ABSTRACT

WATTS, DAVID QUENTIN. Vocalizations of Aye-Ayes (*Daubentonia madagascariensis*) Made in the Presence of a Novel Object or Human. (Under the direction of Dr. Lisa M. Paciulli and Dr. John Meitzen).

Animals produce a variety of vocalizations to communicate their state of being. Common vocalizations indicate fear, contentment, and readiness to mate. In this study, the vocalizations of five captive Duke University Lemur Center (DLC) aye-ayes (*Daubentonia madagascariensis*) were examined as a novel object or human was placed in their enclosure for five minutes. The aye-ayes were housed singly in one of two enclosure sizes (one 2.08 meters taller than the other), and there were two phases of the study. In phase I, the aye-ayes were presented with a novel object, a stainless-steel box wrench, for five minutes. In the second phase, a novel human was presented for five minutes. The vocal responses of the aye-ayes were recorded with a Sennheiser ME66 microphone, and the sound and resulting spectrograms were examined *via* the program Adobe Audition. The aye-ayes made significantly more vocalizations during the human trials than they did during the baseline trials ($p = 0.0364$), and the number of vocalizations appears to be related to the shorter enclosure height and decreased distance between the aye-ayes and the novel human. In addition, "huffs" were made significantly more than any other vocalization ($p = 0.0010$). These results demonstrate that humans were of more interest or concern to the aye-ayes than objects. In addition, since huffs are emitted by aye-ayes when they are agitated (Stanger & Macedonia 1994), the results indicate that the aye-ayes were agitated when the new person was in their enclosure. However, huffs also seem to be similar to a dog clearing their nose (pers. obs.), which then allows for more fresh scents to be breathed-in (Steen *et al*. 1996). Thus, huffs may be a sound made when investigating the environment rather than a vocalization. In the future, taller enclosures are recommended for aye-ayes, as well as additional research exploring how different people and objects affect the aye-ayes. Such information can better inform captive animal enrichment programs, husbandry, and the health of the endangered aye-aye.

Vocalizations of Aye-Ayes (*Daubentonia madagascariensis*) Made in the Presence of a Novel Object or Human

by
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BIOGRAPHY

After many years in another career, I decided to return to school and change my career path to the natural sciences. Following many years of additional education, I am now poised to receive my Master's degree in Biology.

ACKNOWLEDGEMENTS

I would like to thank the Department of Biological Sciences, the College of Sciences, and NC State University for giving me the opportunity to obtain an advanced degree in biology and to perform research that will hopefully lead to a better understanding of the life of an endangered and little known species. Also, I would like to express my gratitude to my main advisor, Dr. Lisa M. Paciulli, for supporting me throughout my undergraduate and Master's degrees, and for reading and editing previous versions of this thesis. Thank you also to Drs. Lisa Paciulli, John Meitzen, and Miles Engell, for providing guidance and teaching me skills that I will use throughout my future career. Many, many thanks to the Duke University Lemur Center, especially Dr. Erin Ehmke and the research team, without who the study could not have happened. In addition, without the help of the Undergraduate Research Assistants Colin Hadley, Lindsey Gentry, Kaitlyn Tiffany, Anna Miller, Elaina Savage, Madison Ketchie, Cooper Lamb, Anna Pannick, Caroline Zuber, Andrea Anguisaca, Taeim Kwon, Jackson Helms, Isaiah Byrd, and Kun Lee who helped code the audio recordings, I may still be coding the files myself. Finally, a special thanks to the endangered aye-aye for the years of pleasure they have given me observing, studying, and getting to know them better. May their numbers increase in both captivity and the wild.

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INTRODUCTION

Natural selection favors traits and behaviors that enable proper responses to stimuli and the environment. "Proper responses" are those that increase the life expectancy of an individual, while "improper responses" would be those that decrease life expectancy (Proctor & Vu 2006). An improper response to a stimulus, such as a predator, could mean the death of the individual, while not responding correctly to a food cue could mean hunger or starvation. The reverse is also true - exhibiting proper anti-predator behavior could mean surviving another day and responding correctly to a food cue leads to satiation and energy. In many species, anti-predator behaviors and food cues are learned both from individual experiences and the population's predation history (Brown & Warburton 1997).

How an individual should respond to an unfamiliar stimulus is less clear. There may not be a behavioral repertoire to fall back upon considering that the stimulus was previously unknown (Meagher *et al*. 2015). This may explain why the way individuals act in the presence of a novel object varies between species, within species, and among individuals. Approaching something new could lead to a negative result (e.g., sickness, death) or it could lead to a positive result (finding a new food source) (Powell *et al*. 2004). Correspondingly, when a new object or human is in the environment for the first time, some organisms exhibit fearful behaviors, while others exhibit curious behavior patterns.

Acting fearful is a state of responding defensively to, or with avoidance of, the unexpected (King *et al*. 2003). For instance, upon seeing a novel umbrella with blue and white triangular patterns, horses (*Equus ferus caballus*) exhibited several signs of fear including snorting, snuffing, and avoidance (Leiner & Fendt 2011). Orange-winged Amazon parrots (*Amazona amazonica*) took longer to approach, and spent very little time near, novel objects such as stuffed chickens or a miniature palm tree (Meehan & Mensch 2002). Likewise, dogs (*Canis familiaris*) exhibited fear when presented with a novel remote-controlled moving toy car (Ley *et al*. 2007). The dogs took longer to approach the device and spent less time near it, unless they were given a selective serotonin reuptake inhibitor (SSRI). The SSRI maintains serotonin (a neurotransmitter associated with anxiety and stress) in the brain's synapses longer than usual, so the dogs were less stressed around the new toy car (Ley *et al*. 2007). These studies show that a high level of fear often causes anxiety and leads the animal to avoid the novel object or human

(Englerova *et al*. 2019). Exhibiting fear in response to novel stimuli protects the animal from potentially harmful situations (King *et* al. 2003).

One reason animals respond adversely to novel situations is prior negative experience. Verdolin & Harper (2013) found that mouse lemurs (*Microcebus murinus*) who were handled more frequently by keepers were more anxious and tended not to explore or exhibit curiosity in the presence of novel objects than those who were handled less often by keepers. The subjects who were handled less also tended to explore novel objects such as miniature wooden chairs, plastic keys, and a stuffed toy frog more than the subjects who were handled more often. Likewise, Rushen *et al*. (1999) reported that aversive handling of domestic pigs (*Sus scrofa*) by one person caused an increase in blood cortisol (a stress hormone) concentrations when the pigs were in the presence of other, non-handling humans. The increase in cortisol potentially led to a "fight-or-flight" response in the pigs, who stopped exploring their environment or foraging for food. Crane and Ferrari (2017) found that damselfish (*Pomacentrus chrysurus*) that were neophobic, or fearful of new things, foraged less in a predator-free environment than did their non-neophobic counterparts. Thus, responding fearfully to non-threatening situations could affect stress levels and food intake, both of which can negatively affect health (Frazao 1999, Macgeorge *et al*. 2005).

Tightly intertwined with fear is feeling curious. Curiosity is a desire to know something or an interest leading to inquiry (Byrne 2013). A low-level of fear can facilitate curiosity (Englerova *et al*. 2019). Curiosity seems to be a trait selected for more in generalist species (Byrne 2013). Generalists are animals that do not require specific food types or environmental conditions because they can feed on many items and live in different habitats (Dennis *et al*. 2011). Since the survival of a generalist can be aided by many different types of resources, knowledge of the location of resources and their conditions at specific times is of the utmost importance to the animal. This strategy may make a generalist more inclined to be curious than fearful when coming across a new stimulus. For example, when a generalist like the white rat (*Rattus norvegicus*) was placed in a maze containing food and water in a fully satiated state, the rat still explored its environment and features of it. Later, when the rat was placed in the maze again while hungry or thirsty, it returned to the previously discovered locations of food and/or water (Byrne 1993). Rhesus macaques (*Macaca mulatta*) are also generalists, and eat a varied diet of fruits, insects, flowers, and soil (Hauser & Marler 1993). Rhesus macaques are curious and exploratory when presented with novel baby or dog toys (Englerova *et al*. 2019). Likewise, when generalist ring-tailed lemurs (*Lemur catta*) were presented with a 1900 cm^3 fluorescent orange stationary object or a similarly sized black and white moving object, the lemurs readily approached and investigated the objects (Hall *et al*. 2018). The rapid approach of the ring-tailed lemurs to the objects lends support to the idea that curiosity of one's environment is a key survival strategy of generalist animals (cf. Polizzi di Sorrentino *et al*. 2014).

When fearful of novel stimuli, animals often exhibit behaviors such as freeze/stop movements (King *et al*. 2003), becoming muscularly tense (Leiner & Fendt 2011), fleeing (Leiner & Fendt 2011), or attempting to intimidate the fear-inducing stimulus (McLennan & Hill 2010). Both fear and curiosity can also be exhibited through vocalizations. For instance, dogs "bark" when startled by an unexpected sound (Lord *et al*. 2009), as will threatened coyotes (*C. latrans*) (Lehner 1978). Similarly, dogs, coyotes, and other canids howl, a locating call, when separated from group members (Lehner 1978). Asian small-clawed otters (*Aonyx cinerea*) use two vocalizations when curiously exploring their environment; one vocalization is a locator call and one is an alarm call (Lemasson *et al*. 2014).

Many nonhuman primates (lemurs, monkeys, and apes) have fear vocalizations. Silky sifakas (*Propithecus candidus*) produce a "zzuss" vocalization most likely as a response to a predator (Patel *et al*. 2012). Black fronted titi monkeys (*Callicebus nigrifrons*) have three distinct calls given in specific order to relay descriptions to group members about the type and location of predators in their environment (Casar *et al* 2013). Vervet monkeys (*Chlorocebus pygerythrus*) emit four different alarm calls depending on the type of predator encountered (Seyfarth *et al*. 1980). For example, a "bark" is given when a leopard (*Panthera spp*.) is spotted, while a "rraup" call is sounded when a Martial eagle (*Polemaetus bellicosus*) is detected (Seyfarth *et al*. 1980). In addition, unhabituated chimpanzees (*Pan troglodytes*) "roar pant-hoot" when startled by researchers (McLennan & Hill 2010).

Similarly, curiosity and puzzlement can elicit specific vocalizations when an animal is exploring an environment or puzzled by an object new to a particular location. Cotton-top tamarins (*Saguinus oedipus oedipus*) utter a type of chirp when they relax and explore their environment (Cleveland & Snowdon 1982). Goodall (1986) described Gombe chimpanzee (*Pan troglodytes*) puzzlement vocalizations as a "huu" sound. Once alerted to the unique object by the "huu," other chimps also emitted "huu" sounds while staring at the object.

While there have been extensive studies on the vocalizations of non-human animals and their meanings (cf. Ey *et al*. 2007), little has been published on the vocal responses of animals to novel objects or humans. Even less is known about the vocalizations of prosimians in these settings. Aye-ayes are endangered nocturnal insectivorous Strepsirrhines (Randimbiharinirina *et al.* 2018) belonging to the family Daubentoniidae (Gray 1863). They have several distinguishing features including long thin middle fingers on each hand, large ears, a bushy tail, and ever-growing incisors (Gould & Sauther 2006). Aye-ayes utilize their long thin middle finger to tap-forage for beetle larvae, their prey of choice, inside tree branches (Erickson 1994). This study aims to contribute to this gap in knowledge by examining the vocalizations of a prosimian primate, the aye-aye (*Daubentonia madagascariensis*). Here we examined the vocalizations of the aye-aye when a novel object or human enters their enclosure for five minutes. Specifically, the research questions were: Do aye-ayes vocalize more when presented with a novel object or a novel human, and which vocalizations will they make in response to each? It was hypothesized that the aye-ayes would vocalize more when presented with a novel object than a novel human because they see humans (e.g., their caretakers) every day. It also was hypothesized that the aye-ayes would produce a disturbance-fear vocalization referred to as "drumming" when the novel object was present.

METHODS

Subjects:

The study was conducted at the Duke Lemur Center (DLC) in Durham, N.C. The DLC had nine aye-ayes (*Daubentonia madagascariensis*), and five were housed alone. Because it would have been difficult to differentiate between vocalizations made by more than one individual in the same enclosure, these five aye-ayes in separate enclosures were selected for study. The demographics of the subjects are listed in Table 1.

Table 1: Demographics of the aye-ayes in the study.

Subject	Sex	Provenance	Age	Enclosure Type
Grendel	Male	Captivity	6	Tall
Lucrezia	Female	Captivity	15	Short
Medusa	Female	Captivity	14	Tall
Nosferatu	Male	Wild	32	Short
Poe	Male	Wild	31	Tall

Procedures:

At the DLC, the aye-ayes are on a reverse light cycle, meaning that white lights in the ceilings of the enclosures are on from approximately 11:00 p.m. to 11:00 a.m., and the room is dark from approximately 11:00 a.m. to 11:00 p.m. All trials were conducted between 11:30 a.m. and 5:00 p.m. Permission to conduct this study was granted by Duke University's Institutional Animal Care and Use Committee (IACUC) Protocol number A202-16-09.

Data collection occurred in two phases. The procedure for both phases included a baseline audio-recording of the aye-ayes for five minutes before an object or human was introduced. Next was the "experimental" period, when a novel object or person was placed in the enclosure for five minutes, and the aye-ayes were audio-recorded again.

During phase I, to elicit vocalizations from the aye-ayes, a novel object, a Sears Craftsman stainless steel box wrench (Figure 1), was placed in the enclosure for five minutes. During phase II, a novel person, a DLC volunteer whom the aye-ayes had never seen, was in the enclosure for five minutes. Each phase of the study was repeated three times for each aye-aye. In order to limit potential stress on the aye-ayes due to having novel objects and/or people in their enclosures, one trial was conducted on each individual per month. There were six trials per aye-aye for a total of thirty trials overall.

Figure 1: Example of a box wrench. *Photo courtesy Sears, Inc.*

Following the methods of Kessler *et al*. (2015), a Tascam DR-680MKII Portable Multichannel Recorder and a Sennheiser ME66 condenser microphone were placed through a hole in the center of the ceiling in the aye-ayes' enclosures and turned on during the trials. The aye-ayes were housed in one of two types of indoor enclosures. The first enclosure type consisted of cinder block walls arranged in a hexagonal form. The maximum floor space measured 17.5 feet (5.3 meters) long by 15.2 feet (4.6 meters) wide. This enclosure was 16.2 feet (4.9 meters) tall with a volume of approximately 3,230 cubic feet and will be referred to here as the "tall" enclosure. The other type of enclosure also consisted of cinder block walls, but they were arranged in a rectangular shape measuring 20.2 feet (6.2 meters) by 14.7 feet (4.5 meters). The distance from the floor to the ceiling in the second enclosure type was 9.4 feet (2.9 meters) with a volume of 2,791 cubic feet, and this enclosure type will be called "short."

Audio-recordings of the two five-minute segments (baseline and experimental) from each trial were imported into Adobe Audition audio software (Adobe Software 2019). The resulting audio and spectrograms were used by a team of fifteen undergraduate research assistants (URA) to identify vocalization types and durations. The vocalizations were coded as one of four main types that adult aye-ayes make: aack, eeep, drum, and huff (Stanger & Macedonia 1994). Aacks are emitted by female aye-ayes when they are in estrus and have a peak frequency of ~10 kHz with an average duration of 0.5 seconds (Figure 2). Eeep vocalizations are used as a locator to other aye-ayes and have a peak frequency of ~15 kHz and last approximately one second (Stanger & Macedonia 1994) (Figure 3). Aye-ayes drum when disturbed (Stanger & Macedonia 1994). On a spectrogram, a drum vocalization is seen as a pattern of two peaks occurring back to back over a varied duration (Figure 4). The two peaks have an average duration of 0.5 seconds together with the first frequency peak being lower than the second. Finally, huff vocalizations mean that an aye-aye is agitated (Stanger & Macedonia 1994). Huffs have an average peak frequency of 20 kHz with an approximate duration of 0.5 seconds (Stanger & Macedonia 1994) (Figure 5).

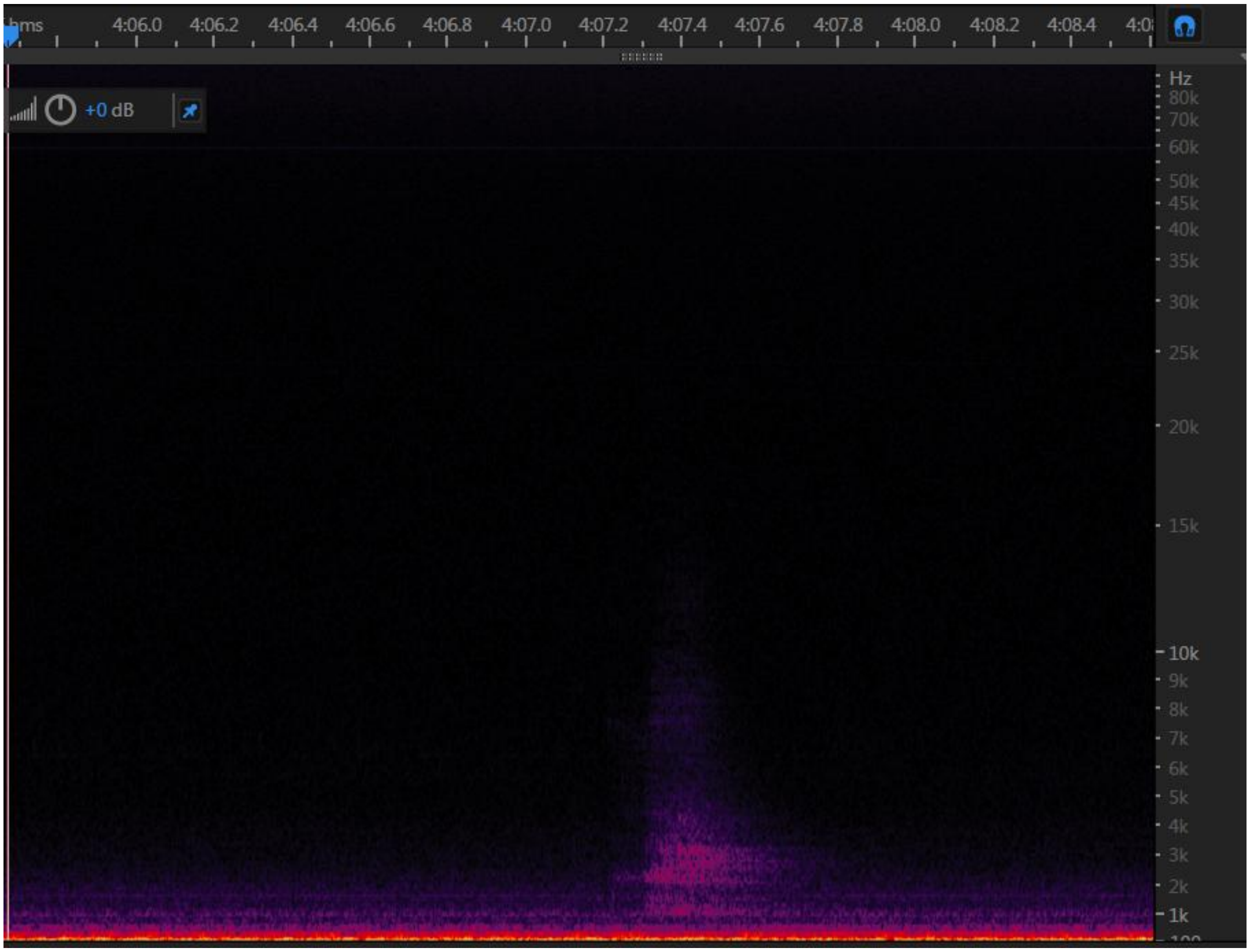

Figure 2: Sonogram of an aye-aye "aack" vocalization.

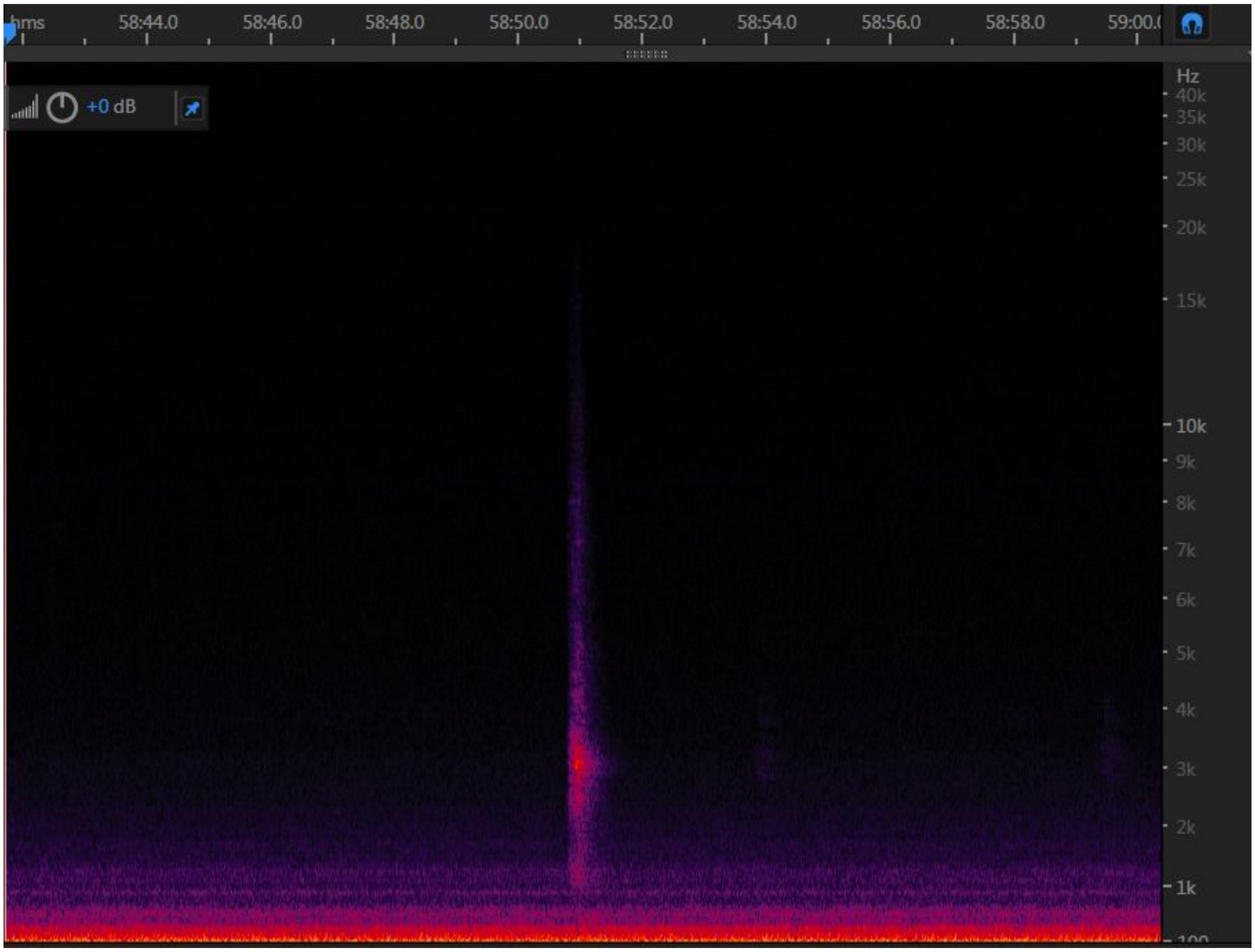

Figure 3: Sonogram of an aye-aye "eeep" vocalization.

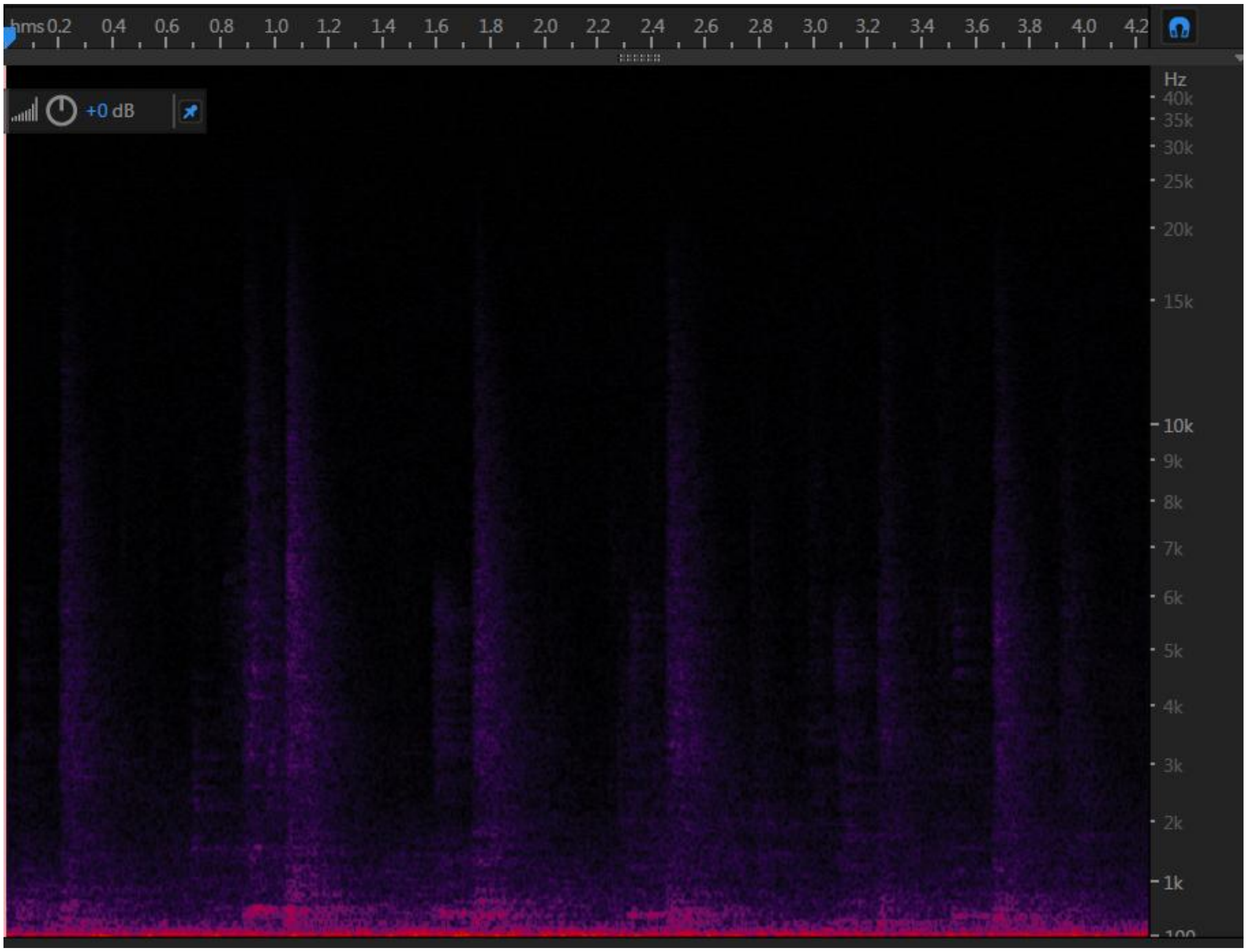

Figure 4: Sonogram of an aye-aye “drum” vocalization.

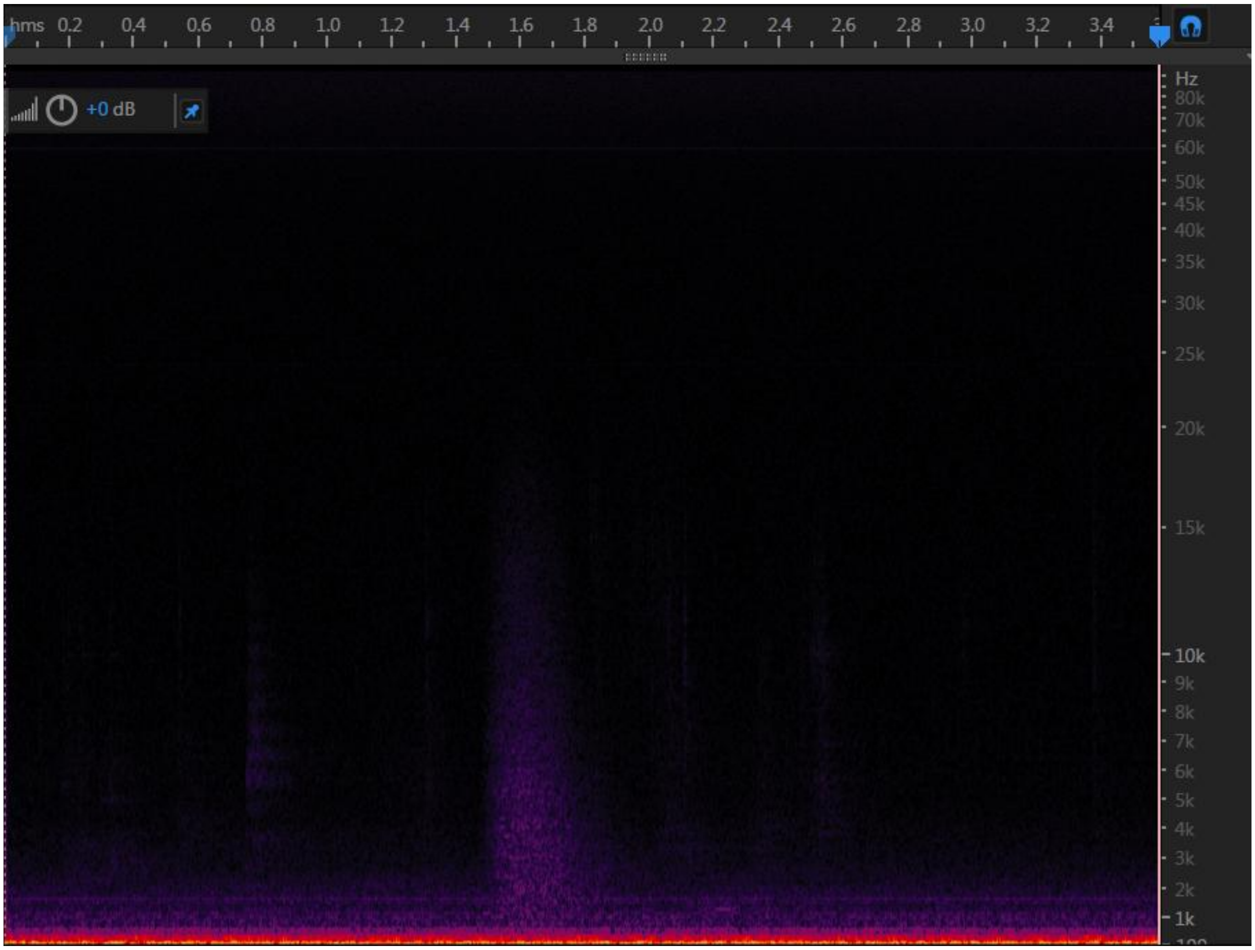

Figure 5: Sonogram of an aye-aye "huff" vocalization.

For each vocalization identified, the following information was entered onto a Google Form: aye-aye's name, trial date, trial type (baseline or experimental, object or human), vocalization type, and the start and end time of the vocalization (in minutes and seconds). Each URA had first to correctly identify aye-aye vocalizations in 12 training audio-files that increased in the number and complexity of vocalizations before coding the audio-files. After, URA's were randomly assigned audio-files to code, and each audio-file was coded by at least two URAs. When the data from two coders differed in either the identification and/or timing of a vocalization, a third person coded the file. At least two coders had to agree upon the identity and the start and end times of a vocalization within 0.5 seconds for the vocalization to be included in the data set. In other words, at least two coders had to agree upon a vocalization's occurrence and timing for it to be counted.

Analyses:

The types and frequencies of vocalizations were tallied, and figures and statistical analyses were done in PRISM GraphPad software (GraphPad Software 2020). Six repeated measures analysis of variances (ANOVA) were carried out because each subject received more than one treatment (baseline and experimental). Four two-way repeated measures ANOVAs (Figures 6 - 9) and two mixed effects models for ANOVAs (Figures 10 and 11) were conducted. The two factors in the repeated measure analyses were: trial number vs. novel object or human, baseline vs. experimental trial, vocalization type vs. object or human, tall or short enclosure vs. object or human. The two factors in the mixed effects model analyses included wild vs. captive birth location and male vs. female sex.

RESULTS

The aye-ayes vocalized a total of 88 times during the study, 29 times during the baseline period and 59 times during the experimental period. Significantly more vocalizations were made during the experimental period than the baseline period during the presentation of the novel human ($p = 0.0212$). Each aye-aye vocalized between zero to seven times during the novel object trials and up to nine times during the novel human trials. To determine if the aye-ayes vocalized more or less during any of the three presentations of the object or person, the number of vocalizations during the first, second, and third trials for each aye-aye were compared. There were no statistically significant differences in the number of vocalizations made among trial numbers for either experimental phase (object: $p = 0.6803$, human: $p = 0.3039$) (Figure 6). Thus, data from the three novel object trials for each aye-aye were pooled for subsequent analyses, and the same was done for the three human trials per subject.

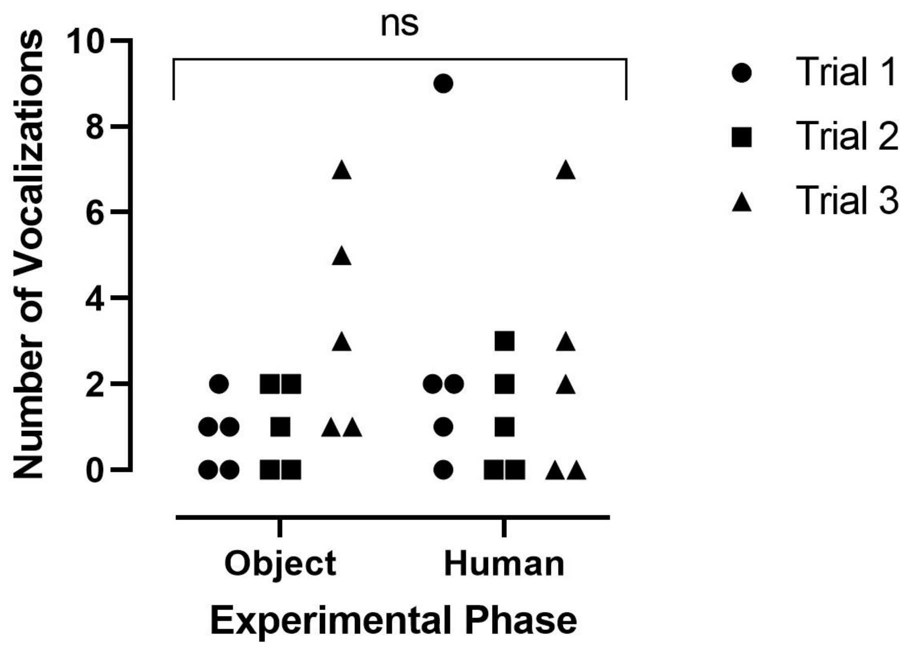

Figure 6: The number of vocalizations produced by each aye-aye during each novel object trial and each human trial. There were no statistically significant differences in the number of vocalizations made among trial numbers for either experimental phase (object: p = 0.6803, human: p = 0.3039).

Two of the aye-ayes vocalized more during the novel object trials than the baseline trials, while two vocalized fewer times. One individual vocalized the same number of times during the novel object and baseline trials. In contrast, all but one individual vocalized more during the novel human trials, resulting in significantly more vocalizations during the human trials than during the baseline trials (p = 0.0364), (Figure 7).

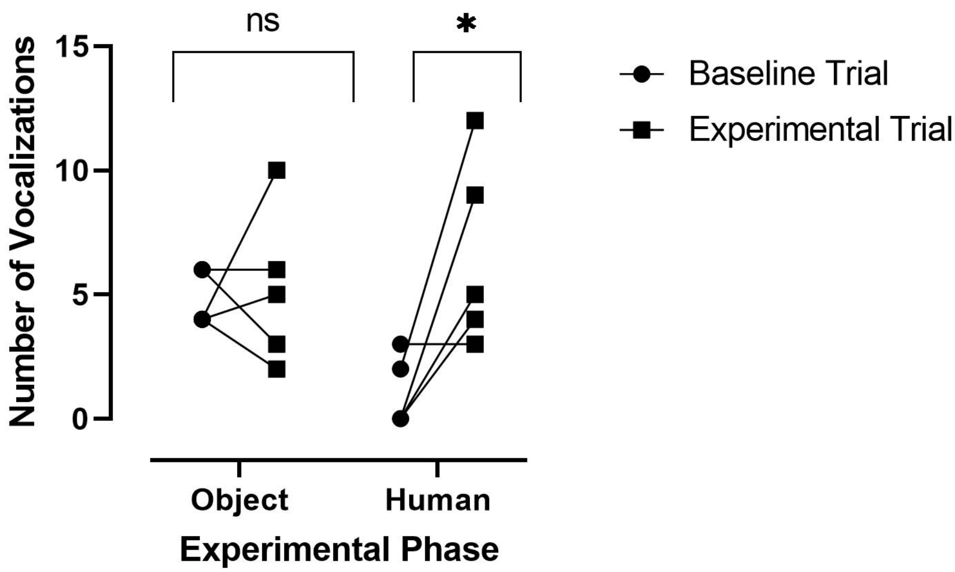

Figure 7: The number of vocalizations made during the baseline and experimental periods. The aye-ayes made significantly more vocalizations during the human trials than they did during the baseline trials (p = 0.0364).

Figure 8 shows that when presented with the novel object, the aye-ayes "huffed" 26 times, and huffed 31 times when the novel human was presented. The only other vocalization produced by the aye-ayes was a drum (two times) when the novel human was in the enclosure. The aye-ayes made significantly more "huffs" than any other vocalization (p = 0.0010).

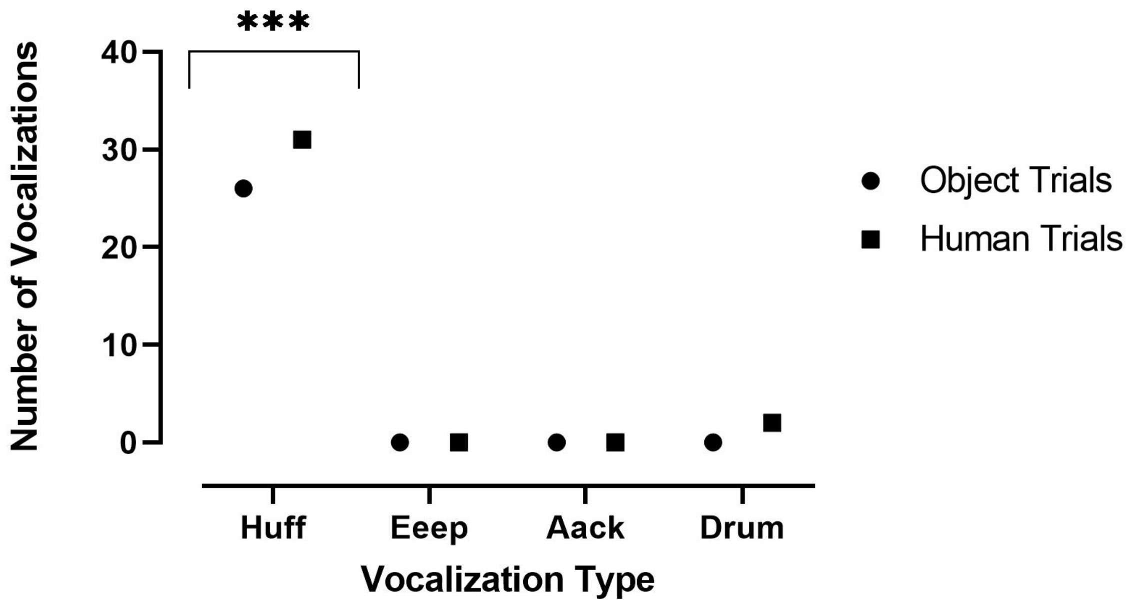

Figure 8: The number of each type of vocalization made by the aye-ayes during the novel object trials and novel human trials. Significantly more "huffs" were made than any other vocalization (p = 0.0010).

In addition, two of the aye-ayes in the taller enclosures vocalized more when presented with the novel object than they did when presented with the novel human. In contrast, the two aye-ayes in the shorter enclosures vocalized more when the novel human was in the enclosure than when the novel object was present (see Figure 9). These results were not significantly different (tall enclosure p = 0.6819, short enclosure p = 0.6819).

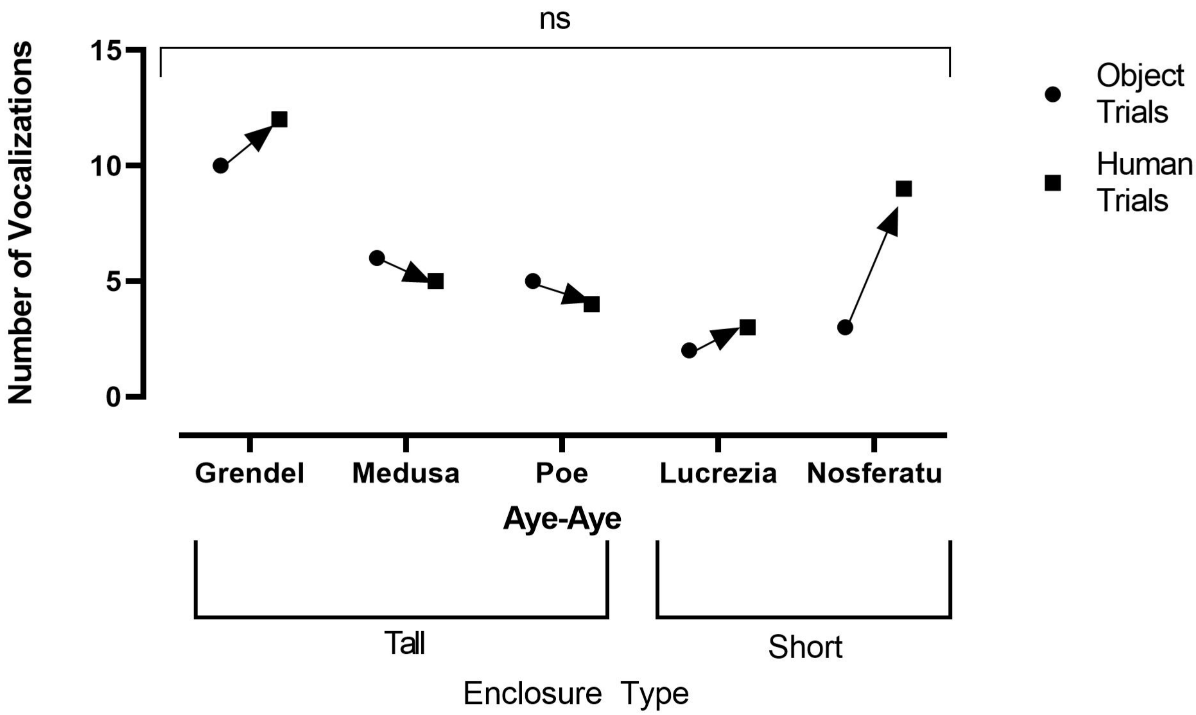

Figure 9: The number of vocalizations produced by each aye-aye in the two different types of enclosures (ns, tall: $p = 0.6819$, short: $p = 0.6819$).

Given this study was conducted in a captive setting with two aye-ayes who were born in the wild, the effect of birth location was examined. Figure 10 demonstrates that there was no statistically significant difference between the number or type of vocalizations made between the captive vs. the wild born aye-ayes ($p = 0.7701$).

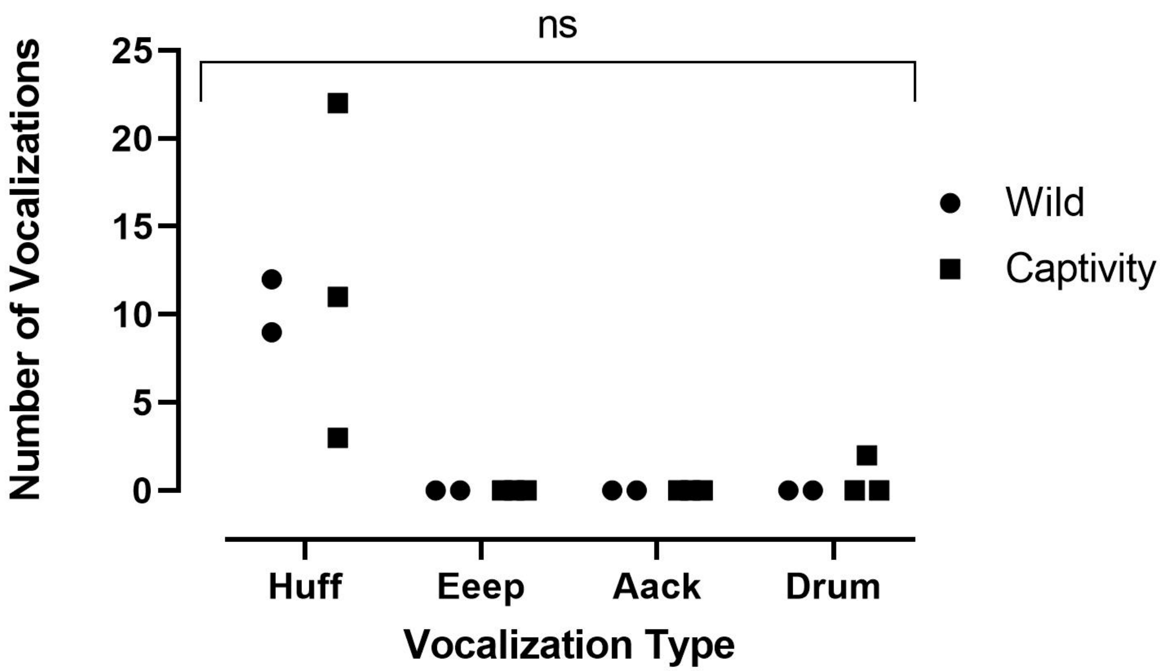

Figure 10: The number and type of vocalizations produced by each aye-aye based on birth provenance. There was no statistically significant difference between the vocalizations made between aye-ayes born in captivity vs. those born in the wild (p = 0.7701).

Furthermore, Figure 11 shows the number of vocalizations produced by the male and female aye-ayes. While not statistically significant, males vocalized three times more than females when the novel human was present (males: 25 vocalizations, females: 8 vocalizations, p = 0.5903).

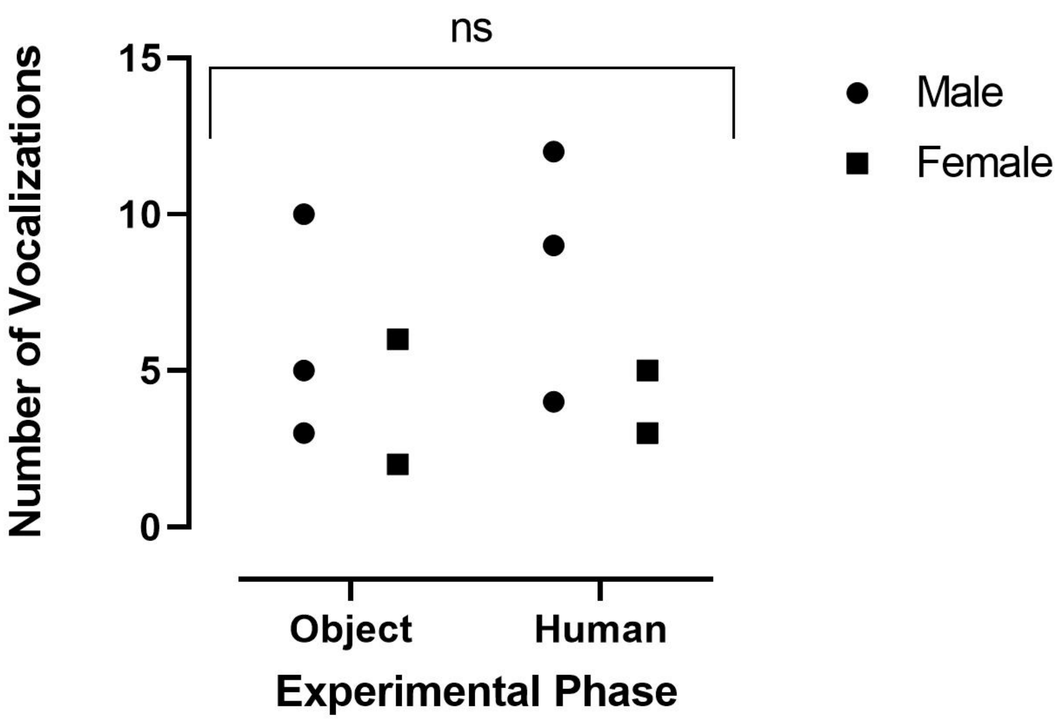

Figure 11: The number of vocalizations made during the experimental phases. Males vocalized three times more than females when the novel human was present, but the results were not statistically significant (p = 0.5903).

DISCUSSION

The hypothesis that aye-ayes would vocalize more when the novel object was in the enclosure than when the novel human was present was not supported. The aye-ayes vocalized seven more times when the novel person was in the enclosure than when the object was there. The aye-ayes may have vocalized less at the novel object since they were aware that the box wrench was inanimate and could neither help nor harm them. Leliveld et al. (2017) found that domestic pigs also vocalized more to a novel human than to a novel object, such as a star-shaped cuddly toy. Kriegeskorte et al. (2008) demonstrated differences in Rhesus macaque responses to photographs of humans and photographs of inanimate novel objects. When an image of a human face was presented, the macaques exhibited greater brain activity than when presented with images of inanimate objects (Kriegeskorte et al. 2008).

At the Duke Lemur Center (DLC), humans assigned to the husbandry unit regularly interact with the aye-ayes, bringing them food and enrichment items, and talking to the aye-ayes throughout the day. It is possible that when the aye-ayes saw the novel person, they were expecting something of interest from them, and thus, vocalized more. Comparably, de Passillé *et al.* (1996) found that Holstein calves initiated contact with a handler with whom they previously had consistent positive encounters and avoided a handler with whom they had had negative encounters. This familiarity with humans could also explain why the aye-ayes made significantly more vocalizations when a human was in the enclosure than they did during the baseline trials when no human was present. The second hypothesis, that the aye-ayes would produce more "drums" than any other vocalization, was also not supported. In fact, the aye-ayes only made two types of vocalizations during any of the trials - two drums and 66 huffs. Aye-ayes drum in response to general disturbances (Stanger & Macedonia 1994), so it is possible that the aye-ayes felt that they were disturbed only twice. More importantly, the aye-ayes huffed frequently during the study. Aye-ayes huff when they are agitated or when they explore new arboreal areas (Stanger & Macedonia 1994). A huff is similar to the sound of a dog "clearing" its nose (pers. obs.). When dogs clear their noses, it allows for additional and fresher scents to be breathed in (Steen *et al*. 1996). When an aye-aye huffs, s/he blows air out of his/her nose. Aye-ayes, like most lemurs, are scent-oriented, and much of the information they receive about their environment is based on the scents they encounter (Price & Feistner 1994). Like dogs, aye-ayes may "huff" to clear their noses and draw in more scent to gather as much information as possible. This would allow them to learn more about an object, person, and/or environment (Stanger & Macedonia 1994). It is likely then that the aye-ayes huffed when the novel human was present to learn more about her. This also calls into question whether huffs are a vocalization or just a sound produced to clear the nose. Vocalizations are scream, plea, whimper, sneeze (drum), snort (huff), and screech (eeep) (Stanger & Macedonia 1994), and the data from this study demonstrate that huffs may not fit this description. Screams, pleas, and whimpers were not observed during this study.

The aye-ayes vocalized twice as many times during the experimental period than they did during the baseline period. This implies that something new, be it an object or person, was a strong enough stimulus to elicit a response from the aye-ayes. A similar result was found when a novel object, a $0.4m^3$ iron green and white striped truncated pyramid, was presented to Holstein Friesian Cattle (*Bos primigenius*). The cattle vocalized more when the object was present than when it was absent (Boissy & Bouissou 1995).

In addition, it could be argued that a novel object is only truly "novel" the first time it is seen. This would mean that after the initial exposure, the novelty of both the object and the human decreased during each successive trial. However, there were no significant differences in the number of vocalizations the aye-ayes made during subsequent trials, which implies that neither the object nor person lost its "novelty" to the aye-ayes. Thus, either enough time had passed in the thirty days between trials to maintain novelty of wrench and human, or regardless of the lack of novelty, the object and human stimuli were strong enough to elicit a similar response in each of the successive trials.. Certain stimuli (e.g., predators, abundant food sources, changes in temperature) are strong enough to elicit responses each time an individual encounters them (Mitchell & Biro 2017). Both new-ish objects and humans may be strong enough stimuli to elicit vocalizations from the aye-ayes.

The effect of enclosure size on aye-aye vocalizations is worth examining. Aye-ayes in the taller enclosures vocalized more when presented with the novel object than they did when presented with the novel human, while the aye-ayes in the shorter enclosures did the opposite, vocalizing more when the novel human was in the enclosure. In the wild, aye-ayes are arboreal and spend most of their time at least ten meters (32.81 feet) up in the trees (Ancrenaz *et al.* 1994). They also avoid lower canopy levels and remain hypervigilant when resting below the ten-meter (~32 feet) level (Ancrenaz *et al*. 1994). Although both of the captive enclosures are higher than or similar to the height of the aye-aye's preferred height of ten meters in the wild, the two meter (6.56 feet) difference in height between the tall and short enclosures may have meant that the aye-ayes in the shorter enclosures felt more threatened and/or that the new person was physically too close to them. This could be why the aye-ayes in the shorter enclosures were investigating potentially threatening unknown humans more vigorously than their counterparts housed in the taller rooms.

Although this study was conducted in a captive environment, two of the aye-ayes were born in their natural habitat of Madagascar. Both the wild and captive born groups made similar types and numbers of vocalizations. One would normally expect wild born individuals to be more wary of humans than their captive born counterparts and to thus vocalize more in their presence. However, this was not the case, and could be due to the length of time the wild born colony members have been in captivity. Nosferatu and Poe both arrived at the DLC in December 1987 (Glander 1994), which means that they had been in captivity for several decades by the time this study was conducted. Most wild caught lemurs take one week to five months to become habituated to their captive environments and keepers (Setchell & Curtis 2011). Thus, thirty years was ample time for the two wild-born aye-ayes to become habituated to humans and no longer feel the need to investigate changes in their enclosures any more than captive born individuals.

Males and females vocalized a similar number of times when the novel object was present. Although there was no significant difference in the total number of vocalizations made by males or females when the human was present, the males vocalized three times more than the females did (males: 25 vocalizations, females: 8 vocalizations). This trifold difference could be related to aye-aye males being more territorial than females (Randimbiharinirina *et al*. 2018). Since males need to protect their territory, they are likely to be more sensitive to disturbances in their environment. Placing a novel human in a male aye-aye's territory (i.e., captive enclosure) could be enough of a threat to cause males to investigate the source of the environmental disturbance more than females would.

<u>Limitations and Future Research</u>:

There were some limitations of the study. First, the sample size of five subjects is small. However, there are only 53 aye-ayes in captivity in the world, with 23 in the United States, and nine of these residing at the Duke University Lemur Center (ZIMS 2020). While the sample size is small, it represents approximately ten percent of the world's captive aye-aye population. Nonetheless, continuing the study with additional aye-ayes would increase reliability of the current study.

Second, sampling aye-ayes all from a single institution could potentially skew the results. Given that the DLC aye-ayes are accustomed to certain interactions and treatment by DLC staff, they may react to a new person differently than aye-ayes at other facilities would. Repeating the study with aye-ayes housed at other facilities would help determine whether the responses vary by institution and/or whether aye-ayes have a species-typical vocal response to a novel object and/or human. The use of only one novel person and one novel object could have limited the type and number of vocalizations emitted as well. For example, would the aye-ayes have reacted differently to a large male or a tiny, fluffy toy in their enclosures? Having a varied demographic background of novel humans wearing similar as well as different clothing, and introducing objects of different shapes, sizes, and types could result in more or fewer vocalizations than those elicited in this study.

Another limitation is that only audio recordings of vocalizations were used to determine the aye-ayes' responses to items placed in their enclosures. One easy way to better understand how aye-ayes react to novel objects and humans would be to also video record the trials. In this way, the aye-ayes' vocal responses could be paired with their behavioral responses. For instance, seeing whether the aye-ayes moved closer or farther away from a person or object would yield important information. Distance between a subject and a novel object has been used as an indicator of how fearful the subject is of the stimulus (e.g., King *et al*. 2003).

An additional limitation is the uncertainty of explanation for the increase in vocalizations made toward the novel human. It is unclear whether the aye-ayes vocalized more because there was a stranger in their enclosure, because that stranger was more odiferous than the steel wrench novel object, if they were more interested and/or excited to see a person because they associate people with bringing items of interest, or if it was a combination of these and other reasons. One way to elucidate which of these interpretations are correct would be to design more studies with various controls in place to test one variable at a time. For instance, recording the aye-ayes' vocalizations when presented with a human-scented wrench or an unscented human (or mannequin) could help determine how aye-ayes respond to different smells and animate vs. inanimate variables differently. Introducing various combinations of objects and humans could clarify some of the ambiguity in the interpretations.

Moreover, a clearer understanding of aye-aye vocalizations in general would help elucidate the function of each different type of aye-aye utterance. For example, knowing whether the "huff" is a communicative vocalization or if it is a sound made when air is cleared from the nose to better inhale scents would help determine the function of this response. Because there have only been three studies on aye-aye vocalizations, Ramsier and Dominy (2012), Stanger and Macedonia (1994), and Winn (1994), the true function and meaning of aye-aye vocalizations are still poorly understood. A better and deeper comprehension of the meanings of aye-aye utterances would allow for improved interpretations of when and why they are emitted. For example, when an aye-aye drums or huffs, how stressed does this indicate the caller is? Such information could be useful not only to analyze the findings of the present study, but it could also be used in an applied sense. Knowing the meaning of specific aye-aye vocalizations could help the husbandry staff of DLC and other institutions to know what causes the aye-ayes stress. Likewise, more detailed information on vocalizations could help provide a better roadmap for understanding aye-aye stressors in wild habitats in Madagascar, and potentially serve to better inform *in-situ* conservation efforts. Moreover, reducing aye-aye stress could potentially increase their reproductive success, as stress usually is negatively correlated with fertility (*cf.* Dobson & Smith 2000).

Next, given that there was a notable, although not significant, difference in the aye-ayes' vocal responses depending on enclosure size, it would be important to examine the effects of different sizes and shapes of housing on the behavior of the aye-ayes. Determining the optimal enclosure design for housing captive aye-ayes is another critical factor for reducing stress and increasing reproductive success (Ross *et al*. 2009). Having healthier aye-ayes would also allow for fewer medical interventions (Smith & Corrow 2005). Fewer medical interventions also results in less stressed animals, as many captive animals such as lemurs usually need to be grabbed, netted, darted, and/or combinations of these for medical attention, which causes excessive stress (Lambeth *et al*. 2006).

Along the lines of creating larger and more ideal enclosures for the aye-ayes, studying the effects of different objects in the aye-ayes' and any lemurs' enclosures could help improve environmental enrichment practices. Environmental enrichment enhances the quality of captive animal care by providing stimuli for psychological and physiological well-being (Shepherdson *et al.* 1998). Belz *et al*. (2003) found that environmental enrichment lowered stress response hormones such as cortisol in captive laboratory rats. By presenting aye-ayes with various and varying objects in their environment, vocal and behavioral responses to the objects could be used to facilitate a better understanding of which objects are favorable and which are not.

Improved enrichment practices result in healthier animals, and the aye-ayes would be no exception (Schapiro *et al*. 1993). Aye-ayes are curious (Haring *et al*. 1994). In the wild, they satisfy their curiosity through natural means by exploring unknown areas and/or encountering new individuals. In captivity however, aye-ayes are usually limited in their ability to fully satisfy their need to explore, as some individuals can spend all or most of their adult lives in one or two enclosures, seeing only one or two other adults (e.g., Endora and Ozma pers. obs.). Various forms of enrichment can introduce more exploration opportunities into a captive animal's environment.

Finally, interpreting the results of this study are challenging as there is a paucity of publications on aye-aye behavior. Given that aye-ayes are rare, nocturnal, and cryptic, they are difficult to habituate and study in the wild (Petter 1977). Also, an often unstable Malagasy political government coupled with uncertain or unenforced conservation policies have likely damaged the aye-aye wild habitat (Schwitzer *et al*. 2014, Waeber *et al*. 2016). These issues also have made it more difficult to increase the captive holdings of aye-ayes, which means that captive births are the only source of captive population growth (Reuter *et al*. 2015). The aye-aye interbirth interval (IBI) is approximately two to three years for this singleton-producing species (Petter 1977). A slow growing captive collection seems to be the best source of aye-aye study subjects which can establish baseline information about their behavior.

<u>Conclusion</u>:

In summary, the aye-ayes vocalized significantly more when the novel person was in their enclosure than when the novel object was present. This suggests that new people are more concerning to the aye-ayes than new objects. Also, all except two of the vocalizations produced were “huffs,” which have been reported in the literature to be emitted when aye-ayes are agitated (Stanger & Macedonia 1994). However, because huffs also seem to be similar to a dog clearing their nose (pers. obs.), which then allows for more and fresher scents to be breathed in (Steen *et al*. 1996), huffs are potentially not a true vocalization, but rather may instead be a sound made as an environmental investigatory technique. Further, the size of the enclosure and distance between the aye-aye and person appeared to affect the number of vocalizations and correspondingly, the stress levels of the aye-ayes. The greater distance between the aye-aye and novel human resulted in fewer vocalizations, indicating that the aye-ayes were less stressed than when they were in enclosures with less distance between themselves and the person. Taller enclosures are thus recommended for aye-ayes, as well as additional research exploring how aye-ayes react to different people and objects. Such information can better inform captive enrichment programs, husbandry, and the health of the endangered and elusive aye-aye.

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