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The University of Southern Mississippi

Predatory Nature and Food Preferences among Captive Otolemur garnettii

by

Morgan Ruby

A Thesis

Submitted to the Honors College of The University of Southern Mississippi in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science in the Department of Biological Sciences

May 2017

Approved by

Dr. B. Katherine Smith Department of Anthropology

Dr. Ann Marie Kinnell

Department of Anthropology and Sociology, Chair

Dr. Ellen Weinauer Honors College, Dean

Abstract

Observing the predatory nature in primates has yielded knowledge pertaining to their biology and evolutionary pathways; however, not many studies have focused on the complexities of their food preferences. This thesis focuses on food preferences among Garnett's Greater Bushbaby (Otolemur garnettii): a small-bodied nocturnal primate native to Central and Southern Africa. Presented food options were raisins: dried mealworms and raisins: live mealworms. The population consists of fifteen bushbabies housed in The University of Southern Mississippi (USM) Primate Behavior Research Facility. Ten trials of three different experiments were performed to identify the dominant hand and food preferences among the USM bushbaby population. Five trials compared bushbaby preferences of live mealworms to raisins, while the other five compared bushbaby preferences of dried mealworms to raisins. The bushbabies showed a preference for raisins over dried mealworms and showed no preference between raisins and live mealworms. Results indicate that the USM population of bushbabies mimic the wild diet of 1:1 ratio of insects to fruit. Additionally, bushbabies would often use their mouth to grab the food directly rather than one or both of their hands; however, when hands were used, many subjects showed hand dominance. The resemblance of the captive population food preferences to the typical wild diet indicates that the USM population has not altered from their wild behavior in regards to diet. Our findings are intended to provide expanded insight on the food preference and predatory instinct of captive O. garnettii, furthering the knowledge regarding the preservation of natural diet in captive bushbabies.

Keywords: *Otolemur garnettii*, bushbabies, food preference, handedness, USM population, wild diet, captivity.

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Chapter I: Introduction

Behavioral observations in primates have yielded knowledge pertaining to their biology and evolutionary pathways. Research on primates typically involves behavioral studies focusing on topics including, but not limited to, cognition (e.g. tool usage), competition in social interactions, and food availability (Cheney et al., 1986); however, little is known for nocturnal primate food choices due to lack of visibility. It is necessary to observe sample populations and diet representing food options to make broader inferences into predatory behavior regarding food preferences among primates.

The University of Southern Mississippi (USM) Primate Behavior Research Facility houses fifteen *Otolemur garnettii* bushbabies in individual, compartmentalized cages. Ages among the bushbaby population range from 2 to 22 years old. General life expectancy among captive bushbabies is considered to be up to 15 years (Bearder, 1987). Few studies focus on captive *O. garnettii* due to the limited number of facilities containing this species of bushbaby. Some of the bushbabies housed in this facility were involved in previous research incorporating handedness and stress levels; however, food preferences were not tested (Hanbury et al., 2013). The presented research observes food preference and predatory patterns among *O. garnettii* housed at the USM Primate Behavior Research Facility to determine if the captive population's dietary preference resembles that of a natural diet. Findings will enhance overall knowledge of the USM population, compare this population to their wild counterparts, and better understand *O. garnettii* placement into the broader primate lineage.

The following food preferences were observed among the USM population:

• Raisins versus dried mealworms

• Raisins versus live mealworms

It was hypothesized that the captive population would choose the raisin over the dried mealworm due to the lack of mobility of the insect and would choose the live mealworm over the raisin due to their predatory nature generated by the mobile insect. By not showing a preference between the raisin and live mealworm, the captive population would be mimicking their natural diet in the wild. Hand dominance – if applicable – among the primates, and spatial proximity of food options to dominant hand was also observed to ensure that this did not bias results. It was hypothesized that the food position in relation to dominant hand would not overpower food preference. This thesis will give insight into food preferences and predatory instincts of the observed captive *O. garnettii* population within the USM population. Furthermore, knowledge gained will influence other comparative research projects between the captive USM population and their wild counterparts.

Chapter II: Literature Review

2.1 Otolemur garnettii Background

Due to the specialized lateral movements and foraging skills among extant bushbabies, their evolutionary pathway – suborder Strepsirrhini – is estimated to have diverged from the primates in the Late Cretaceous Period (see Figure 1) (Milton, 1993; Pozzi et al., 2014). This splitting of lineages and the observed increased specific dietary niche of modern bushbabies resulted in a more diversified primate lineage.

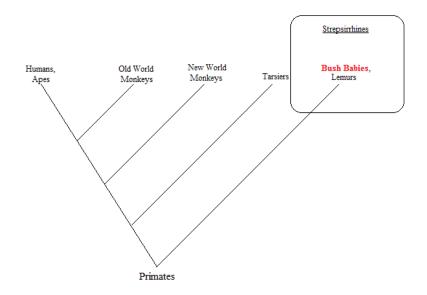


Figure 1: Primate phylogeny tree highlighting the placement of bushbabies in relation to other primates.

There are four subspecies (ssp.) of *O. garnettii* in the Galagidae family; bushbabies found within the USM facility fall within the *O. garnettii garnettii*. Common names for this subspecies are Garnett's Greater bushbabies, small-eared Greater Galago, or Northern Greater Galago bushbaby. *O. garnettii* bushbabies are mid- to high-canopy dwelling nocturnal primates native to coastal and tropical forest regions of Africa – more specifically, Kenya, Somalia, and United Republic of Tanzania (see Figure 2) (Butynski et al., 2008). Due to the abundance of this

subspecies and geographical range, *O. garnettii* are at the lowest risk of endangerment (Butynski et al., 2008). *O. garnettii garnettii* have short, round ears and a bush tail that doubles their total body length; this particular subspecies have a relatively small body size (less than 1000g), when compared to other *Otolemur species*. Weighing in at 998g, Hercules is the largest captive bushbaby within the USM facility. With the males slightly larger than the females, a slight-sexual dimorphism is also present. Furthermore, *O. garnettii garnetti*, exhibit polygynandry, meaning that both sexes breed with multiple partners and do not mate for life with a single partner.

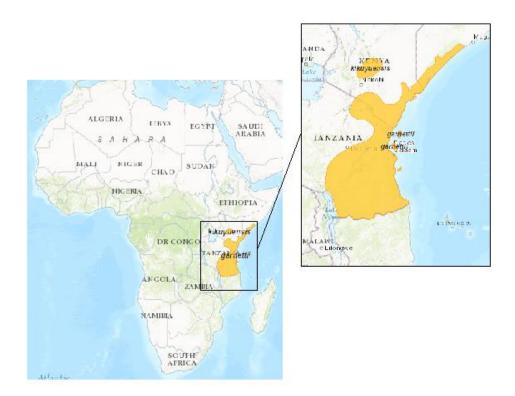


Figure 2: O. garnettii habitat distribution (Adapted from Butynski et al., 2008)

With the distinct crying—yell that sounds like a human infant, verbal communications among bushbabies are considered their most recognizable feature – hence the name "bushbaby" (Becker et al., 2003). Each species has a distinct set of calls specific to a particular task, such as a mother communicating with her adolescent (Becker et al., 2003). Pheromone communication by urine washing has been predominantly observed in males (Tandy, 1976). Urine is spread on hands and feet and then distributed to secondary objects through physical contact (Tandy, 1976). Bushbabies typically locomote on all fours limbs, but will occasionally stand bipedally. Furthermore, bushbabies may be left- or right-handed as well as ambidextrous (Hanbury et al., 2013). These evolutionary aspects allow bushbabies to grasp objects – including food – while maintaining balance.

2.2 Food Preferences

O. garnettii bushbabies are omnivores. Proteins promote muscle growth and sustenance; while fiber, sugar, and fat are necessary for energy and hormone regulation (Jolly, 1985). The natural diet of *O. garnettii* has been observed to consist of half fruit and half insect (Nash and Harcourt, 1986). However, it has been observed that primates have an inclination towards a more variable diet. For example, a primate may be frugivorous one month, while the same primate may prefer a more insect-based diet the following month (Chapman and Chapman, 1981). This is considered to be a result of food abundance and food preference among primates rather than a limited food source.

The food preferences observed within this study will be based on the following two comparisons:

- Raisin versus dried mealworm
- Raisin versus live mealworm

Mealworms are protein rich and contain 35%-60% fat on a dry mass basis, and 6.3%-8.4% fiber content (Finke, 2002). Dry mealworms have more protein and less water than live mealworms (Finke, 2002). For one seedless raisin, a typical serving for USM bushbaby population, it contains 1.6 g of carbohydrates (0.08g dietary fiber, 1.2 g of sugar), and only 0.06g of protein (USDA, 2012).

2.3 Handedness

The evolution of cognitive ability among primates has resulted in behavioral complexity. The transition from olfactory dependency to visual reliance has resulted to a more holistic understanding to spatial location of objects (Jolly, 1985). With a higher sense of the spatial patterns of the environment, primates are able to use more precise movements to pick up objects. Subsequent advancements of these capabilities coincide with increased size and complexity of the cerebral cortex of the brain, leading to the capability of tool usage (Jolly, 1985).

O. garnettii are sociable and active since they are accessing the left hemisphere of their brain (Hanbury et al., 2013). A few research studies by Hanbury have been primarily on the usage of left or right handedness. When capturing prey, seventeen bushbabies were observed to be highly lateralized with 59% of the subjects using the left hand and 41% using the right hand (Hanbury et al., 2010). There was no advantage concerning prey capture between left and right handed bushbabies (Hanbury et al., 2010); however, adult males tend to favor the left hand and adult females favor the right hand (Milliken et al., 1991). When retrieving food, many bushbabies used their mouths entirely with no hand movement; out of twenty-three bushbabies, 74% used their mouths (Hanbury et al., 2012).

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Chapter III: Methods

3.1 Subjects

Fifteen subjects at the USM Primate Behavior Research Facility participated in this study. The bushbabies were fed daily at approximately 10:00 AM. Bushbabies are housed and fed inside their individual, bi-level 77x77x152 cm PVC-coated wire mesh cages. The bushbabies' daily diet consisted of LabDiet[®] 5048 Certified Primate Diet, various amounts of fruits and vegetables, and usually one insect a day. Water was provided ad libitum. Mealworms were the prominent insect presented to the bushbabies; however, crickets, superworms, and nightcrawlers were occasionally fed.

The experiment was performed approximately one hour prior to their normal diet which remained the same. Raisins were chosen to be the presented fruit while live and dried mealworms were the presented insects – all of which are in their current diet. The experiment began each day at around 9:00 AM and continued for thirty-three consecutive days with two exceptions. One day was skipped mid-way through the second experiment due to a vet visit, and a video clip was lost for one subject's last trial. The outlier trial was redone almost three months later.

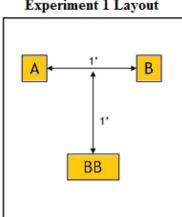
Ten trials of three various experiments were performed in order to identify the dominant hand and food preferences among the captive bushbaby population. All subjects received one raisin concurrently with one mealworm in shallow containers. Five trials compared live mealworms to raisins, while the other five compared dried mealworms to raisins. Food options were presented in alternate locations to ensure that dominant hand did not alter

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results. Furthermore, the first food item chosen was recorded along with the hand used, when applicable.

3.2 Experiment Layouts

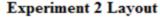
The first experiment was the simplest format, with the raisin and mealworm equal distances away from the bushbaby, one foot in front of the bushbaby in addition to being one foot away from each other, approximately (see Figure 3). The first food item chosen was recorded along with the hand used – when applicable.



Experiment 1 Layout

Figure 3: Spatial location of bushbaby (BB) and food items (A) and (B) for experiment 1.

To further determine the bushbabies' food preference, a slightly more difficult pathway was initiated for the second experiment. One food item was placed one foot in front of the bushbaby while the other was 0.5 inches away from the other food, all forming a linear line in front of the bushbaby (see Figure 4). Similar to the first experiment, the hand usage and food chosen were also recorded.



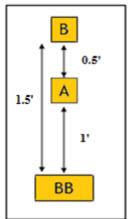


Figure 4: Spatial location of bushbaby (BB) and food items (A) and (B) for experiment 2.

Considering that most bushbabies are apt to use the mouth exclusively for food retrieval, the final experiment was more complex and was executed to mainly promote hand usage and to rely more on their smell receptors. The spatial location of the food items mirrored the first experiment, with the food items placed one foot in front of the bushbaby while simultaneously being one foot away from each other (see Figure 5). The food items were placed in separate Munchkin[®] snack catchers, to induce primarily hand usage and their sense of smell.

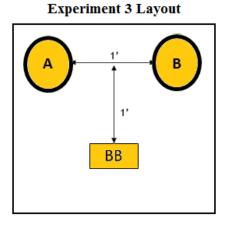


Figure 5: Experiment 3 spatial location of bushbaby (BB) and food items (A) and (B) in duplicate of experiment 1, but inside snack catchers.

3.3 Statistical Analysis

All statistical analyses were performed in Microsoft Excel. Paired T-tests were performed to find the two-tailed P value for all results. For food preference, the results for dried mealworm versus raisin and live mealworm versus raisin were tested for all experiments separately and combined for each. For handedness, the results for dominance between mouth usage and hand usage were tested, along with the dominance between left- and right-handedness; statistical significance was set for P<0.05 for all tests.

Chapter IV: Results

4.1 Food Preference

The results in this section depict the preference for dried mealworm versus raisin and live mealworm versus raisin. Each separated into results for each experiment and the overall results.

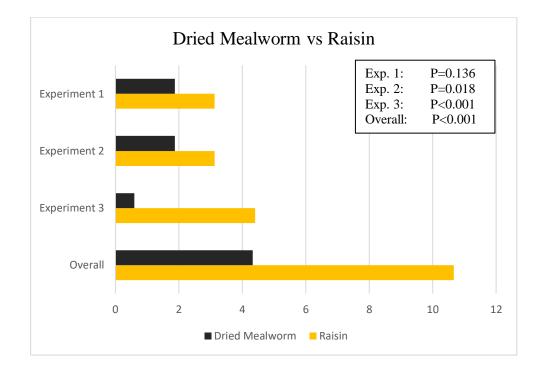


Figure 6: Food preference of dried mealworm versus raisin for all three experiments separately and combined.

A comparison of choices between dried mealworm and raisin for all experiments individually and the overall results are presented in Figure 6. For Experiment 1, there were no significant differences (p=0.136). Experiment 2 and 3, raisins were chosen significantly more than dried mealworms (p \leq 0.05). Overall, the results showed a significant difference, with the subjects favoring the raisin over the dried mealworm (p \leq 0.05).

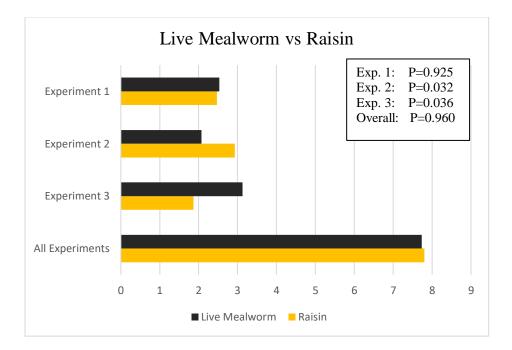


Figure 7: Food preference of live mealworm versus raisin for all three experiments separately and combined.

Food choice results between live mealworm and raisins for each experiment and overall are depicted in Figure 7. For Experiment 1, there was no significant difference. Experiment 2 and 3 showed significant difference; however, Experiment 2 reflected raisin was significantly chosen over live mealworm ($p \le 0.05$) and Experiment 3 showed that live mealworm was chosen significantly over raisin ($p \le 0.05$). The overall results of all experiments displayed no significant difference between live mealworm and raisin.

4.2 Hand Utilization

For the first and second food item choices in all experiments, the hand usage was recorded as either left-handed, right-handed, or none (mouth). The results indicate whether the food was grabbed with a particular hand or simply selected with the mouth.

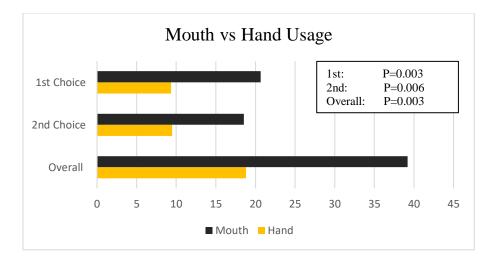


Figure 8: How often mouth or hand was used to choose 1st item, 2nd item, and overall.

The use of hand or mouth on each food choice is depicted in Figure 8. There was a significant difference in favor of mouth over hand usage for both first and second choices, $(p \le 0.05)$.

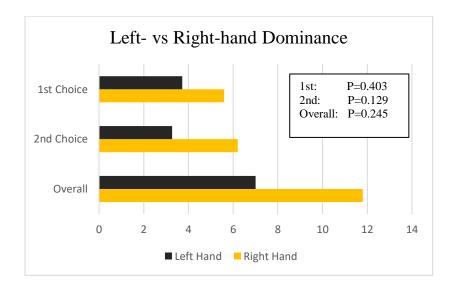


Figure 9: For had usage, number of times left and right hand was used for the 1st choice, 2nd choice, and overall.

In Figure 9, although, subjects tended to be more right handed, there was no significant difference between using left or right hand for all subjects. This figure shows overall hand usage of the USM population, while the preferred hand usage of each bushbaby separately can be seen in Figure 10.

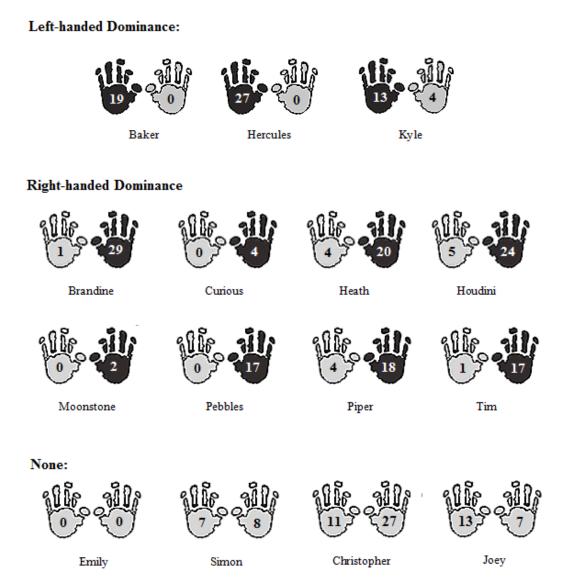


Figure 10: Hand dominance of all bushbaby individuals at the USM Facility. Blacked out hands indicate dominance while numbers on palms represent amount of times hand was used in food choice.

As depicted in Figure 10, most bushbabies displayed hand dominance. Only three subjects displayed left-hand dominance (20%), while eight displayed right-hand dominance (53.33%). Three bushbabies did not show a significant difference between left or right (20%), while one never used their hands and therefore hand dominance was not determined (6.66%).

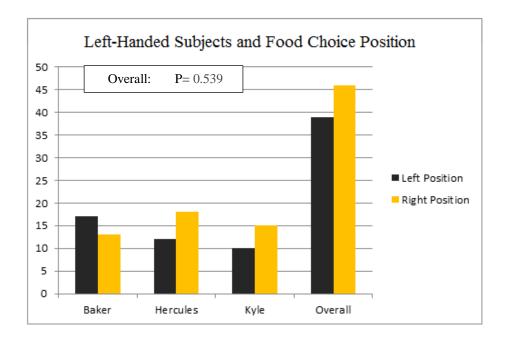


Figure 11: Left hand dominant subjects hand usage correlation with food choice.

As depicted in Figure 11, there was no significant difference in food choice positions for left-handed subjects. Food position had no significant bias in relation to left side placement.

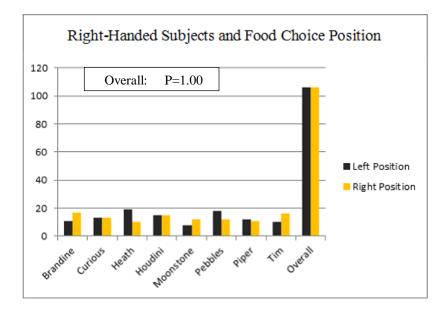


Figure 12: Right hand dominant subjects hand usage correlation with food choice.

In regards to right-handed subjects, in Figure 12, there was no significant difference in food choice positions. Food position had no significant bias in relation to right side placement.

Chapter V: Discussion

The primary purpose of this study was to evaluate food preferences of the captive USM bushbaby population and to compare preference composition to that of their natural wild diet. The following food preferences observed:

- Raisins versus dried mealworms
- Raisins versus live mealworms

It was hypothesized that the captive USM population would choose the raisin over the dried mealworm and the live mealworm over the raisin. Significant differences were observed for preferences of the raisins over the dried mealworms. No significant differences were witnessed among preferences between the raisins and the live mealworms, rejecting the hypothesized food preference. For hand usage, it was hypothesized that there would be hand dominance observed among the subjects. However, food experiments were spatially designed to not allow hand dominance to bias food preferences (i.e., food was chosen based on preference and not spatial proximity to dominant hand). There was no significant difference for left- and right-handed subjects when comparing chosen food positions.

5.1 Food Preference

When data from all experiments were combined, the USM bushbaby population preferred the raisin over the dried mealworm. However, when data are divided by experiment:

- Differences within experiments 1 and 2 fall just beyond the set significance parameter.
- Differences within experiment 3 showed significant difference.

The significance observed within experiment 3 requires the bushbabies to rely on their olfactory senses to determine contents within the enclosed container. Overall results among the three experiments indicate significant difference in food preferences, with raisin being preferred over dried mealworm. These results were as expected, due to predatory nature of bushbabies.

Although their wild diet is 1:1 fruit to insect, this study suggests that their predatory instinct requires the mealworm to be moving and resembling actual prey. The main finding was that there was no significant difference for any of the experiments for raisin versus live mealworm. The results for food preference are congruent with previous research by Nash and Harcourt (1986) on the 1:1 bushbaby diet of fruit and insects observed in the wild. Therefore, the findings within this study suggest that food preferences among the captive USM bushbaby population emulate that of a wild busy baby diet.

5.2 Hand Utilization

The USM subjects exhibited a statistical significance of using their mouth over hands for food choice. These results reflect the findings by Hanbury et al. (2012) where 74% of the USM bushbabies used their mouths. As hypothesized, there was displayed hand dominance with individual subjects – with 20% showing left and 53.33% right handedness. However, there was no significant difference between which hand was dominant as a group. Bushbabies that did not suggest a dominant hand either directly used their mouth or may be considered ambidextrous; this was roughly 27.33% of the USM population. These results contrast with Hanbury et al. (2010), where he observed 59% using left hand and 41% using right. This difference in results may be due to small population sizes for both studies, and some different individuals in the current USM population than in 2010. Left dominant and right dominant subjects did not choose

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more food items on their dominant side; therefore, it can be assumed that subjects chose food due to preference.

Chapter VI: Conclusion

The captive USM bushbaby population chose the raisin over the dried mealworm. This indicated that this was due to absence of predatory nature from the immobile dried mealworm. Unlike what was hypothesized, they resembled their wild diet by exhibiting a 1:1 ratio of the raisin to the live mealworm. The resemblance of the captive population food preferences to the typical wild diet indicates that the USM population has not deterred from their wild behavior in regards to diet. For hand usage, there was a hand dominance in most subjects, but did not bias food preference. Findings are intended to provide expanded insight on the food preference and predatory instinct of captive *Otolemur garnettii*, furthering the knowledge regarding captive bushbabies and other captive primates. Further research should have more trials, refined experimental methods, and further testing on the primal instinct.

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Appendix A:

IACUC Approval Letter



INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE ANIMAL SUBJECTS RESEARCH APPLICATION FORM

ANIMAL RESEARCH APPLICATION PROCEDURES

Federal regulations and University policy require that any research involving animal subjects conducted in affiliation with The University of Southern Mississippi be submitted for IACUC review and approval.

- Any necessary appendices must be completed and attached to the bottom of this form as indicated.
- Submit a completed copy of this form electronically to <u>iacuc@usm.edu</u>.
- Submit a physical copy of the signature page (located on the IRB website) to IACUC, 118 College Dr. #5116.

Last Edited January 12th, 2015

| Section 1: InvestigAtor information | | | | | | | |
|---|----------------------|-------|------------|---|----------------|--|--|
| Project Title: Examining the Health and Wellbeing of Captive Housed Protocol # (Renewal Applicants Only): | | | | | | | |
| Otolemur garnetti | | | | | | | |
| Principal Investigator: B. Katherine Smith, PhD 7221 | | | | USM Email: bonnie.smith@usm.edu | | | |
| FIID | | /221 | | | | | |
| Campus ID: | Department: Anthropo | ology | Office Pho | ne: 601-266- | Lab Phone: n/a | | |
| 956996 | and Sociology | | 5476 | | | | |
| Alternative Contact Fu | | | | Funding Agency or Sponsor (if applicable) | | | |
| Name: n/a Organization: n/a | | | | | | | |
| Phone: n/a Grant #: | | | | | | | |

| | Name | Project Role | Experience/Training |
|--|----------------------------|--------------|--|
| List all USM affiliated investigators, laboratory personnel, and | B. Katherine Smith, PhD | PI | PhD in primate behavior and health, over 10 years experience working with multiple species of captive housed primates |
| instructional staff. | see attached list | | |
| | | | |
| | | | |
| | Name | Project Role | Experience/Training |
| | | | |
| List all Non-USM affiliated | | | |
| investigators. | | | |
| | | | |

Describe any additional training needed and how it will be conducted.

Smith will train graduate and undergraduate research assistants in behavior sampling (both real time and camera observations) and noninvasive biological sampling (feces, urine, saliva). Interobserver reliability will be tested at 95%. Proper PPE will be worn during training and data collection.

SECTION 2: Protocol Procedures

Protocol Abstract: Describe the protocol briefly in non-scientific, non-technical language. (This description may be used for press releases and in response to Freedom of Information Act (FOIA) requests.)

The primary aim of this study is to examine both the health and stress related to captivity and the relationships between diet, health and nutrition among captive Garnett's Bushbaby (Otolemur garnetti). In addition, this study will propose and test the efficacy of more closely approximating native bushbaby diets and more naturalistic and social housing by reducing the amount of processed foods and increasing the amounts of insects and vegetables in their diet and housing them in larger, more naturalistic enclosures in social housing. These changes are expected to reduce stress, stereotypic behaviors, and self-injurious behavior. Additionally, their overall health is expected to improve by giving them a more naturalistic diet. This research will contribute not only to improvements in captive managament of primates, but will also provide the foundation for larger research projects that will examine the role of insectivory and nocturnality in the evolution of primates.

Detail the protocols' procedures and goal(s) in two to three paragraphs.

All bushbabies are housed at the University of Southern Mississippi Bushbaby Facility. There are currently 15 bushbabies, ranging from 1-21 years old. All bushbabies are currently singly housed in 2.5x2.5x5 ft. cages and are fed a diet consisting of Purina Primate Maintenance Chow (5045), and are treated once a day with fruit, nuts, or vegetables.

The bushbabies will be switched to a more naturalistic and species appropriate diet. This diet has been created based off of recommendations from the Association of Zoos and Aquariums' Bushbaby Species Survival Plan (SSP). The diet consists of a 50/50 balance of insects (mealworms, crickets, superworms, nightcrawlers, and waxworms) and both starchy and leafy vegetables. They will be supplemented with minimal fruit, harboiled eggs, and Mazuri callitrichid and insectivore gel and pellets. All of these foods replicate the natural diet that has been observed being consumed in the wild (wild Otolemur garnetti have also been observed consuming small birds, reptiles, and fish). During this time, both behavioral and hormonal monitoring will occur to ensure that the bushbabies are positively adapting to the changes.

After a one week period of new diet introduction, the bushbabies will be "introduced" to potential cage mates, by placing individual cages next to one another, to allow acclimitization.

After another week, the bushbabies will be introduced into one another's cage (m/f pair housing, with potential m/m housing - it has been noted in zoo populations that males tend to have less agonistic behaviors toward one another than females who are pair or group housed).

Behavioral and hormonal monitoring will continue during this time. In order to assess hormonal correlates, fecal samples will be collected daily, to be later assayed for a variety of stress and health related hormones, including cortisol, DHEA, and testosterone. Moreover, fecal samples will be analyzed to examine nutritional digestibility of fat, fiber, and protein fractions.

Monthly heel sticks will be administered to monitor blood glucose, as many bushbabies in captivity have been shown to have diabetes.

| Animal Disposition (check all that apply): | Disposition of Animal Carcasses: |
|--|--|
| | |
| | |
| External transfer to Non-USM Facility (must be processed by AR) | AR Processed |
| Internal Transfer to Another USM Protocol/AR Holding Colony (must be processed by AR) Released back into the wild Return to owner/client Euthanasia (indicate drug and method): | Other (explain below): Any bushbabies that die of natural causes will first be examined by Dr. Smith and Dr. John Bailey to determine cause of death. Dr. Tom Ricks will always be notified and consulted. Dr. Marie Danforth will then use dermestid beetles to clean the carcass, to allow for a comparative skeletal collection that will be used for teaching various Anthropology |
| | courses. |
| | |
| | |

| Hazardous Materials Summary (check all that apply and fill out any necessary appendices): | | | | | | | | |
|---|--|---|--|--|--|--|--|--|
| | | | | | | | | |
| Non-USDA Restricted Animal Pathogens | USDA Restricted Pathogens (S | See Appendix H) | | | | | | |
| CDC Select Agents (See Appendix H) | Hazardous/Toxic Chemicals (S | See Appendix J) | | | | | | |
| Human Pathogens (See Appendix H) | Mutagens/Carcinoger | ns (See Appendix J) | | | | | | |
| Recombinant DN/RNA (See Appendix H) | Radioactive Materials | s/Isotopes (See Appendix I) | | | | | | |
| Transgenic Animals | Volatile Anesthetic Gasses (Se | ee Appendix J) | | | | | | |
| | | | | | | | | |
| Required Laboratory Biosafety Level: | | | | | | | | |
| | | | | | | | | |
| BSL I BSL II | | | | | | | | |
| BSL III BSL IV (Non-USM facility only) | Note: This refers to the level of biocontainment pr | | | | | | | |
| Animal Biosafety Level: | variety of biological agents (examples: Escherichia disease and dengue fever, BSL III includes West N BSL IV includes smallpox and a variety of hemory | Nile virus and eastern equine encephalitis virus, | | | | | | |
| | have BSL IV or ABSL IV coverage. Contact Lynn determine what level of BSL coverage is available | - | | | | | | |
| BSL I BSL II | | | | | | | | |
| BSL III BSL IV (Non-USM | | | | | | | | |
| facility only) | | | | | | | | |
| Animal Procedures (check all that apply and fill of | out any necessary appendices): | Animal Source (check all that apply | | | | | | |
| Annual Procedures (check an that apply and third | fut any necessary appendices). | and fill out any necessary appendices): | | | | | | |
| | Unalleviated Pain/Distress (USDA | | | | | | | |
| Cat. E studies) | Contum of Wild Animals (Ann. D) | Other Approved Protocol: | | | | | | |
| | Capture of Wild Animals (App. B) Breeding Colony (App. C) | In House Breeding Colony | | | | | | |
| | (App. C) | | | | | | | |
| | Long-Term Restraint (App. D) | Commercial Vendor | | | | | | |
| | Major Survival Surgeries (App. E) | Privately Owned/Client (App. K) | | | | | | |
| | Non-Survival Surgery (App. E) | Private Farm/Ranch | | | | | | |
| Noxious stimuli | Survival Surgery (App. E) | USDA Licensed Dealer | | | | | | |

| Other Non-Surgical Procedures Anesthetic/Analgesic/Tranquilizers/Sedatives | Wild Caught/Trapped (App. B) | | | | | | |
|---|---------------------------------------|--|--|--|--|--|--|
| (App. F) | Other (explain below): | | | | | | |
| Special Diets Paralytics (App. F) | | | | | | | |
| | | | | | | | |
| Water Restriction Antibody/Ascites Production | | | | | | | |
| (Appendix G) | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| List and describe all non-surgical animal procedures/manipulations (e.g., weighing, dos | ing injections) | | | | | | |
| | ing, injections). | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Bushbabies have been found in captivity to suffer from both obesity and diabetes. In ord | | | | | | | |
| bushbabies will be weighed monthly. Additionally, skin pricks will be used monthly for bushbaby has high blood glucose, this monitoring will occur bi-monthly). | blood glucose tests (though if the | | | | | | |
| bushbaby has high blood glucose, this monitoring will occur bi-monthly). | | | | | | | |
| The bushbaby colony at USM has a long history of stereotypy and self-injurious behavior | or (SIB). Often these SIB's will lead | | | | | | |
| to Dr. Tom Ricks needing to prescribe antibiotics, pain medication, or "wrapping" (whe | | | | | | | |
| wounded area). Moreover, the SIB's have led to the need for amputation by Dr. Ricks, the | | | | | | | |
| necessary. | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Describe the restraint method (physical or chemical) that will be used for each of the ab | ove procedures. | | | | | | |
| | F | | | | | | |
| | | | | | | | |
| Restraint is achieved by a catch method, where a research assistant (wearing protective | aloves) will catch the bushbaby | | | | | | |
| around their midsection and hold them, while another research assistant doses or wraps. | | | | | | | |
| and leapers, sometimes a net must be employed to catch them in midair. | | | | | | | |
| and reapers, sometimes a net must be employed to eaten menn in midan. | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Describe the restraint method (physical or chemical) that will be used for blood sample | collection (where applicable). | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Venous blood samples will only ever be taken by Dr. Ricks during surgery and/or a cher | ckup in his office. Glucose | | | | | | |
| monitoring will occur at the bushbaby research facility. Smith has been trained in collec | ting blood spots, and will use a | | | | | | |
| micro-lancet to stick the heels of the bushbabies in order to allow for monthly glucose n | nonitoring. | | | | | | |
| | | | | | | | |
| | | | | | | | |
| Briefly describe what post-mortem procedures (necropsy, histology, etc.) will be perform | med. | | | | | | |
| | | | | | | | |
| | | | | | | | |
| As there is no veterinary school on campus, Drs. Smith and Bailey will perform a necro | nsy. If veterinary intervention is | | | | | | |
| required, Dr. Ricks will be asked to assist. | psy. If veter mary intervention is | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

SECTION 3: RESEARCH Justification

Briefly summarize the scientific literature and/or previous research results, the curriculum/course, and/or the testing standards, regulations, or guidelines that are the basis for this animal use protocol.

There is very little known about wild Otolemur garnetti, as there have been a great deal of taxonomic changes over the years. However, it has been shown in multiple captive species, inlcuding non-human primates that switches to a more naturalistic diet and more naturalistic housing have both reduced stereotypic behaviors, and improved overall health (Clubb and Mason, 2003; Dierenfeld, 1997; Lukas, 1998; Mallpur and Chellam, 2002; Smith, 2008; Smith, 2012).

| List the databases that | DATABASE | DATE CONSULTED | SEARCH TERMS | |
|--|----------------|-------------------|--|--|
| were consulted to search for previous studies in this area, the last date each | JSTOR | 10/13/15 | bushbaby, captivity, captive management | |
| was consulted, and key search terms used (minimum of two databases). | Web of Science | 10/13/15 | bushbaby, Otolemur, captivity | |
| List the databases that | DATABASE | DATE CONSULTED | SEARCH TERMS | |
| were consulted to search for non-animal based alternative methods of | Web of Science | 10/13/15 | captive primate, health, wellfare, noninvasive | |
| research (a minimum of two database are required). | PubMed | 10/13/15 | captive primate, health, wellfare, noninvasive | |
| | | | | |
| List the databases that | DATABASE | DATE CONSULTED | SEARCH TERMS | |
| were consulted to search for alternative methods for | PubMed | 10/13/15 | stress, allostasis, noninvasive | |
| painful/distressful procedures (minimum of two databases required). | Web Of Science | 10/31/15 | stress, allostasis, noninvasive | |
| | | | | |

Briefly describe why each species/strain/stock/breed listed above was chosen for use in this protocol.

The Bushbaby Facility is a preexisting facility here at USM. I have inherited the lab and am looking to improve their health and wellbeing. There is a high rate of stereotypy and SIBs, which need to be remedied immediately.

Briefly describe how the number of animals per experiment/control group was arrived at (i.e. statistical sample size calculation, basis for determining the student: animal ratio etc.).

There are currently 15 bushbabies living in the facility.

Briefly describe the justification for not alleviating pain/distress (required for all USDA Pain Category E procedures).

All efforts to alleviate pain and distress will always be used. Only behavioral and noninvasive biological collection will ever occur.

Briefly describe the justification for using death as the end point of the study.

n/a

SECTION 4: Animal Selection & Housing Details

Complete the following information for all requested animal species. Refer to the USDA categorization pain descriptions at the bottom of this chart if you are uncertain about any animal categorization.

| Criteria | 1 st Species | 2 nd Species | 3 rd Species | 4 th Species |
|---------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Common Name | Garnett's Bush Baby | | | |
| Scientific Name (Genus species) | Otolemur garnetti | | | |
| Strain/Stock/Breed | n/a | | | |
| Age | 1-21 years | | | |
| Weight Range | 600-1000 g | | | |
| Sex | 9.6 | | | |

| Special Requirements | n/a | | |
|-----------------------------------|--------------------------|--|--|
| Number Purchased/Donated | 0 | | |
| Number produced in-House | 15 | | |
| Number from Other Protocols | 15 (holding protocol) | | |
| Number Trapped/Wild Caught | 0 | | |
| Number Obtained by Other Means | 0 | | |
| Total Number of Species | 15 | | |
| Number in USDA Category B | 0 | | |
| Number in USDA Category C | 15 | | |
| Number in USDA Category D | 0 | | |
| Number in USDA Category E | 0 | | |

USDA Pain Category Definitions:

Category B: Animals "bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes." (i.e. no use) Category C: Procedures that cause minimal, transient, and/or no pain/distress when performed by competent persons using recognized methods. (i.e. no pain)

Category D: Procedures that cause more than minimal/transient pain/distress where the pain/distress is alleviated by the use of anesthetics, analgesics, or tranquilizers. (i.e. pain alleviated)

Category E: Procedures that cause more than minimal/transient pain/distress WITHOUT the use of anesthetics, analgesics, or tranquilizers to alleviate the pain/distress. (i.e. unalleviated pain) must be scientifically justified (See 3.5.4).

Animal Facilities: Enter the IACUC approved building and room numbers where animals will be housed as applicable.

| Species | Housing | /Holding | Non-Surgical Procedures | | Survival | Surgery | Non-Survival Surgery | |
|----------------------------|--|-------------------------------------|----------------------------|------------|--------------|---------|-------------------------|---------|
| | Buildin g | Room(s) | Buildin g | Room(s | Buildin g | Room(s) | Building | Room(s) |
| Otolemur garnetti | Bushbab y Researc h Facility | 101, 102, 103,104, 105,106 | | | | | | |
| | | | | | | | | |
| Name(s) of Preferred Anima | l Sources (lea | ave blank if | not applic | able or no | preference |) | | |
| Species | Preferre | d Source | USDA License No. | | Address | | Pho | one |
| | | | | | | | | |

| The remainder of this section should be filled out only for protocols involving non-aquatic animals. | | | | | | | | | |
|---|------------------|--------------|-------------|-----------|------------------|-------------|-----------------------|--|--|
| Cage Type: | | | | Тур | pe of Bec | lding: | Co-habitation: | | |
| | | | | | | | | | |
| Aseptic Microisolator Indoor rur | /pen/stall | Shoebox | | \square | Contact | | Group housed | | |
| Metabolism Microisola | ator 🔀 | Wire Botto | om | \square | Non-con | tact | Individually | | |
| Outdoor run/pen E (explain below): | ird Housing | | Other | | None | | housed | | |
| | | | | | | | | | |
| | | | | | | | | | |
| Feed Preparations (check all that apply): | | | | | Feeding | Procedures | 3: | | |
| | | | | | | | | | |
| | rradiated | Medicated | /Treat | | Ad li | oidum | | | |
| Purified/Chemically Defined | emi-purified 🔀 | Standard (| Commorcial | | Contr | olled feedi | ng regimen | | |
| Diet | | | Johnnereiar | | Food restriction | | | | |
| | | | | | | | | | |
| Water Provision: | Water Compo | osition: | | | | Water Re | estrictions: | | |
| | | | | | | | | | |
| Automatic Provision | Acidified | | Autoc | lave | d | Ad lib | oidum | | |
| Bowl/tank/trough | Medicated | l/Treated | Munic | cipal | Тар | Contro | olled watering regime | | |
| Water bottle | Water bot | tle | R/O | | | Water | restriction | | |
| | | ell/Pond/etc | | | | | | | |
| Describe any non-standard environmenta | | | | noise | or light | ing require | ements) | | |
| | - Faraniecers (K | | , i | | , or ingit | | | | |
| As all bushbaby species are nocturnal, they require a reverse lighting schedule. Humidity and temperature must be kept steady, as even minor fluctuations cause stress and distress to the animals. | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

| SECTION | 5: | Checklist and A | Attachments |
|---------|----|-----------------|-------------|
| | | | |

The following documents must be attached to this form:

CITI Common Course Certificate

CITI IACUC Certificate

List of all references cited in this study and the basis for scientific research

The following documents must be attached if applicable:

Letter from dissertation or thesis committee indicating approval of research proposal

Permission letter from external organization participating in the project (if applicable) on official letterhead

Appendix A – Protocol Flow Sheet/Experimental Design Table/Course Syllabus/Testing SOP

Appendix B – Trapping/Capturing of Wild Animals

Appendix C – In-house Breeding Colony

Appendix D – Long-term Restraint

Appendix E – Surgery

Appendix F – Anesthesia/Analgesia

Appendix G – Antibody Production

Appendix H – Biological Hazards Summary

Appendix I – Radiation Hazards Summary

Appendix J – Chemical Hazards Summary

Appendix K – Owner informed Consent Form

Appendix L – Other

Appendix M – Aquaculture

Instructions for Attaching Documents:

1) Place the cursor where you want the attachment to appear.

- 2) Select the "Insert" tab at the top of MS Word.
- 3) Select "Object," located on the far right of the tool bar (PC) or the bottom of the list (MAC)
- 4) Select the "Create from File" tab and <u>check the box that states "Display as Icon."</u>
- 5) Browse to the location of your document, and double click on it.
- 6) Repeat these steps for each document to be attached.

Note for Mac Users: Word for MAC is unable to attach .pdf files, so you will have to first save the CITI certificates or any other .pdf files you intend to attach as a .doc or .rtf file before attaching them. There are several ways to accomplish this. You may use Adobe to open the file and then select "File" and "Save as" and change the file type to an .rtf or .doc format. Alternatively, you may also download or create your own .pdf to .doc application.

Attach all relevant documents in this section:

RA List for IACUC.pdf

References.docx

CITI\citiCompletionReport5147293.pdf

CITI\citiCompletionReport5147293 (2).pdf