (mainly peanuts, celery and oranges on the days studied), and standard monkey biscuits. Food enrichment was distributed mainly in the night time with the front foraging board, and information was recorded by Center staff.

Table 2. Ethogram of behaviors for recording both scan and focal animal sampling.

BEHAVIOR	DESCRIPTION
Travel	Any motion that moves all four appendages in any direction for at least one body length, bouts separated by stationary position for more than 5 seconds
Resting	Animal trunk is motionless and stationary with hindquarters in a seated position, tail, head and limbs can be moving, bouts separated by any travel (one body length) or other activity
Resting-Non-active	Rest without movement of any body parts, eyes closed, bouts separated by change in behavior for more than 10 seconds
Standing	Extended hindlimbs while grasping something though not moving, different from resting by body position, separated by 5s
Nursing	By mother or infant, this behavior involved either the mother allowing the infant to access to the nipple or the back and forth movement of the head of the infant on the nipple of the mother, separated by stopping behavior
Feeding	Animal visibly consumes food, bouts separated by 5s
Drinking	Animal uses water bottle and/or consumes liquid, bouts separated by 5s
Huddling/Huddling-non-active	Resting (resting non-active) in contact with another animal, bouts separated by 10s
Social	Any behavior involving interaction with another animal aside from huddling or play, bouts separated by 15s
Play	Any behavior involving solitary-locomotor, solitary object play and social play, meeting the five criteria defined by Graham and Burghardt 2010
Rub	Pressing face, back or neck against a surface, repeatedly, separated by 5s
Scratch	Rapid movements of hands and feet along fur, separated by 5s
Grooming	Combing through hair with hands, feet or teeth, bouts separated by 5s
Other	Any behavior not covered in other categories

Caging

Each cage is 1.2m by 1.2m by 1.8m and contains several PVC® (plastic) perches at different heights (UP (upper perch), MP (middle perch), LP (lower perch), CFP (cage front perch) (Figure 9). There is a food trough (FT) for feed and water containers. The ladder (L) and vine (V) provide environmental enrichment as well for the monkeys to use as substrates or manipulate.

Nest Boxes

There are 4 nest sites: 35.6cm³ opaque white plastic box at the front of the cage (“O” in Figure 9, opaque box), 35.6cm³ plastic mesh box upper left corner (“M” in Figure 9, mesh box), 35.6cm³ covered black plastic mesh box upper (“C” in Figure 9, covered box) and a plastic bucket turned horizontal without bottom on the right inside (“B” in Figure 9, bucket). Dense-Foliage Type Nest Boxes: nest boxes B and M are most similar to the more open nesting sites of dense foliage. Tree-Hole Type Nest Boxes: nest boxes O and C are most similar to closed, covered nesting sites within tree holes.

Data Collection

Location and behavioral data were collected between 21 June 2012 and 23 August 2012. Behavioral data coding is explained in Table 2. Supplies used to observe and record primate behavior included a plastic chair, unlit stopwatch, clipboard, pen and paper. These minimal supplies were used in order reduce stress on the animals. I was located as far as space allowed from the cage (approximately 2m) and made minimal noise to reduce stress. Red lights were available throughout the colony during the night time period and were sufficient for data recording and visibility of individual animals.

Animals were identified using a combination of physical features, especially head stripe patterns, due to the lack of sexual dimorphism (Figure 10) . Location information was recorded based on the assigned locations labeled in Figure 9. When an animal spanned two or more surfaces, the piece of the cage that contained more than an estimated 50% of the body was recorded. Behavioral recordings were supplemented by *ad libitum* recordings. *Ad libitum* data included reactions to staff, myself, noises, when food was distributed and which kind, and which group members were resting with each other as well as other pertinent information. Scan sampling was conducted as described by Altmann (1974) for 5 cages from each species every 20 minutes over a 1 hour period from 06:30 to 07:30 on Mondays and Wednesdays and hourly from 11:00 to 17:00 on Tuesdays and Thursdays. Fifteen-minute focal animal sampling (Altmann 1974) was conducted on randomly selected individuals, through a number assignment and using a random generator online (<http://www.random.org>), within each species Monday through Thursday between scan sampling. Information on individuals that were added or removed during the study was included in the analysis. The monkeys were acclimated to my presence and all four boxes prior to the start of the study.

Statistical Analysis

Standard descriptive statistics were computed using (Excel®) conducted on frequencies (for scan sampling data) as well as durations (for focal animal sampling data) of behaviors and locations. These included the means and standard deviations for locations and behaviors. Analyses included the use of non-traditional locations for resting as well as the four nest boxes described above. Mann-Whitney and Kruskal-Wallace comparisons were used for location preference between species using focal and scan data

to compare all three species on location preference and behavioral budget. Chi-square goodness-of-fit test was run on scan sampling data for the morning to determine location preference during this time period by species and group. The analysis for Mann-Whitney comparisons was conducted using *R* (R Development Core Team 2013). The location preference analysis was stratified by group size (2-3 individuals vs. 4 or more individuals) to account for group size confounding variables. Behaviors and locations are written as mean \pm standard deviation percentage of time or scans.

RESULTS

In this analysis, there were 14 *A. vociferans* individuals, 17 *A. nancymae* individuals and 14 *A. azarai* individuals. The infant born during the study to *A. azarai* was left out due to her constant presence on her father. However, the individuals that were removed were included due to the amount of data collected on both before removal.

Focal Animal Sampling Results

Behavioral Budget

All three species spent the majority of their time resting in a 24-hour period ($75.3 \pm 1\%$), followed by travelling ($9.2 \pm 8\%$) and feeding ($7.3 \pm 8\%$) (Figure 11). Due to the difficult nature of observing primates in the dark, even with the help of red lights, out of sight data were included ($4.0 \pm 6\%$). The out of sight category included information regardless of whether location was known (hiding in a box) or unknown (loss of visual contact or unable to locate without disturbance).

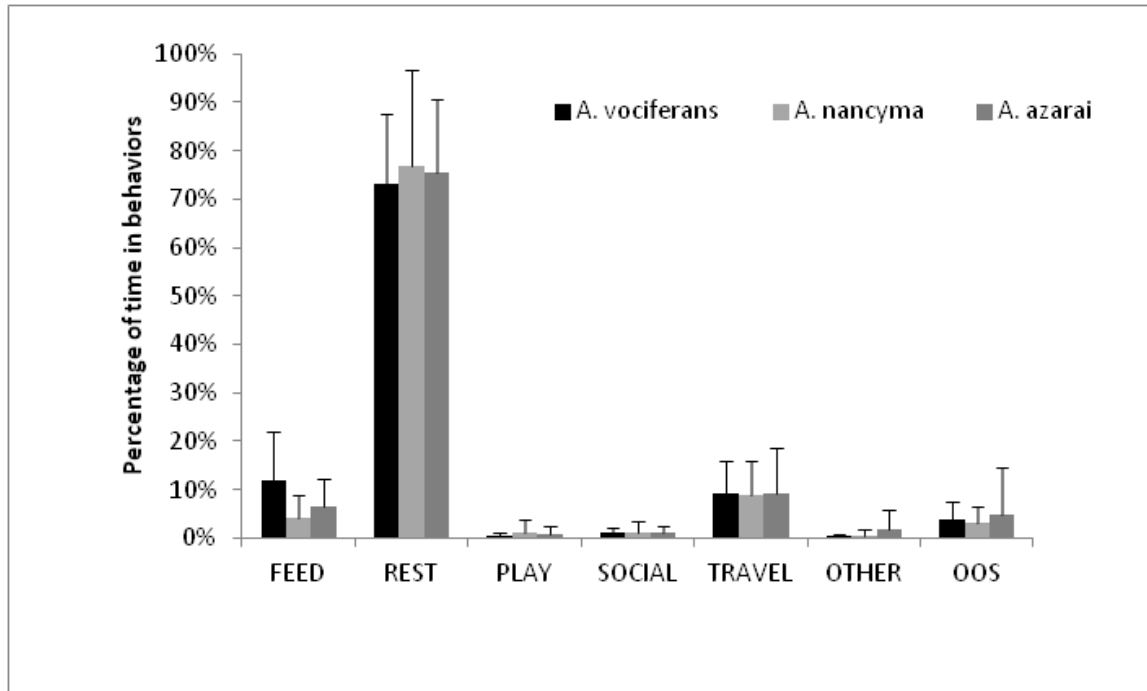


Figure 11. Percentage of focal time spent in each behavioral category for each species of owl monkeys.

Play Behavior

All three species of owl monkeys employed mainly social play, especially chasing and wrestling, with other conspecifics. *A. nancymae* exhibited social play most often ($1.2 \pm 2.5\%$), followed by *A. azarai* ($0.8 \pm 1.9\%$) and *A. vociferans* ($0.2 \pm 0.8\%$) (Figure 11). Collectively, juveniles played ($2.2 \pm 2.8\%$). One juvenile *A. nancymae* played 9.0% of the focal time. Object play was only observed *ad libitum* on a few occasions. Once when a juvenile manipulated, examined and repeatedly dropped a small rubber duck and on three occasions juvenile males in the upper mesh nest box swung back and forth on a plastic vine, chewed on or hung from the vine.

Social Behavior

All three species spent approximately 1% of their time socializing, which included nursing. Huddling was not included in the social category of behaviors. Huddling and huddling-non-active were combined into the resting category for the

behavioral budget.

Cathemeral versus Nocturnal

A. azarai subjects were significantly less restful ($77.3 \pm 15\%$) during the dusk period (1200 to 1500) compared to both *A. nancymae* ($93.3 \pm 11\%$; Mann-Whitney, $n_{A.azarai}=14$, $n_{A. nancymae}=17$, $U=209$, $p=0.00035$) and *A. vociferans* ($97.9 \pm 3\%$; M-W, $n_{A.azarai}=14$, $n_{A. vociferans}=14$, $U=190.5$, $p=2.049e-05$). *Ad libitum* observations showed several groups of *A. azarai* beginning to eat biscuits and move around during this period of time, while the other two species were watchful, but did not move from their original resting site. All three species rested during the morning (0600 to 1200). *A. vociferans* rested the most ($97.0 \pm 6\%$), followed by *A. nancymae* ($96.9 \pm 10\%$) and the *A. azarai* ($90.5 \pm 14\%$). However, the differences were not statistically significant (M-W, $n_{A.azarai}=14$, $n_{A. nancymae}=17$, $U=160$, $p=0.0746$; M-W, $n_{A.azarai}=14$, $n_{A. vociferans}=14$, $U=113$, $p=0.4803$).

Nesting Sites

The nesting sites of the three species of owl monkeys in the current study can be categorized by 2 types: Tree Hole Type Sleeping Sites and Dense Foliage Type Sleeping Sites.

Tree Hole Type Sleeping Site

Tree hole type sleeping sites included the covered nest box (“C” in Figure 9) and the opaque nest box (“O” in Figure 9). For the covered and opaque nest boxes (resembling tree holes) there was no significant difference between the three species (Kruskal-Wallis, $H_{adj}=3.2$, $p=0.2$). For the covered nesting box, *A. vociferans* ($25.5 \pm$

Table 3. Average percentage (+SD) Focal Time in Nest Box by Type.

Species	Tree-Hole Type	Foliage-Type
<i>A. vociferans</i>	25.5 \pm 25.7	18.4 \pm 18.8
<i>A. azarai</i>	22.5 \pm 26.0	13.6 \pm 19.1
<i>A. nancymae</i>	20.1 \pm 30.7	8.1 \pm 18.0

26%) and *A. nancymae* (20.1 \pm 31%) did not show a significant preference compared to *A. azarai* (22.5 \pm 21%; U=102, n_{A.azarai}=14, n_{A. vociferans}=14, p=0.8743; U=79, n_{A.azarai}=14, n_{A. nancymae}=17, p=0.1123) (Table 3).

Dense Foliage Type Sleeping Site

Dense foliage type nest boxes are more open than the tree hole type and include the open bucket (“B” in Fig. 9) and the mesh box (“M” in Fig. 9). For the open bucket and mesh box there was a significant difference (Kruskal-Wallis, H_{adj}=10.1, p=0.006). Contrary to expectations *A. azarai* did not show a greater preference for foliage type nest boxes (15.4 \pm 20.3) compared to *A. vociferans* (18.4 \pm 18.8; M-W, n_{A.azarai}=14, n_{A. vociferans}=14, U=107, p=0.696). Compared to *A. nancymae* (18.0 \pm 8.1), *A. azarai* preferred foliage type sites (M-W, n_{A.azarai}=14, n_{A. nancymae}=17, U=48.5, p=0.004425) (Table 3). These results include the use of these nesting sites over a 24-hour period and include any activity within the boxes, other than resting.

Alternative Nesting Site

Unexpectedly, the floor was a common resting site for the focal animal sampling. The floor was used more often (15.1 \pm 23.9%) than the bucket (3.1 \pm 9.5%) for all species. Compared to the opaque nesting box (6.5 \pm 18.1%), the bucket (6.3 \pm 18.5%)

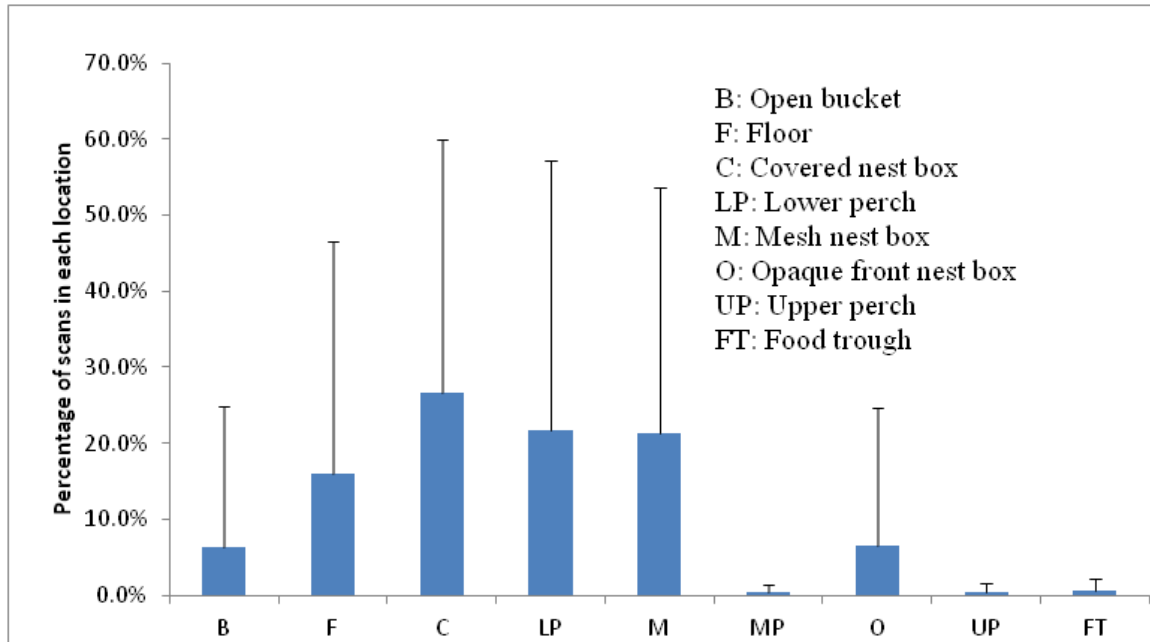


Figure 12. Percentage of focal observation time in each site for resting behavior, all three *Aotus* species.

was used less often as a resting site and may not be considered a sleeping site despite my interpretation of this structure as one of the nesting sites (Figure 12).

Group Size

To determine the reason for groups' preferences of the floor or lower perch as a sleeping site rather than a box designed for that purpose, I analyzed the impact of group size. Four out of the 5 groups with more than 4 group members spent time in locations other than sites specifically for resting. Groups comprising 2-3 individuals used the nest boxes significantly more often than groups of 4 or more ($n_{\text{group}2-3}=28$, $53.9 \pm 22.7\%$, $n_{\text{groups}4+}=17$, $6.8 \pm 7.6\%$, Mann-Whitney, $U=457$, $p=2.94\text{e-}07$). The only group that used a nest box with 4 or more members was the newest large group of *A. azarai* which welcomed a newborn midway through the summer. Prior to the birth of the infant, the group used the covered nest box, which shows both site fidelity afterwards as well as a

possible reason to the use of other sites by more established larger groups with larger (non-infant) group members.

Scan Sampling Behavior Results

Nesting Behavior

Groups tended to rest huddled together with most other members of the group ($92 \pm 5\%$) during the morning scans. *Ad libitum* data showed they often maintained their position within body contact of the other members of the group whether they were alert (eyes open) or not.

Nesting Site

To understand the most likely nesting sites used for sleeping I analyzed the scan sampling data between 0600 and 1200 hours (daylight), the time during which the owl monkeys were most likely resting in their sleep site. These scans showed that for all three species the most commonly employed resting site was the covered nest box (785 out of 2889 scans, $27 \pm 4\%$). *A. azarai* spent the majority of scans in the covered nest box ($32 \pm 39\%$). *A. nancymae* spent $23 \pm 32\%$ and *A. vociferans* $26 \pm 30\%$ of scans in the covered nest box during this time period. During these scans, *A. nancymae* preferred the tree-hole type nesting site more often than the dense foliage type sites (tree hole=378, dense-foliage=167, $\chi^2 = 81.7$, $p = 1.6E-19$); *A. azarai* preferred the tree hole type sites (tree hole=360, dense foliage=250, $\chi^2 = 19.8$, $p = 8.4E-06$); *A. vociferans* preferred the dense-foliage type sites (tree hole=368, dense-foliage=242, $\chi^2 = 26.0$, $p = 3.4E-07$).

Alternative Sleeping Sites

During morning scans, the floor ($16.0 \pm 30.6\%$) and the lower perch ($21.7 \pm 35.4\%$) were common resting sites. The second most common sleeping site during the morning scans for *A. azarai* was the lower perch ($30.0 \pm 37.5\%$) and followed by the mesh box ($25.6 \pm 35.9\%$). *A. nancymae* spent the majority of scans on the lower perch ($29.7 \pm 42.8\%$), followed by the covered nest box ($22.7 \pm 31.7\%$) and the floor ($18.5 \pm 30.2\%$). *A. vociferans* used the floor the most ($26.9 \pm 42.1\%$), followed by the covered nest box ($26.1 \pm 30.5\%$) and mesh box ($23.4 \pm 32.1\%$) (Table 4).

Table 4. Frequency, chi-square and p-values for all groups by species for the top 6 locations used for resting during the morning scan sampling data collection.

Group #	Bucket	Floor	Covered Box	Lower Perch	Mesh Box	Opaque Box	Mean	Chi ²	P
<i>A. vociferans</i>									
1	0	3	0	23	127	0	25.5	500.6	5.9E-106
2	0	236	0	0	24	0	43.3	1038.6	2.7E-222
3	93	0	99	0	3	0	32.5	373.0	2.0E-78
4	0	0	102	0	28	0	21.7	386.4	2.6E-81
5	82	0	32	0	0	0	19.0	293.8	2.2E-61
<i>A. nancymae</i>									
1	0	2	51	0	0	132	30.8	464.6	3.5E-98
2	0	2	121	2	0	1	21.0	571.6	2.7E-121
3	0	136	70	0	3	0	34.8	462.9	8.0E-98
4	1	12	0	21	147	0	30.2	554.7	1.2E-117
5	0	7	0	295	13	0	52.5	1346.8	4.7E-289
<i>A. azarai</i>									
1	0	1	161	1	31	0	32.3	637.5	1.6E-135
2	0	9	3	115	0	0	21.2	502.1	2.9E-106
3	1	20	0	185	0	55	43.5	604.5	2.1E-128
4	0	0	104	3	22	1	21.7	392.0	1.6E-82
5	0	0	13	1	180	3	32.8	795.3	1.2E-169

Site Fidelity

As demonstrated in Table 4, site fidelity was extremely high for all of the groups. All 15 groups spent more than 50% of the summer in one sleeping site during the morning scans, and 8 of the 15 groups spent more than 75% in one site. None of the groups used all possible nest sites for resting. Each group from each species showed a strong preference for one nesting site. Only one group of *A. nancymae* preferred the opaque nesting box. One group of *A. vociferans* and *A. nancymae* strongly preferred the floor while one group of *A. nancymae* and two groups of *A. azarai* preferred the floor for the entire summer.

DISCUSSION

I compared three owl monkey species to illuminate potential differences in nest usage for captivity. Differences in the selection of certain sleeping sites is likely impacted by several factors, including predator avoidance, protection from the weather and parasites, social cohesion, human avoidance, light reduction and thermoregulation (Kappeler 1998; Anderson 1998; Anderson 2000; Obaldia et al. 2011). In owl monkeys, differences in circadian activity and sleeping site usage witnessed in the wild are thought to influence differences in nest box usage and behavioral budgets in captivity. Owl monkeys employ a variety of types of sleeping sites in the wild and in captivity, and their selection can vary by species, therefore species differences can be important in considering optimal cage designs for captive animals.

Owl monkeys use dense foliage and tree holes as nest sites (Aquino and Encarnación 1986; Garcia and Braza 1993). Although *A. azarai* have rarely been observed

using open branches to sleep (Garcia and Braza 1993). The smaller, more nocturnal *A. vociferans* and *A. nancymae* use tree holes more often than the larger, cathemeral *A. azarai*; (Aquino and Encarnación 1986; Garcia and Braza 1993; Puertas et al. 1995; Fernandez-Duque and Erkert 2006).

In my study, the three owl monkey species employed several sites within their cages for resting/sleeping, including both concealed and open areas of the cage. Six sites in the cages were most frequently selected for resting during the morning: the floor, lowest perch, covered nest box, mesh box, open-ended bucket and opaque nest box (Figure 11). Based on studies of wild owl monkeys, the covered nest box (C, Figure. 9) and opaque box (O, Figure. 9) most closely resemble tree holes (Tree Hole Type Sleeping Sites), while the open-ended bucket (B, Figure 9) and mesh box (M, Figure. 9) resemble dense foliage type sleeping sites found in studies on wild owl monkeys (Dense Foliage Type Sleeping Sites) (Aquino and Encarnación 1986; Garcia and Braza 1993).

Dense Foliage Type Sites

Aquino and Encarnación (1986) identified 4 types and 11 subtypes of sleeping sites for owl monkeys. Of the 4 types, 2 were inside of trees and 2 were combinations of plants such as thickets and epiphytes (Aquino and Encarnación 1986). The plants (epiphytes, hemiepiphytes, hemiparasites, lianas) and thickets (bamboo, etc.) can provide some concealment though not as much as the hollows within branches or trunks of trees (Aquino and Encarnación 1986). In the wild, the *A. nancymae* were the most diverse and used all 4 types (tree holes in trunks, tree holes in branches, dense foliage in epiphytes and hemiepiphytes and dense foliage in thickets). *A. vociferans* were observed mainly in tree holes and occasionally in dense foliage of hemiepiphytes (Aquino and

Encarnación 1986; Puertas et al. 1995). *A. azarai* mainly use dense foliage sleeping sites and occasionally open branches (Garcia and Braza 1993; Fernandez-Duque et al. 2008).

In the current study, *A. azarai* preferred the more open, foliage-type nest boxes when compared to *A. nancymaae* but not *A. vociferans*. *A. vociferans* and *A. nancymaae* regularly employed open sites such as the floor and lower perch as well as the less concealing nest boxes.

Tree Hole Type Sites

A. nancymaae has been shown to use all sleeping sites and *A. vociferans* has been shown to predominantly use tree holes in the wild (Aquino and Encarnación 1986; Puertas et al. 1995). However, tree holes are the most common nesting sites for both *A. nancymaae* and *A. vociferans*, though not for *A. azarai* (Aquino and Encarnación 1986; Garcia and Braza 1993). *A. azarai* have not been observed to employ tree holes like *A. nancymaae* and *A. vociferans* (Garcia and Braza 1993). The focal animal sampling showed that *A. nancymaae* and *A. vociferans* did not prefer the tree-hole type nest boxes when compared to *A. azarai* in the current study (Table 3). *A. vociferans* used the tree-hole type boxes (25.5%) only slightly more often than the other nest sites (18.4%). Although, *A. nancymaae* did use the tree-hole types (20.1%) more often than dense foliage type sites (8.1%). Regularly, *A. azarai* employed the tree-hole like sites (22.5%). Two of the five groups (one of which had a pregnant then later lactating female and the other a male-female pair) regularly used their covered mesh box throughout the summer (50.7% and 43.5%, respectively).

Open Sites

The use of open branches for sleeping sites is not as common in owl monkeys as it is in other primate species which employ ends of branches, cliffs or simply high areas for sleeping during the night, without any foliage or plant coverage (Anderson 1998; Kappeler 1998; Anderson 2000). Although the use of open branches is rare in *Aotus* spp., it has been observed in *A. azarai* (Garcia and Braza 1993). This differs from the current study in which lower, exposed sites such as the floor and lower perch were commonly employed by all species as a regular nesting site ($32.1 \pm 34.3\%$, focal animal sampling). The lack of visual obstruction or height preference in these sleeping sites is unclear. One possible explanation is the distance from sunlight or overhead lights. Of the groups that regularly employed the floor, they were positioned directly below the lower perch, which may have provided some coverage. The use of the lower perch (LP in Figure 9) is the most perplexing as it is not covered in any way. One possible explanation for the use of this site rather than higher perches (such as MP and UP in Figure 9) is the position of the perch. The lower perch (LP) is the furthest from the front of the cage compared to the other perches. This is consistent with the use of the floor, in which all groups that employed either the floor or the lower perch were the furthest away from the front of the cage. Human avoidance can be a strong consideration in designing spaces within cages for these owl monkeys and has been observed in other studies (Obaldia et al. 2011).

Available Space

Space considerations (i.e. size of site relative to size of group) may play an important role in site selection for family groups; thus group size may explain differences among groups rather than innate species differences. In captivity, there is limited space

within cages in which to rest. In the wild, most owl monkey live in family groups of fewer than 5 individuals with an average home range of 9.2 ha (Aquino and Encarnación 1994; Wright 1994; Fernandez-Duque et al. 2008). The amount of space inside the tree hole is important in order to fit the entire group. In tree holes the amount of space available inside the tree holes in the wild is often much larger (0.6 to 13.4 m³) than those found in the current study (35.6 cm³) (Aquino and Encarnación 1986). In the current study, groups with fewer members tended to use more nest boxes (53.9%) than larger group sizes (6.8%, based on focal animal sampling data). Space available within the nest boxes may be a consideration for these larger groups. Husbandry techniques and human interaction in captivity may also influence the use of nest boxes in different ways that are difficult to measure. For example, *A. azarai* may be more likely to use the covered nest boxes in captivity than use tree holes in the wild due to the amount of human intervention.

Owl Monkey Nest Use in Captivity

In captivity, Obaldia et al. (2011) added a simple T-shaped PVC nest box to the cages that already held a stainless steel nest box and noticed an improvement in sexual reproductive success (birth of viable offspring). Owl monkeys may use the additional nest box for reasons such as hiding from humans, other pairs of owl monkeys, and shade from the overhead lights (Obaldia et al. 2011). In the current study, owl monkeys regularly used nest sites that vary in size, shape and position within a cage. However, there were areas of the cage that the owl monkeys rarely used to rest. These include any of the perches other than the lowest one and the floor. In one study on captive owl monkeys, all monkeys spent over 69.4% in their nest boxes, usually huddling, during the

light period (Jones and Simpson 1982). Although the owl monkeys in the current study spent the majority of scans huddling (92.4 \pm 5%, scan sampling data from the morning 0600-1200) only 46.1% of my morning scans found owl monkeys in one of the four nest boxes. This differs from Jones and Simpson (1982), because several groups from each of the three species in the current study did not sleep in any type of nesting box and instead preferred the floor or lower perch, both of which are exposed to more light and viewing by humans, strongly suggesting that preference of a family group to nest huddled together takes precedence over the coverage in a sleeping site. Anderson (2000) posits that social cohesion and maintenance of social bonds may be a critical factor in determining which individuals sleep together, usually huddled and touching a large portion of the torso. The current study results agree with this assessment in family groups of all three species of owl monkeys. The owl monkeys likely preferred to sleep in a group in a more exposed position than individually within a nest box.

Nesting Sites for Other Species in Captivity

Nesting supplies are an important part of environmental enrichment to elicit species typical behavior (Anderson 1998; Baker et al. 2006). One survey of zoos and laboratories that house primates found that 50% provide bedding/nesting materials and 36% provide nest boxes (Baker et al. 2006). Nest boxes have been provided to primates species such as owl monkeys (*Aotus* spp.: Jones and Simpson 1982; Obaldia et al. 2011), common marmosets (*Callithrix jacchus*: Goncalves et al. 2009), pygmy marmosets (*Cebuella pygmaea*: Genoud et al. 1997), red-bellied tamarins (*Saguinus labiatus*: Caine et al. 1992) and mouse lemurs (*Microcebus murinus*: Perret 1998). In the wild, these species typically use tree holes (Kappeler 1998). In addition to protection from predators and

inclement weather, tree hole/nest box usage has been shown to provide thermoregulatory advantages (Genoud et al. 1997; Anderson 1998; Perret 1998; Radespiel et al. 1998).

Although no research has explored the thermoregulatory indications of sleep site sharing in *Aotus* spp. this may explain why groups remained in close contact during the daylight hours, even when the nest box was not employed. However, as previously noted, this cannot be separated from the importance of social cohesion and sentinel behaviors in huddling at sleeping sites (Anderson 1998). In one study on captive red-bellied tamarins (*Saguinus labiatus*), one group consistently chose the nest box that was the most concealing, highest available and had the most overhead coverage (Caine et al. 1992). While the owl monkeys in the current study chose the sleeping site that matched this description (the covered mesh box, M, Figure 9) they also employed most other sites.

Site Fidelity

In the current study, all of the groups showed high site fidelity and employed only one or two nesting sites (including the floor and lower perch) throughout the summer. Only one group of *A. vociferans* split time between two different nesting sites (covered mesh box 50.8% and open-ended bucket 47.7%, during morning scans). This particular group occasionally moved due to the presence of personnel from the bucket to the covered mesh box. All other groups spent over 50% of morning scans at one nesting site for the entire summer. Another nocturnal primate, mouse lemurs (*Microcebus murinus*) showed similar site fidelity and stable sleeping groups among females (Radespiel et al. 1998). Hairy-eared dwarf lemurs (*Allocebus trichotis*) also show high site fidelity of tree holes with multiple individuals (Biebouw et al. 2009). Both male and female *L. mustelinus* showed high site fidelity (Rasoloharijaona et al. 2008). *L. edwardsi*

also showed high site fidelity, limiting to only 2 to 3 sites over a season (Rasoloharijaona et al. 2003). Factors in the wild such as tree height/breadth, foraging distances, territorial boundaries, available nest sites (Radespiel et al. 1998), ambient temperature (Fernandez-Duque 2003), moonlight (Fernandez-Duque 2003) and predators (Kappeler 1998; Anderson 1998) may influence the site fidelity among primates. In several of the previously mentioned studies, the authors suggest sleeping sites as a possible limiting resource that can be defended, especially in when predation risk is high (Radespiel et al. 1998; Rasoloharijaona et al. 2003; Rasoloharijaona et al. 2008).

Nest Site Behavior: Sleep

Sleep is one of the most important activities to occur within the nest box during the daylight hours for anthropoid primates such as owl monkeys (Anderson 1998). In one study, *A. azarai* spent between 9.5 hours and 12.5 hours sleeping (Sri Kantha and Suzuki 2006). Although my study did not focus on sleep, resting behavior included huddling and huddling-non-responsive, this likely included physiological sleep. All three species spent over 73% of focal sampling resting, though not always in one of the nest boxes. The use of tree holes and/or nest box may allow for longer sleeping bouts and total sleep time for nocturnal versus diurnal primates, especially in response to different predators available at night compared to day (Sri Kantha et al. 2009). Sri Kantha and Suzuki (2006) also found the male carrying a 7-8 week old infant spent 2 hours longer sleeping than normal. In this study, the male *A. azarai* rested 78.1% prior to the birth of the infant and 85.2% after birth. The extra burden of carrying an infant full time likely contributes to the increase in resting even during the active period.

Other Nest Site Behavior

In the current study, there are a few behaviors that are mostly exclusive to nest box usage including marking, tail twining, and huddling.

Marking and Tail Twining

Marking behaviors such as urine washing and anal rubbing has been observed in wild *A. azarai* (Garcia and Braza 1993), but was not common in this study. There were two instances of possible marking behavior involving the nest boxes: a male from one *A. azarai* family rubbed inside of several nest boxes on two different days (0.1% focal animal sampling), and once a female *A. azarai* rubbed the inside of the enclosed nest box during the morning scan. Increased scent marking is often associated with the presence of conspecifics and territorial boundaries (Wolovich and Evans 2007; Fernandez-Duque 2012). In captivity, the cages and nest boxes are sanitized bi-weekly. If marking behavior occurred, it would likely have been within the time period immediately following sanitization, but would not be associated with entering or exiting a sleeping as suggested by Aquino and Encarnación (1986). Tail twining was a rare behavior, only recorded *ad lib* on two occasions, in *A. vociferans* and *A. azarai*. Tail twinning has been observed in wild *A. nancymae*. Although considered part of huddling behavior in this study the behavior may have had a social function.

Huddling

The most common nest site behavior in the morning (0600 to noon) was huddling. Morning scan sampling data analyses revealed that during this time period, *A. vociferans* huddled 98 +/-4%, *A. nancymae* 95 +/-5% and *A. azarai* 84 +/-8%. Often the entire group huddled together with at least one side of the body contacting another

individual. In groups with juveniles, the juveniles were often in the middle or infants were on the back of the father. The only occasion in which a juvenile was not huddled with his parents during the morning hours resulted in the removal of this individual due to fighting. This is similar to the spacing of social sleeping baboons in which the males were closest to the danger area (trunk of a tree) while juveniles and females were further out on branches (Anderson 2000). The natural emigration of young from the natal group is restricted in captivity and is usually abrupt. In the wild, subadults emigrate from their natal group usually between 3 and 4 years of age after reaching sexual maturity between 18 and 24 months (Dixon 1994; Fernandez-Duque and Huntington 2002; Fernandez-Duque 2012). The juvenile male in this study was only 1 year and 10 months when fighting and removal from the natal group occurred. The female was later determined to be pregnant, which may have contributed to her nesting site choices and behaviors. Interestingly, *A. vociferans* were the only species observed to lay ventrolaterally on the floor or in the bucket during the restful periods (*ad lib*). Although body position was not part of this study, it may be important in selecting a nest site (Anderson 2000). There is no information on the positions of individual owl monkeys in the wild.

Behavioral Budget

Cathemeral and Nocturnal Circadian Rhythms

Aotus spp. are considered predominantly nocturnal across most species (Wright 1994). However, research on wild *Aotus azarai* has shown that these monkeys are more cathemeral than nocturnal, especially at different points during the lunar cycle (Fernandez-Duque 2003; Fernandez-Duque and Erkert 2006). Cathemerality allows for the owl monkeys to exploit different resources and could impact nest site preference

(Fernandez-Duque 2003; Fernandez-Duque and Erkert 2006). *A. azarai* in this study were much more active during the “dusk” period from noon to 1500 compared to both *A. nancymae* and *A. vociferans*. Often *A. azarai* groups were observed moving around, handling food and playing during the dusk period. However, all groups were very active after the final lights and skylights were shut off at 1500. In addition, all *Aotus* spp. were inactive during the period prior to dusk. Factors that influence cathemeral activity of *A. azarai* include moonlight and ambient temperature (Fernandez-Duque 2003; Fernandez-Duque and Erkert 2006; Fernandez-Duque 2012). The brighter the moonlight, the more active *A. azarai* were during the night and on nights following a warmer day with a brighter moonlit nights (Fernandez-Duque 2003). In captivity, these influences are constant throughout the year and may not impact the behavior of the owl monkeys. In this study, the light cycle and intensity of the lights never changed. However, during the dusk period, overcast skies caused the colony room to be much darker and likely impacted behavior.

Play Behavior

Play behavior included rough and tumble play and solitary object play as defined in Graham and Burghardt (2010). Play behavior was observed most often with juveniles and sub-adults (up to 3.5 years) (1.7% of behavioral budget, focal animal sampling). According to the focal animal sampling data, one group of *A. azarai* with two juveniles was playful (2.4%, focal animal sampling). These two juveniles played by chasing each other around the cage, occasionally grabbing at the others tail and rolling over onto their dorsum on either a perch or the floor. The most playful individual (9.0%, focal animal sampling) was a 4 month old male in a large group (5 individuals) of *A.*

nancymaae. The amount time spent engaging in play behavior was similar between males and females (0.9% and 0.5%, respectively). Play behavior is common in primates and often seen most often in juveniles compared to other age groups (see review, Graham and Burghardt 2010). Play behavior is mentioned only *ad lib* in studies on *Aotus* spp. in the wild (Wright 1978; Aquino and Encarnación 1986; Wright 1994) but has been observed in captivity (Jones and Simpson 1982). The lack of information on play behavior in owl monkeys in the wild is likely due to the difficulty of observing nocturnal species, especially in higher canopy. In the current study, play behavior was found in all three species. *Ad libitum* observations showed many instances of play occurring other than during the focal-animal period. Several instances include object-play with food, tails, and plastic toys in the cage as well as social play such as rough and tumble play involving several instances of chasing between juveniles. Captive settings may impact the amount of play seen in primates. Cage substrates may impact the amount and type of play seen in captive groups of tamarins (Caine and O'Boyle 1992). For example, the flatter surfaces found in cages, which are not common in the wild, provide a safe surface for rough-and-tumble (in this case, "wrestling") play behavior (Caine and O'Boyle 1992). Although there is little research on the activities inside the nests of cavity-nesting primates, it is possible that a variety of social interactions, including play, are more common in nest sites where safety and protection are available.

Food Behaviors

Social behaviors often involve food. Food-sharing is a common behavior in owl monkeys (Wolovich and Evans 2007; Wolovich et al. 2006; Wolovich et al. 2010). Food sharing was observed in this study and often including begging and stealing, especially

with high value items such as fruit over the abundant Lab Diet® monkey biscuits. Food sharing is most common between parents and offspring and males with lactating females (Wolovich et al. 2007; Wolovich and Evans 2007; Rivera et al. 2007). One instance of food sharing involved a male-female pair of *A. vociferans* in which the female had acquired an entire banana peel, from which the male took pieces while she ate.

Consuming insects is an important behavior for owl monkeys in the wild and occasionally involved food sharing behavior in captivity (Wright 1994; Wolovich et al. 2010). Eating usually took place on a perch or the floor. However, during the dark hours, after 15:00, the owl monkeys employed a variety of spaces in which to eat, including all of the nest boxes. Under the focal animal sampling analysis, *A. vociferans* was observed feeding the most often (12.0%), then *A. azarai* (6.4%) and *A. nancymae* (4.2%). Feeding behavior included environmental enrichment feeding as well as the standard produce and chow diet. When food enrichment such as a foraging board was made available, the owl monkeys would work on the device until most of the foraging material had been consumed. Social behavior was approximately the same across all species (1.2%, focal animal sampling) and included nursing, food sharing, allogrooming and playing.

Nursing

Nursing is rarely seen in the wild due to the difficulty of observing *Aotus* spp. during their active period (Rotundo et al. 2005). Nursing was observed on several occasions in *A. vociferans* and *A. azarai*. Rotundo et al. (2005) recorded the mean duration of nursing as 69s (range: 31-160s). The bouts in this study were often much shorter (<30s) and occasionally recorded *ad lib* during the scans or observations of other groups. Nursing almost exclusively took place during the morning or dusk periods. The

oldest infant was a male (13 months at end of study) *A. azarai* that was observed nursing. Rotundo et al. (2005) observed nipple contact and possible nursing after 8 months. The youngest was a female *A. azarai* (born July 19, 2012, during the study). Consistent with previous research, this female was not observed away from either parent until 4 ½ weeks (Rotundo et al. 2005). Occasionally the infants were inside a nest box, alone, during the night hours when the group was feeding. One male (2 months old at the start of the study) was often in the mesh box playing with on the vine or eating. This behavior resembles infant parking which is found in several strepsirrhines and involves a stable nesting site (Kappeler 1998). When not in one of the nest boxes, most infants were sitting with or on a parent, which is consistent with observations of wild owl monkeys (Dixson 1994).

Strengths and Limitations

In captivity, the available resources and logistics of colony management such as sanitation and durability are necessary considerations in designing and implementing nesting sites (Baker et al. 2006). In the wild, substrates available to owl monkeys for nesting include trees and foliage (Aquino and Encarnación 1986; Fernandez-Duque 2012). In the current study, natural foliage was unavailable, which may have limited the results. Other limitations include the limited sample sizes for each species (*A. nancymae* 17, *A. vociferans* 14, and *A. azarai* 14). Finally, group size as well as the size of the individuals likely dictate whether a tree hole or dense foliage site is used in the wild as well as in captivity, and group sizes in captivity necessarily. Therefore, the lack of larger nesting sites available may have been a limitation. Nonetheless, because one of the broader objectives of this study is to improve habitats for captive species, these results can be generalized, given other limitations, to similar, captive situations.

Understanding species differences in captive species is important to any environmental enrichment program (Baker et al. 2006). There was one minor difference between *A. nancymae* and *A. azarai* in the use of foliage type sleeping sites. The information from the current study on nest box preference in *Aotus* spp. is valuable to understanding both resting behavior as well as the amount of nesting material that is needed to house these species in captivity. The finding of very little statistical differences between nest site usage supports the use of a similar nest box for all species of owl monkeys and can help aid in management. Although there are species differences between wild owl monkeys (Aquino and Encarnación 1986; Garcia and Braza 1993; Puertas et al. 1995), these differences may not be strong considerations when designing caging systems in captivity. The current study is one of few that systematically analyzed the differences between individual species of owl monkeys in captivity.

CONCLUSIONS

Nest box usage in primates is critical to understanding both wild and captive groups. Environmental enrichment is designed to elicit species-typical behaviors in captivity. Understanding the differences between related species of primates will aid in the housing and enrichment available. Most environmental enrichment considerations are designed for diurnal, multi-male/multi-female groups such as macaques and chimpanzees (Baker et al. 2006). The aim of this study was to determine the role of nest boxes in housing captive owl monkeys and the differences between three species of owl monkeys.

In this study, there were few significant differences between *A. nancymae*, *A. vociferans* and *A. azarai* and the use of different nest boxes. Factors such as group size,

lack of influences such as weather, predators, moonlight and temperature changes, in captivity compared to the wild, may impact the preferences of these species for tree holes or dense foliage. In considering caging materials, areas further from the front of the cage may be an important factor. Future research could explore the preferences of location and size, as seen in other studies (Caine et al. 1992).

There was a clear difference between the activity patterns. *A. azarai* showed more cathemeral behavior than *A. vociferans* and *A. nancymae*. Activity patterns and the availability of a dusk/dawn period may be important in housing colonies of owl monkeys in the future. The amount of ambient light through sources similar to moonlight might also be considered for colony enrichment.

In conclusion, although different owl monkey species use different sites in the wild they do not appear to show strong sleeping site differences in captivity. Also, evidence for cathemerality for *A. azarai* was suggestive but not conclusive in the current study.

APPENDIX A: REGARDING IACUC APPROVAL

From: Michelle Lane <ml48@txstate.edu<mailto:ml48@txstate.edu>>

Date: Fri, 3 Feb 2012 13:58:11 -0600

To: Becky Northcut <bnorthcut@txstate.edu<mailto:bnorthcut@txstate.edu>>

Subject: Re: IACUC

Dear Leilani,

I agree with Ms. Northcut. If the protocol is only observational, and you will in no way affect the monkeys' behavior, then you do not need an IACUC.

Best,

Dr. Lane

--

Michelle Lane, Ph.D.

Associate Professor

IACUC Chair

School of Family and Consumer Sciences

Nutrition and Foods Program

Texas State University-San Marcos

On 2/3/12 11:29 AM, "Northcut, Becky"

<bnorthcut@txstate.edu<mailto:bnorthcut@txstate.edu>> wrote:

Leilani,

If it's observational only, it's unlikely you'll need approval. However, I am copying the IACUC chair, Dr. Michelle Lane, so she can confirm. If you do need an approval, your faculty member will have to submit the protocol.

Sent from my Verizon Wireless 4G LTE Phone

Northcut, Becky

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VITA

Leilani Case was born October 25, 1985. After completing high school at the LBJ Science Academy in Austin, Texas she attended the University of Wisconsin-Madison where she received her Bachelor of Science degree in Zoology in December 2007. She worked for the University of Texas system with a variety of animal species after graduation and continued working for the University of Texas at Austin while attending graduate school in the Anthropology department at Texas State University-San Marcos, which she entered in August 2011.

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