A Thesis Presented to

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The Effects of Crowd Size and Keeper Presence on the Stereotypic Pacing of Mexican Gray Wolves (*Canis lupus baileyi*)

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Forward

The keepers at the Seneca Park Zoo were noticing their three Mexican gray wolves pacing more than usual lately. While this phenomenon isn't uncommon in populations of zoo animals on the whole, patrons were starting to take notice of the constant laps. I noticed it myself on the first day I went down to meet the keepers and my subjects after one of the keepers contacted my advisor and offered an opportunity for behavioral research. Even as I took data in the ensuing June of 2015, I heard so many people saying "oh look! The wolves are running their laps again" or, "there they are running again". I knew pacing wasn't a normal behavior in captive animals, but it wasn't until I completed my literature review that I found out how detrimental it could be.

Stereotypies are purposeless, repetitive, and invariable behaviors done by animals, usually captive ones. Pacing is one of the more obvious examples of stereotypies found in zoos, exhibited frequently by carnivores. Humans also participate in stereotypies, like when we pace talking on the phone, twirl our hair, chew on toothpicks, or drum our fingers absentmindedly. Why are they pacing then? Animal behavior researchers have a few different theories, but almost all will say that these behaviors are because of poor welfare conditions or some sort of adverse stimulus in their environment. Poor welfare can be anything from an enclosure that's too small or barren, repeated stressful events, or simply boredom. Zoos use the presence and frequency of stereotypical behavior as a mental health yardstick, with the most common remedy being the addition environmental enrichment (toys, new scents, puzzle boxes, or novel ways of delivering food) to engage the animal's senses and alleviate boredom. The problem with stereotypies is that if left unchecked, they will start to become routine to the animal, a behavior they are compelled to do, and it becomes difficult to get them to stop so much that enrichment no longer has any

effect. Stereotypies are likened to a scar, a visible reminder of damage past. Chronic stereotypies have physiological effects too, ranging from self-neglect, self-harm, to impaired reproductive success.

The Mexican gray wolf is an endangered subspecies of the gray wolf, native to the Southwest United States and Northern Mexico and once hunted to near-extinction by ranchers. An intensive breeding and reintroduction program has helped grow the wild population from a handful of survivors to around 100 individuals at last count in 2014. U.S. Fish and Wildlife Service controls all Mexican gray wolves in captivity, and any individual can be selected for the breeding program or reintroduction. USFW also set down strict rules governing the care of these captive wolves, most of which aim to limit exposure and familiarization with humans. I find there is an inherent irony with deliberately placing wolves in very public, human-filled zoos, but USFW may very well have their reasons. The problem here is such: if the wolves are pacing often, it could have negative effects on not only their mental and physical well-being, but potentially lower their reproductive success, crucial to a species that needs all the new individuals it can muster.

I took this on initially because it was a research project with all the pieces laid out for me to put together; I didn't have to pull a thesis out of thin air when I was being asked to study the question directly. However, the more I watched them, "my boys" as I affectionately referred to them; this research took on a more noble pursuit. If the data analysis showed the pacing herein was at a problematic level, I could spur some sort of change for the betterment of Chico, Diego, and Durango. Better yet, if pacing was related to either the amount of people in front of the exhibit or the presence of keepers, there's a concrete direction to shape this policy. The more I sat and watched, the more I cared about them, their situation.

The keepers shared this same sentiment and told me there was a rule against behavior modification, or training of the wolves. Most of the zoo animals are trained (using food) to go to a separate area of their enclosure so the keepers can clean the main area. Since the wolves can't be trained, cleaning has to occur with the wolves and keepers in the enclosure together. Extra keepers are taken as "guards" to hold up rakes and shoo away any that come too close. Chico and Diego seemed to be whipped into frenzy and would run in circles in the area the keepers weren't occupying. Durango, ever aloof, would try to approach the keepers, as if curious about their presence. This pacing would continue after the keepers were gone and it took Chico and Diego a while to settle down. This policy seemed obviously stressful to the wolves, which the keepers knew, but lamented that their hands were tied; they could put food in the off exhibit areas and hope the wolves went in, but this didn't work. The ones I spoke to were perplexed by the USFW's policies and limits on human interaction, yet it was USFW that put them in a zoo instead of a sanctuary. With this in mind, I set out to see if not only the pacing was problematic, but if their exposure to crowds or keepers had any effect.

I sat two hours a day, for an entire month, taking behavioral observations every minute on all three. With that amount of time sunk in, you get to know each of the wolves not only by looks, but personality. Chico was the white counterpart to Diego's darker coat, and seemed to act the part of the sentry. Chico was frequently atop the large center log that jutted out like Pride Rock from The Lion King, usually resting and keeping an eye on the crowds below. He would frequently pace at a trot along the perimeter of the enclosure at a brisk pace. Durango was the aloof alpha who mostly slept off exhibit and paced the least, but was the most curious about the keepers coming into the exhibit, approaching them with interest. Diego, the darkest of the three,

stuck close to Chico, but had the most pronounced pacing route as every lap was ended by stepping up on a particular rock and pausing before starting a particular lap.

On a bittersweet note, the wolves were moved out of the zoo in November of 2015. Chico and Durango now reside at the Living Desert Zoo in Southwest California, while Diego has been chosen to be integrated into the breeding program because of his desirable genetics. Now, there are two gray wolves (*Canis lupus*), brother and sister, living in their former exhibit. The data herein are a snapshot of the wolves' tenure at Seneca Park Zoo, though if these new wolves start exhibiting similar behavior patterns, this research may be useful. I would think Chico and Durango would be happier in their new location, considering it's in an area more like their native range on the American Southwest (not like snowy and cold upstate new York), but I'm curious as to how they're doing in their new home. Maybe one day they'll be on the range with the wild brethren, and Diego will be the proud father of a new generation of wolves helping to bolster their numbers.

Now the culmination of almost a whole year's worth of constant, steady work has presented you with this, my finest academic accomplishment to date. Though I was nervous that I wouldn't have the right data, or the ability to analyze it properly, I was guided by the best professors I could ask for, every step of the way. The project was left to be wholly by my own design, but I wasn't far from any advice I needed in creating it. For that, I give my heartfelt thanks to my committee for helping see me through, and answering my constant spur-of the moment questions. I hope this first foray into research proves to be the start of a love for discovering, a desire to grow as a scientist and inquiring mind, and a passion for helping animals in any capacity I can.

Abstract:

Stereotypies are repetitive behavioral patterns that serve no apparent purpose often performed by captive animals in response to poor welfare conditions. In carnivores, the most common stereotypy is pacing. In this study, three Mexican gray wolves (*Canis lupus baileyi*) were observed at the Seneca Park Zoo (Rochester, NY) to evaluate frequencies of pacing by individuals as well as investigate if the crowd size in front of their exhibit or keeper presence had any effect of the frequency of pacing. It was found that two individuals had significantly different rates of pacing/day ($H = 34.56$, $df=2$, $P < 0.005$), over 10% of their total activity budget ($Z = 7.38$, $P < 0.005$). The presence of any amount of people in front of the exhibit significantly increased pacing for all three wolves ($\chi^2 = 48.608$, $df = 8$, $P < 0.005$), but no difference was found between non-zero crowd levels. Keepers were not found to have a significant effect on pacing, and in fact significantly more pacing occurred while keepers were not present. Some suggestions for reducing pacing would be to educate the public on the sensitivity of the wolves to large crowds and large noises, move the wolves to a quieter, less travel area of the zoo, or place them in a private facility designed to house wolves in larger, naturalistic habitats.

Literature Review

Introduction to Stereotypies

Stereotypic behaviors are repetitive actions done by captive animals that serve no apparent function and often become routine for the animal. They are usually normal behaviors or patterns that are performed in an abnormal context, grow increasingly difficult to abolish over time, and may even grow independent of the original stimulus with repetition (Mason 1991). These behaviors are found largely in captive farm, zoo, or domestic animals, leading many to believe that the presence of stereotypies are indicative of poor welfare conditions that fail to meet the standards of their natural environments (Mason 2004).

Stereotypies are prevalent in almost all species, including humans. Perhaps the best example in humans is autism spectrum disorders, in which repetitive, invariable behavior patterns are critical to diagnosis and seem to parallel animal stereotypies (Lewis 2007). Indeed, some believe that chronic stereotypical behavior is an animal equivalent to autism and/or schizophrenia, and recent research shows both disorders and stereotypies share a common mechanism of neurophysiological damage promoting these behaviors (Garner 2003, Martin 2008). While wild animals exhibit stereotypical behaviors to some degree, captive animals exhibit these behaviors more often and frequently, sometimes to concerning extremes (Veasey 1996, Mason 2004).

The definition of what a stereotypical behavior is leaves it wide open for many behaviors to be classified as stereotypies. However, what sorts of stereotypic behaviors manifest is dependent on the species, housing, and life history strategies; even individuals differ in their stereotypical repertoires (Mason 2004, Clubb 2006). In general, it's been observed that

carnivorous, hunting animals engage in more locomotory stereotypies such as pacing, while herbivores display oral stereotypies, such as chewing on their enclosures and mimicking foraging behaviors (Mason 1997). These behaviors can be as simple as head swinging in elephants or cribbing horses (wherein horses will bite a solid object, arch their neck, and suck in air) or as elaborate as backflips in deer mice and particular pacing regiments of carnivorous animals (Cooper 2007, Mason 1991, Lewis 2007, Clubb 2003).

Stereotypy Development

There are some ideas as to how stereotypies arise; most commonly it is thought to come about from a sub-optimal environment. Barren enclosures have been shown to induce stereotypies more often than enriched ones and smaller enclosures more than expansive ones (Ödberg 1987). Restrictions on space and amenities can prompt frustration of natural behaviors and under-stimulation can prompt stereotype development in attempt to cope (Broom 1991, Mason 1991). In zoos, this is often the problem as exhibits may not have the space or resources necessary to replicate a suitable environment for each species' needs, and instead opt to address this problem through various enrichment programs to stave off stereotype development.

Stereotypies in captive and domestic animals may also be due to boredom, since environments rarely change day to day, aside from the weather. In the wild, environments are continuously changing in all sensory modalities due to the actions of other animals, which require engaging in the environment and exploration of these changes all of the time. The lack of new sensory stimulus in captivity takes away this drive to explore, leaving an animal with few options of what to do with its time (Mason 1991). Compounding this is the reliability of being fed and sheltered, which further detracts from the need to be out in the environment searching,

and so an animal may turn to stereotypic behaviors to occupy their time (Broom 1983). This is often the first cause addressed when attempting to eliminate stereotypic behaviors by introducing novel items or changing the enclosure in some fashion on a regular basis.

Recent research suggests that stereotypies may also be controlled by genetic factors (Lewis 2007). Many breeding programs in zoos will not breed otherwise healthy individuals that exhibit alarming rates of stereotypies for fear of offspring inheriting the condition; it is even a requirement in the Netherlands to breed stereotypies out of fur farming animals (Mason 2004). Lewis et al. (2007) have found the deletion of particular genes in mice results in distinct stereotypies as well as exposure to particular drugs correlating to higher instances of stereotypies. Homozygous *Gabrb3* knockout mice will circle or chase their tails for hours, while *Hoxb8* homozygous mutants will engage in excessive grooming at twice the rate of wild type controls and is thought to be related to Obsessive Compulsive Disorder (Lewis 2007). Prenatal exposure to the antiepileptic drug valproic acid results in higher levels of stereotypic behaviors in rats that mirror symptoms of autistic individuals; these behaviors can be effectively reduced with enriched environments (Lewis 2007, Schneider 2006). Most of this research is concerned with using mice to model human disorders and devise effective treatments, but in the future these techniques could be useful for identifying at-risk animals and addressing their deficiencies with psychoactive drugs or environmental improvements.

Further still is the idea that stereotypies can be a result of abnormal brain function or damage. An increasing body of work suggests that autistic and schizophrenic stereotypies in humans are similar in their neurophysiological causes as animal stereotypies. This is supported by the fact that particular psychoactive drugs can totally abolish or even induce stereotypies in animals; these drugs can be used as a last resort treatment for particularly ingrained and

problematic stereotypies (Mason 1991, Lewis 2007). Human mental patients and primates with similar brain damage share similar stereotypical behaviors like body rocking (Mason 1991). These stereotypies are often irreversible due to the damage and can persist despite efforts to correct the actions (Mason 1991).

It's evident in trying to explain why stereotypies form that the question becomes more difficult to answer. In all, expressions of stereotypical behaviors vary between species and individuals and there could be a number of motivating factors behind why an individual engages in these behaviors, making this a difficult area of study to form absolutes and laws of behavior (Mason 2004, Clubb 2006). If changing the environment or enrichment doesn't have a significant effect, it may be a lack of sufficient space. Beyond that, if attempts to correct the situation fail, the stereotypies may be the result of brain damage, faulty genetics, or prenatal developmental problems.

The Effects of Stereotypies

Detrimental stereotypies are often linked to high cortisol levels, physical damage such as foot abrasions, self-neglect or self-harm in extreme cases, and may even affect the development of offspring (Clubb 2006, Mason 1991).These behaviors can persist even when housed in better conditions, if prior mistreatment was extreme enough to leave a permanent mark on an animal's well-being (Mason 1991). Many believe stereotypies are a sure sign of suffering and are a non-verbal way for animals to communicate their distress to those who would notice it (Mason 1991, broom 1993). With such potential for harm, it's easy to see why stereotypies are viewed with such concern from caretakers and used for criticism by activists.

Whether or not these behaviors are completely detrimental remains to be seen; some have recently argued that these behaviors may help animals relieve stress by giving then an outlet to their energies as a positive coping mechanism to reduce stress in poor-welfare situations (Mason 2004). These behaviors may be used as "do-it-yourself enrichment" as a way to express natural behaviors where the correct stimulus or substrate may not be present, or to create variety in an otherwise predictable environment (Mason 2004, Broom 1983). Another thought is that through repetition, the behavior may be soothing by being rhythmic and predictable, or used in a way to create order in a disordered environment and gain some sense of control (Broom 1983). Often in these cases, corticosteroid levels decrease during stereotypic activities, rather than increase (Mason 1991). Still, while the action itself may not be detrimental, its performance speaks of something lacking in the environment that should be addressed.

Mason (1991 2004), states that stereotypies may not be exclusively performed in poor welfare conditions, but may actually occur with good welfare conditions. Indeed, some stereotypies are benign and harmless like thumb sucking in humans, and the author's curious predisposition to weave her thumbs repeatedly in and out of knit fabrics for no discernably good reason (Mason 1991). This can be seen in animals that are moved from barren enclosures to enriched ones; these animals actually exhibited more stereotypical behaviors or maintained previous levels (Mason 1991). However, the impact of stereotypies is largely dependent on individual context; what may be stress-reducing for one animal may be stress-inducing in another (Mason 1991).

Use of Stereotypies as a Welfare Indicator

In captivity, levels of stereotypic behaviors exhibited are often used as an indicator of an animal's mental well-being and reflective of the conditions of its environment, an emotional yardstick if you will (Mason 1991, 2004). Since stereotypies arise more often from adverse conditions than acceptable ones, incidences are regarded as a sign that something is wrong with the animal's environment. What level of stereotypic behavior is acceptable, however, is up for debate. Broom (1991) gives a threshold of 10% of an animal's total time spent in stereotypic behavior, while Wiepkema (1983) says conditions should be changed if more than 5% of a population exhibits these behaviors, and Broom and Johnson (1993) say there is no acceptable level of stereotypical behavior and its incidence must be addressed immediately. Again, while some generalities can be drawn, not all stereotypies are negative, and should be evaluated on an individual level before conclusions are drawn.

Correcting Stereotypies

Most often enrichment is used to combat stereotypies, as it is more feasible than time-intensive behavioral correction and more cost-effective for zoos. Enrichment can be novel ways of presenting food items, such as in puzzle boxes or frozen, introducing novel scents such as perfumes, spices, or urine/feces from novel animals, introducing new toys, or simply rearranging the animal's enclosure to encourage exploration. All these ideas attempt to introduce something new to the animal's environment in an attempt to stimulate the senses and engage the mind (Carlstead 2000). These techniques have found some measure of success as they have been shown to reduce cortisol levels, improve immune function, and ultimately decrease stereotypical behaviors (Carlstead 2000). While enrichment may have immediate effects on improving an

animal's well-being; results typically don't last long, requiring more and varied forms of enrichment (Mason 2007, Skibiel 2007). Considering all of the possible positive effects of enrichment compared to the deleterious effects of stereotypies, enrichment should still be considered an option to manage stereotypies. Mason (2007) suggests that when current enrichment techniques are missing their mark, the stereotype to be corrected should be evaluated before an enrichment schedule is selected for optimal effectiveness.

Additional correction of these stereotypies can be achieved by punishment, barring the activity from being performed, reinforcing alternative behaviors, or even antidepressant drugs as a last resort (Mason 1991, 2007). Punishment is not a recommended course of action as it may further complicate the stereotype-inducing stimulus and introduce more stress to the situation (Mason 2004). Removing the substrates necessary to preform stereotypies seems to work well for non-locomotive stereotypies, such as using cribbing collars in horses (which prevents the animal from arching its neck) or removing bars that are chewed on. This doesn't seem as helpful for locomotive stereotypies as those that pace will simply find an alternate route to continue their routine, as did one animal in the author's recent study (Clubb 2006). Correction of these behaviors should also be done in conjunction with addressing the root cause of the stereotyped behavior to truly abolish the problem behavior, or they may simply arise again (Mason 2004). Stereotypies may become ingrained in an animal's repertoire over time (one of its defining characteristics) and may become independent of its original electing stimulus, making it even more difficult to abolish; swift correction is paramount (Mason 2004).

Stereotypies in Carnivores

Of particular interest in the study of stereotypies in captive zoo animals are carnivores, which are far and away the most studied practitioners. Most carnivores, which generally move through expansive territories in the wild, seem to be more prone to locomotive stereotypies and stereotypies in general (Clubb 2006). Pacing especially is so pervasive that it is readily noticed by zoo patrons who often find the behavior a negative reflection of captivity. The most widespread notion is that these behaviors come from being unable to hunt or forage independently, and pacing near feeding times is an attempt to model natural hunting behaviors (Club 2006). Another hypothesis is that far-ranging carnivores such as felids, canids, and ursines may become frustrated at their relatively small enclosures that limit their need to patrol and explore (Clubb 2006). These locomotive stereotypies could also be a manifestation of frustrated attempts at escape, whether escaping the enclosure to seek resources or get away from aversive stimulus within the enclosure (Clubb 2006). Clubb and Vickery (2006) postulate there may be multiple motivation factors behind carnivores' stereotypical behaviors, citing the difficulty in directly testing these hypotheses and the lack of studies involving sufficient experimental control to draw a clear conclusion; most of these hypotheses are supported largely by observational studies that can be over-interpreted. Mason (2007), after meta-analysis of these studies, believe that natural stamina or "exercise physiology" may contribute to higher rates of stereotypies, as some animals cover lots of ground in their daily lives and thus may be more predisposed to stereotypies.

The Visitor Effect

The nature of being housed at a zoological facility brings with it an exposure to larger volumes of humans and urban environment noise that can evoke stress responses in animals, which can the also spur stereotype development (Carlstead 2000). The Visitor Effect, as it's called in literature, presents a Catch-22 for zoos. In order to strengthen conservation through public outreach, exhibits are designed to facilitate more up-close patron-animal interaction, however zoos' commitment to conservation of the animals in their care dictates their well-being above all, to the exclusion of patron-animal interactions that induce stress (Hosey 2000, Fernandez 2009). Balancing fulfilling paying customers' expectations and preserving the health of exhibit animals if often tricky. High visitor densities can elicit stressful and fearful responses from an overabundance of loud noises or a lack of places to hide from the crowds, depending on the exhibit design (Morgan 2007). While data on the Visitor Effect on non-primate species is growing, it is generally concluded that high visitor densities have a negative impact on captive animal's well-being (Sellinger 2005, Davey 2007). This stress can deteriorate an animal's overall health; it can also greatly affect reproductive health potentially reducing the success of captive breeding programs (Davey 2007). Zoos that use these programs to boost conservation efforts or run reintroduction programs can find these consequences particularly deleterious.

This stress response has also been well documented in a few non-primate studies. High visitor density as correlated to higher stress responses has been studied by measuring levels of fecal cortisol in captive Mexican Gray wolves (*Canis lupus baileyi*) (Pifarré 2012) . Higher cortisol levels were present on days with high visitor traffic in front of the wolves' exhibit, accounting for the time lag. On high visitor density days like Saturday and Sunday, the wolves were also found to be significantly more active than on low visitor density days such as Monday

and Tuesday (Pifarré 2012). A similar study that measured behavioral changes in captive jaguars (*Panthera onca*) found that high visitor density as well as visitor intensity (noise level) significantly changed the cats' behaviors, increasing aggression and stereotypical pacing (Sellinger 2005). This study was further supported by a study that measured salivary cortisol levels in other groups of captive jaguars which found increased cortisol levels on days with higher public exposure (Montanha 2009).

Compounding this problem is the fact that keepers are charged with daily maintenance of the habitat, which may be intrusive for the animals depending on the design of their enclosure and any rules that prevent them from being trained to go off exhibit. Stress responses have been measured in laboratory primates and rats and found that even if performed routinely, will elicit physiological responses of similar intensity; in other words, they don't get used to these intrusions (Morgan 2007). While it is difficult to measure heart rates in animals without more intrusion, it can be reasonably inferred that the same happens to zoo animals. More intense stress responses can be evoked if keepers are in the exhibit with the animals and are instructed to act defensively (holding up arms/tools) to keep the animals away as feeding and maintenance are performed in their enclosures; these sorts of protocols are implemented when modification of the animal's behavior (i.e., training to go off exhibit for maintenance) is prohibited by a species survival plan (Morgan 2007). As a result, the routine presence of keepers can stress out animals before the keepers are present, leading to more stereotypic behaviors as a means cope with these repeated intrusions into their space.

To try and balance the need for visitor interaction and animal welfare, more exhibits are being designed with built-in enrichment opportunities and naturalistic spaces to hide in a two pronged approach (Coe 1997). More spaces to retreat from the presence of people when

overwhelming provides a sense of security, while integrated, activity-based exhibits are used to engage animals and potentially draw them into the open for viewing (Coe 1997). Alongside this should be some measure of public education on how to passively view the animals as to not frighten them. The extent to which this sort of policy can be successfully implemented may be limited especially with the high volume of children that visit and a lack of manpower to quiet or remove disruptive patrons (Kreger 1995).

The Challenge of Stereotypies

Stereotypical behaviors are a cause for concern in the world of animal welfare, and this emerging field of study into the way captive animals' behaviors differ from their wild counterparts has illuminated some fundamental issues in the care and keeping of some species. The challenge of stereotypies for study and correction stems from the myriad of stimuli that can create these repetitive behaviors and the nuances of each individual's development as well as innate physiological factors. Development may be further prompted in zoos by the omnipresence of humans, from daily intrusive maintenance by keepers to large crowds pressing in to catch a glimpse. Furthermore, debate rages on as to why animals perform stereotypies, whether the act is a manifestation of suffering, boredom, and frustration or used as a soothing ritual to cope in inadequate environments. Zoos have responded by increasing their enrichment repertoires and designing exhibit spaces that keep animals and public alike engaged. As zoos attempt to balance their financial drive with their conservation efforts, addressing the underlying problems that drive stereotypical behavior can provide animals with better overall physical and mental health while at the same time entertaining guests with enrichment activities and more natural behaviors from the animals. While just beginning to be understood, the study of stereotypies has the potential to improve the lives of all captive animals, domestic, livestock, and zoo animals alike.

In this interest of improving animal welfare, this study attempts to analyze the behavioral schedules of three Mexican Gray wolves (*Canis lupus baileyi*), who by their keepers' admissions have been pacing at a concerning rate. By collecting behavioral data throughout zoo hours, as well as noting keeper presence and estimating crowd size for each data point, the aim of this study is to see if the wolves are indeed exhibiting stereotypical behaviors at concerning frequencies for each individual. On top of that, this study will see if stereotypic behavior frequency is in some way correlated to keeper presence, higher visitor density, or both. The outcome may hopefully shape zoo policy to provide the wolves with a more comfortable environment through more enrichment, remodeling of their space, or encouraging patrons to respectfully (and quietly) observe the wolves.

Introduction:

Stereotypic behaviors, or stereotypies, are repetitive, purposeless, and invariable behaviors performed by animals. These are often normal behaviors in the animal's repertoire performed out of context or in an abnormal fashion that grow increasingly unchangeable and rigid in their practice over time (Mason 1991). They are prevalent in almost all species, including humans, but tend to occur more often in captive animals than wild counterparts, which lead most to believe that the conditions of captivity are closely related with the causes of animal stereotypies, be it in laboratories, zoos, or farm settings (Mason 2004). While multitudes of different behaviors can be classified as stereotypies, each species and individual can differ on what sort of stereotypies manifest (Mason 2004, Clubb 2006). These behaviors can be as simple as head swinging in elephants or cribbing in horses (wherein horses will bite a solid object, arch their neck, and suck in air) or as elaborate as backflips in deer mice and particular pacing regiments of carnivorous animals (Cooper 2007, Mason 1991, Lewis 2007, Clubb 2003).

While there is some debate as to how stereotypies form, they are generally thought to arise from boredom due to lack of a stimulating environment or as a coping mechanism to adverse stimulus in the environment (Mason 1991, Ödberg 1987, Broom 1983). They may be positive actions in the sense that stereotypies may provide "do it yourself" enrichment that can reasonably occupy an animal, or provide a sense of regularity or control in a chaotic environment (Mason 2004, Broom 1983). On the other hand, they may be manifestations of anxiety and stress that, if left unchecked, can cause self-harm, neglect, and even affect the gestational and neonatal development of offspring (Mason 1991, Clubb 2006). Sub-optimal environments that can induce stereotypies are often too small for the needs of the animal to move about and explore, or tend to be barren and without natural substrates on which to preform natural behaviors (Ödberg 1987).

In zoos, exposure to large crowds of potentially noisy humans tends to increase stress responses in animals. The higher the visitor density, the greater stress response is elicited in a phenomenon termed the "Visitor Effect" (Pifarré 2012, Sellinger 2005). This increase in stress can, in turn, increase the frequency of stereotypic behavior used by the animal as a coping mechanism (Davey 2007). This idea presents a fundamental problem for zoos, which are institutions predicated on the notion of bringing people closer to exotic wildlife for the purposes of education and conservation, as well as a business that relies on patron support to fund animal care. While more paying visitors might provide the zoo means to better care for its charges, it can come at the cost of their animals' overall mental and physical wellbeing (Hosey 2000, Fernandez 2009).

Carnivores, the most studied and known for stereotypic behaviors, exhibit more locomotive stereotypies than herbivores, which tend to display more orally-associated stereotypies (Mason 1997, Clubb 2006). The frequency of pacing stereotypies in canids, felids, and ursines is thought to arise from a frustrated ability to get outside their enclosure, whether to hunt and obtain resources beyond their reach or to escape aversive stimulus (Clubb 2006). Aversive stimulus could apply to urban noise, large crowds, and even keepers performing daily maintenance routines if maintenance is intrusive (Morgan 2007). Another postulation is that these carnivores are pre-disposed to travel expansive territories to patrol boundaries and explore; their enclosures simply do not provide enough space to satisfy this need, so they burn off excess energy by pacing. The routes carnivores pace are so set that many will simply go over or around obstacles intentionally placed to discourage their behavior in order to complete the pattern (Clubb 2006).

Often, stereotypies are indicative of some deficit in the captive animal's environment that should be addressed immediately for the mental and physical health of the animal. In zoos, it is often dealt with by using enrichment techniques to stimulate the animal's senses and prompt them to explore novel items placed in their environment. Enrichment may be novel delivery of food items (i.e. frozen in ice, stuffed in cardboard boxes), adding toys or novel items into the enclosure, rearranging exhibit foliage or terrain, or adding novel scents to the enclosure. Though effective at reducing stereotypies for a short time, the novelty soon wears off and enrichment must be varied frequently in order to remain effective (Skibiel et al. 2007). Other techniques to correct stereotypies, such as operant conditioning or punishment, are simply too time costly for zoos to effectively implement, and enlarging exhibit areas can be too costly or impossible due to property constraints. As a last resort, antidepressant drugs can be and have been used to reduce severely protracted stereotypies, particularly with fluoxetine, commonly known as Prozac (Poulsen 1996).

Mexican gray wolves are currently classified as endangered, and all populations of captive wolves are under control by the U.S. Fish and Wildlife Service (USFW). As of February 2016, the USFW published that there were at least 109 wolves in the established reintroduction area, and numbers were steadily increasing thanks to captive conservation efforts (USFW 2014). Wolves in zoo populations are essentially on loan from USFW and get moved around as individuals are needed for breeding purposes or selected for reintroduction. As they are under the USFW control, keepers must follow strict caretaking rules outlined in the Species Survival Plan, some of which conflict with normal keeper routines. Of particular note is the fact that behavioral modification is forbidden to decrease naturalization to humans, which means the wolves cannot

be trained to go off exhibit for maintenance routines, making daily cleaning and feeding an invasive and stressful as keepers are in the exhibit space with the wolves.

This study attempts to analyze the behavior schedules of three Mexican Gray wolves (*Canis lupus baileyi*) to determine the frequency of the keeper-noted pacing, as well as perhaps find a conditional cause. This study also investigated if the wolves were exhibiting abnormal amounts of pacing when there were large crowd sizes in front of their enclosure, and if the presence of keepers prompts pacing as both situations could be stressful enough to produce stereotypies. Our hypothesis is that the wolves are pacing at frequencies that are indicative of poor welfare, and that large crowd sizes and presence of keepers contribute to increased pacing. The hope is that the data from this study can be used to shape policy regarding the welfare of these animals through exhibit space requirements, encouraging patrons to be quiet and respectful when observing, or recommending better placement for these animals.

Methods:

Study Subjects

Chico, Diego, and Durango are three male Mexican gray wolves (*Canis lupus baileyi*) from the Seneca Park Zoo in Rochester, NY. All three are brothers, born in 2007 at the California Wolf Center in Julian, CA, that resided at the Seneca Park Zoo from May 2011 to November 2015. The exhibit area is roughly 50 ft. x 50 ft. with two off-exhibit areas obscured from public view. One area is used for feeding and may be closed when there is no food, while the other off-exhibit area is always open and contains three straw-lined, kennel-like enclosures. The main exhibit area contains mature native tree species, native substrate, and native plants as

well as two dug-in dens situated towards the back to provide naturalistic shelter and various rocks and log features (Figure A).

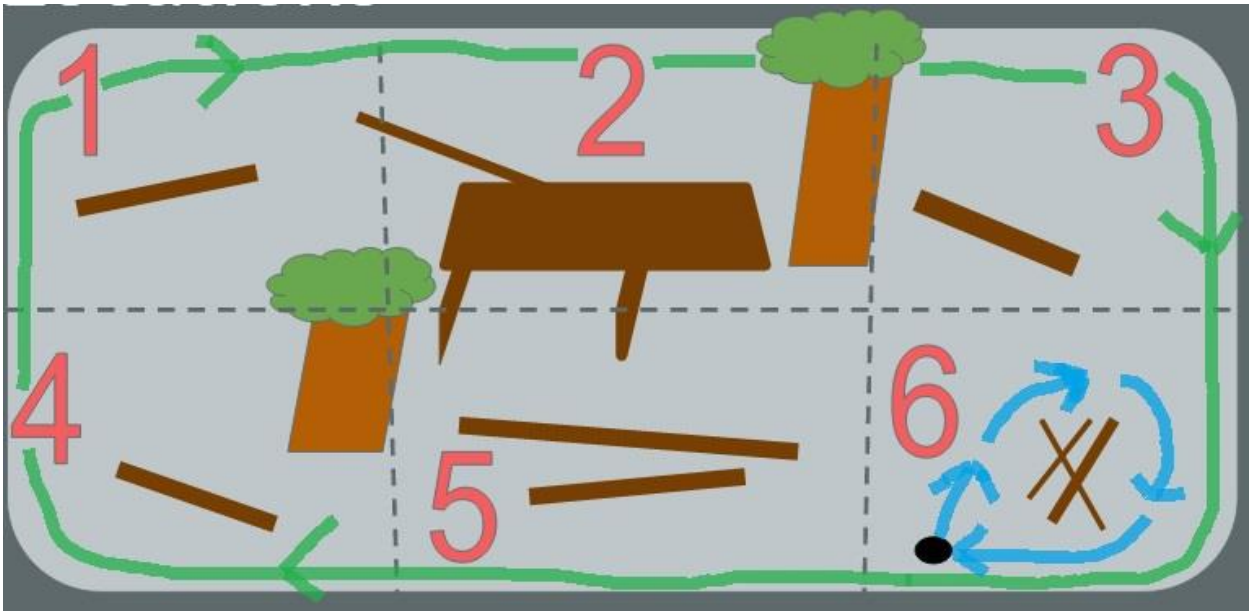

Figure 1: Wolf enclosure diagram provided by Rachael Rogers and Caitlin Hughes. Off-exhibit areas are located west of section1 and east of section 3. Diego's pacing route is marked in dashed blue and Chico's pacing route is marked in solid green. The brown bars represent large logs, with the one in section 2 being a large tree trunk section that serves as an elevated platform for the wolves to climb up on. The black dot in section six indicated the placement of a rock that was integral to the pacing regimen of Diego, as he would step on it every time he completed a loop around the log pile.

All three wolves were relocated in November 2015; Diego currently resides at a wolf conservation facility in NY as a viable breeding candidate. Chico and Durango have been relocated to The Living Desert Zoo in Palm Desert CA, and are still viable candidates for reintroduction. Their old enclosure now houses a non-breeding pair of gray wolves (*Canis lupus)*. This study therefore should be treated as a case study for the wolves, as well as their environment.

Data Collection

All three Mexican gray wolves were simultaneously observed using scan sampling in one-minute intervals for a duration of one hour for a total of two hours each day to reduce timing bias between the morning and afternoon. Observations were limited to the open hours of the zoo (9 AM – 4 PM). Hour time slots were assigned at random via number generator for both morning (9 AM - 12 PM) and afternoon shifts (1 PM - 4 PM) for each day. Data collection was performed in June 2015 by the author, and July and August data were collected by trained volunteer observers from the zoo's teen program.

Instantaneous scan sampling of the behavior displayed by each wolf was recorded via the Animal Behavior Pro iPad app at each time interval based on the ethogram in Table 1 (Newton-Fisher 2012). The app features a customizable ethogram input and an adjustable scan sampling timer that allows for selection of behaviors only when the time has run out. This software was chosen to simplify data conversion to electronic formats, as well as provide an intuitive interface for volunteers to use in their data collection with minimal time delay errors. All data taken by the Animal Behavior App was then exported to Microsoft Excel 2010, formatted, and combined for analysis for MiniTab 17 (Minitab 2010).

The ethogram is designed to focus on common locomotive or active behaviors, as well as be simple for ease of use by volunteers (Table 1). In addition to each behavioral observation, estimates of crowd counts at the wolf exhibit were taken with each scan sample (in the format: 0, ≤5, 6-10, 11-20, 21-50, >50), and notation of the visual/auditory presence of keepers near the exhibit were recorded. People who were at the exhibit fence or nearby and visually engaged with the wolf exhibit were included in the crowd size estimate; toddlers and babies were not included in this count. Other miscellaneous events of note, such as weather conditions, nearby zoo

demonstrations or construction noise were noted *ad libitum*. Any unusual behaviors of note (eating grass, vomiting, tail chasing, etc.) were also marked and keepers notified.

Volunteers were enlisted to assist with data collection from July to August 2015. Each volunteer underwent an intense, 2 hour training on the ethogram, data collection techniques, and recording of data, as well as a reliability assay to assess data validity (threshold of >85%). Volunteers collected data in pairs, one monitored the crowd, the other monitored behavior to help increase the ease of data collection for volunteers and have a second set of eyes to confirm behaviors, crowd counts, and keeper presence to reduce bias. The training protocol used is listed in Appendix A.

Data Analysis

In total, 109 hours of data were collected among all three wolves. Data were then compiled into Microsoft Excel (2010), categorical variables coded, and totaled per individual. In MiniTab 17, the Kruskal-Wallis test was performed to compare pacing/day values among all three to determine if individuals were different. A two proportion test with a Fisher's Exact test was also done for each individual to compare incidences of pacing and total data points to a 10% threshold of pacing as suggested by Broom (1991). All data points of pacing for each wolf were then extracted with associated crowd counts and keeper data for further analysis. Chi square analysis was performed across visitor intensities and keeper presence states to determine if there was a significant difference in frequencies of pacing. For crowd intensity, the crowd intensity data had to be modified as the > 50 category had counts with less than one for some wolves;

therefore the analyses are based on crowd levels up to > 20. A chi-square test for all crowd level categories was implemented, as well as a chi-square test for all non-zero categories.

Table 1: Ethogram used in this study, designed to focus on active behaviors and to be easy to use by volunteers

Category	Behavior	Definition
Active	Walking	Slow locomotion from one point to another
	Trotting	Between a walk and a run, a jogging motion with a two beat pace where the two diagonal legs move synchronously
	Running	Includes bounding; only counted if it is not part of play or aggression
	Play with other wolves	At least 2 wolves interacting, includes jumping or climbing on each other, play bows, and gentle tugs on ears and fur. Does not count as play if there is any baring of teeth, growling, or yelping.
	Aggression	An interaction between at least 2 wolves in which one chases another away from a desired toy or food item or any interaction involving raised hackles, ears laid flat against the head, baring of teeth, growling, or yelping. This should be marked for both the aggressor and the subject of the aggression.
	Climbing	Any locomotion up logs or fence other than that involved in aggression or play
	Digging	Using two paws in a repetitive manner to move substrate
	Interact W/ Keepers	Any direct interaction with the Staff such as following along the fence or play bowing and jumping towards staff members, in or out of uniform.
	Interact W/ Public	Any direct interaction with zoo patrons such as following along the fence or play bowing and jumping towards patrons
	Interact W/ Enrichment	Any engagement with known enrichment items, such as olfactory investigation, playing with an object, or feeding on food enrichment
Stereotypical	Pacing	Walking in a repetitive pattern (such as Chico's loop or Diego's figure 8 or Durango's route up and down the fence). A walking pattern is considered pacing if it occurs three times in a row. Pacing stops when the animal completely changes course or stops moving for longer than 10 seconds.
Passive	Standing	Wolf is stationary and standing for at least 5 seconds (does not include standing while eating or participating in an aggressive or playful interaction)
	Resting	Laying down, sitting, or sleeping, eyes open or closed.
	Feeding	Eating, carrying, chewing, or licking food
	Mark Territory	Urination, scratching (other than digging), or deliberately rubbing on any part of the exhibit
	Sniffing	Standing still, examining an object thoroughly with the nose at close range, may be due to enrichment scents, territory markings, or other.
Not Visible	Not Visible	Subject is unable to be located or cannot reasonably be identified if obscured.

Results:

Each wolf spent most of their time engaged in passive behaviors (standing, resting, feeding, mark territory, sniffing) (Fig. 1.1-1.3). Durango notably spent 38% of the observed time not visible, while Chico was the most visible of all three at only 8% of time spent not visible (Fig 1.3, 1.1). Of all behaviors from the ethogram, all three spent most of their time resting at a 37 ± 8.95 %. Of the active behaviors (anything other than the above listed and pacing), all three spent the most time walking with an average of 12 ± 2% of all observed behaviors.

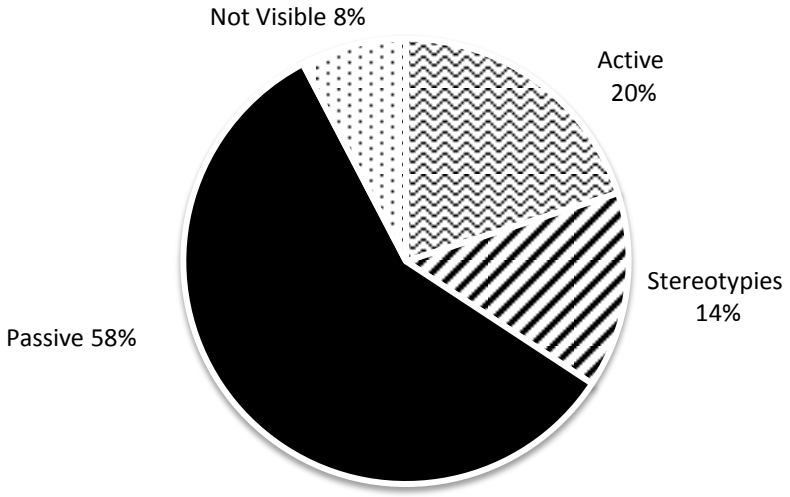

Figure 2.1: Time budget of Chico

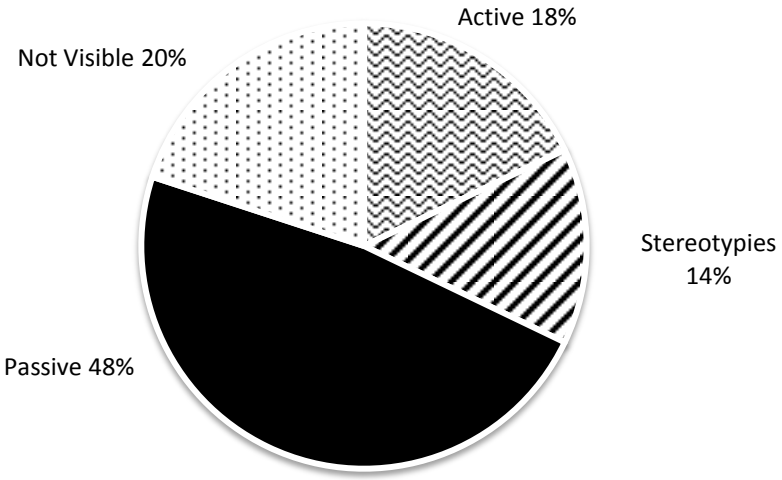

Figure 2.2: Time budget of Diego

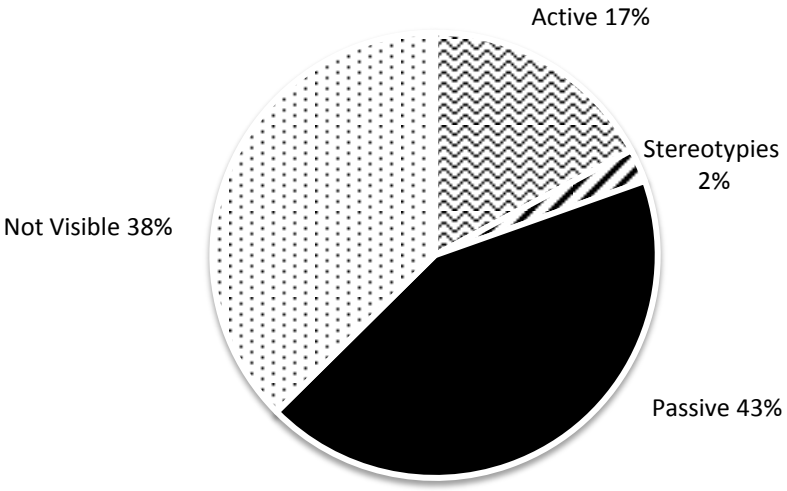

Figure 2.3: Time budget of Durango

Analysis of Pacing Frequency

There was a significant difference in means among all three for rates of pacing per day (H = 34.56, *df*=2, P < 0.005). Durango exhibited only 2.290 ± 4.4 %, while Chico and Diego paced about 13.58 ± 15.5 % averaged together. Therefore, when Durango was removed and the tests were run again, both tests found no difference between the means of pacing instances/day (H =0.01, *df* =1, P= 0.939). Compared to the total behaviors observed, Chico and Diego spent 14% of their total time pacing, and Durango spent only 2% of his total behavior budget pacing. Instances of pacing for Chico and Diego were significantly over the 10% threshold of pacing compared to all other observed data points (Z = 7.38, P < 0.005). However, Durango was found to have levels of pacing significantly under the threshold

(Z = -18.33, P < 0.005, Fig 2.3).

Visitor Effect Analysis

The average crowd size was between 1 and 5 people, with the majority of very large crowd sizes coinciding with zoo events such as live exhibit demonstrations (at both the wolf and nearby Siberian Tiger exhibit). There was a significant difference in pacing across crowd size categories for all three wolves (χ^2= 48.608, *df* = 8, P < 0.005). This significance was still found when Durango was removed to account for the individual differences in observed pacing rates (χ^2= 41.162, *df* = 4, P < 0.005). For all non-zero crowd levels (≤5 and up), no significant difference was found between non-zero crowd levels (Pearson χ^2 = 2.005, *df* = 6, P-Value = 0.921). No significant difference was found when Durango was removed to account for individual differences (χ^2=0.835, *df* = 3, P = 0.841, Figure 3). More pacing seemed to occur at

low to moderate crowd sizes, but this may be due to the fact that there were rarely crowd sizes of 20 or more persons.

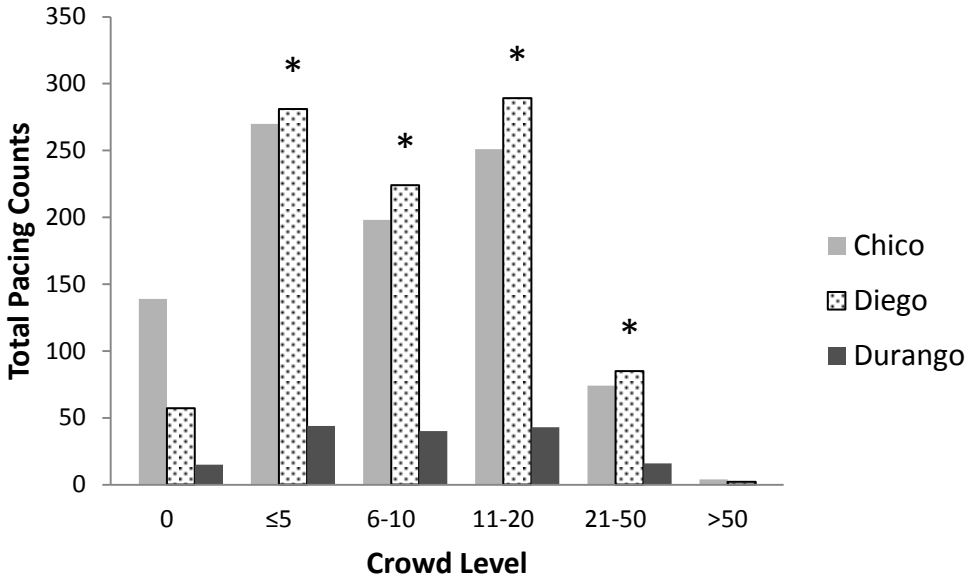

Figure 3: Histogram of total pacing counts over crowd count levels for all three wolves. The >50 category was removed because they had counts less than 1 and a χ^2 test could not be performed with those values. A star (*) indicates a significant difference in that category for all three wolves.

Keeper Presence analysis

Overall, keepers were present only 11% of the total time. While there was a significant difference between categories, pacing occurred more when keepers were not present, contrary to expectation ($\chi^2 = 37.308$, $df = 2$, $P < 0.005$, Figure 4). The significance remained unchanged when removing Durango to account for individual differences in overall pacing rates ($\chi^2 = 32.757$, $df = 1$, $P < 0.005$).

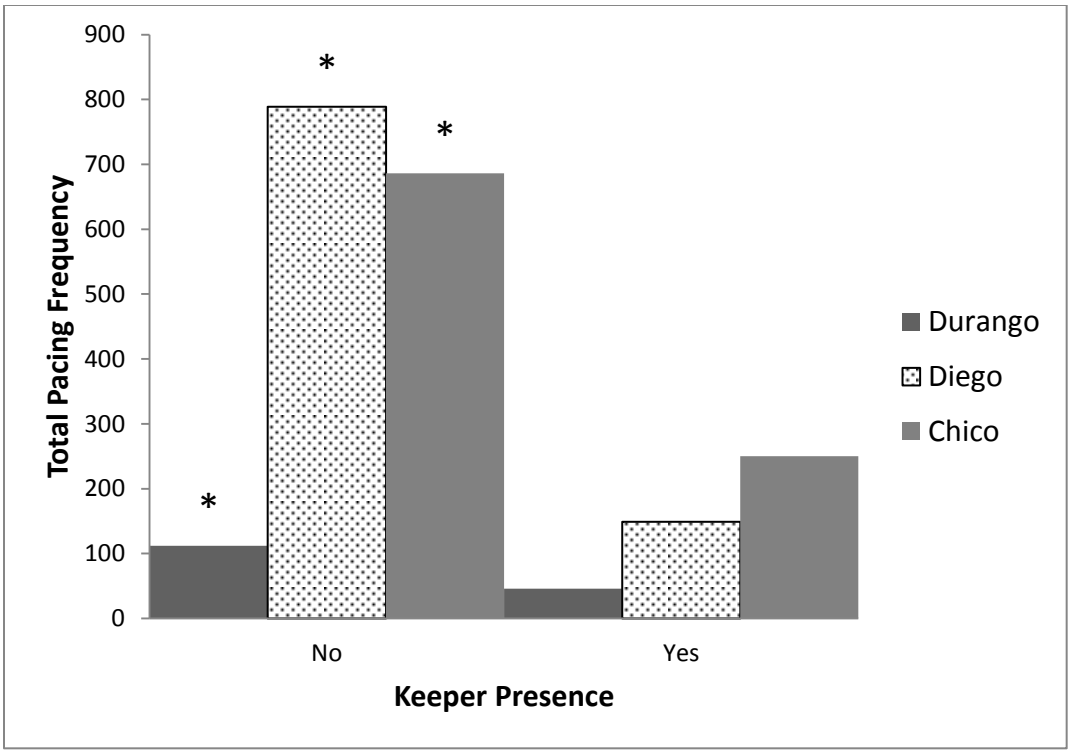

Figure 4: Histogram of pacing frequency by keeper presence

Discussion:

Both Chico and Diego were found to be pacing in total above a 10% threshold, while Durango was significantly under. However, there is no set threshold in this particular field of study to judge what is and is not an abnormal amount of pacing by a standard, agreed upon metric. Wiepkema (1983) states that conditions should be changed if more than 5% of a population exhibits these behaviors; however, Broom and Johnson (1993) do not believe there is any acceptable level of stereotypical behavior. There does not seem to be a clear consensus as to what characterizes an abnormal level of stereotypic behavior in the published literature. For this situation, the 10% behavioral budget threshold was decided upon as it was the most applicable to

the situation from the standards that could be found in literature. It was difficult to find a study that simply measured stereotypies without testing the effects of enrichment, especially since agricultural and lab studies on improving welfare seemed to dominate the topic. The fact that the data coincidentally satisfies the conditions for Wiepkema's (1983) 5% population threshold may simply be due to a small sample size. A standard, published metric for what is a concerning level of stereotypies is necessary if we are to study this topic further. Our results showed that Chico and Diego were pacing significantly above this 10% threshold which can be reasonably interpreted as a sign that there is some aversive stimulus in their environment.

For the wolves, routes for pacing were markedly different. Chico would pace in a trot around the perimeter of the exhibit space in a clockwise fashion. Diego would trot in bottom right corner (sector 6 in Figure 1) in a teardrop shaped route that was also clockwise. Diego's route included touching of a particular rock with a front paw, indicated by the black oval, and then pausing for a brief period before starting another loop (Figure 1). When logs or branches were put in the way of his route to try to prevent pacing, Diego would simply walk over the obstacles or slightly modify his route to avoid them. This illustrates the ingrained, unvaried, and almost compulsive nature of stereotypies (Mason 1991). Further testing could be done to obtain physiological evidence of the effects of pacing on these animals, such as measuring cortisol levels in feces or other signifiers of stress (Pifarré 2012).

It was found that more pacing occurred when crowds were present in any amount, but not significantly different among non-zero crowd levels. Past research has shown that both larger crowd sizes and keeper presence can increase both physiological stress and in turn, stereotypy frequency (Sellinger 2005, Davey 2007). With this and the accounts given by the Seneca Park Zoo keepers who requested this study, it seemed fitting to see if crowd size and keeper presence

had any relation to increased instances of stereotypies. I hypothesized that higher crowd densities and keeper presence would lead to higher instances of pacing in consideration of the wolves' enclosure and their predilection to avoid humans. While crowd size seemed to have an effect on the frequency of pacing in the wolves of the Seneca Park Zoo, keeper presence did not. The statistical analysis of overall pacing frequency supports the anecdotal evidence provided by keepers, as well as observations made by frequent visitors and volunteer staff that the wolves were pacing more often than not. If the abnormal behavior is so pervasive that it is readily noticed and remarked upon by multiple recurring zoo patrons, investigation should be prompted to see if the behavior is occurring at detrimental levels.

While most pacing occurred when crowds were small or moderate, this may be due to the fact that very large crowd aggregates were uncommon or fleeting. Periods of large crowd sizes correlate with higher levels of stress in captive animals which can externally manifest in stereotypies as a response (Pifarré 2012, Sellinger 2005, Hosey 2000). It has been shown that in fennec foxes (*Vulpes zerda*), there is a strong positive correlation between total visitors and amount of pacing per day, however noisy patrons can also elicit stereotypical behavior without large numbers of people present (Carlstead 1991, Fentress 1976). While there was significantly more pacing when crowds were present, it cannot be determined whether the reason behind it was the amount of people or the noise generated. At the Seneca Park Zoo, periods with higher than average crowd counts generally coincided with zoo events such as exhibit demonstrations and animal day (wolf day, tiger day). Sometimes, it would also be due to elementary school groups, summer camps, and the zoo's own day camp program. Data were collected in the summer months when attendance is traditionally greater, so the significant difference in pacing could be seasonal based on attendance. Further research to compare visitor density to pacing

over the course of a year, or at least during busy and slow periods, would help clarify. Anecdotally, when speakers and microphones were used for demos, the wolves seemed very agitated by the volume of the speakers and would become alert and active during the pre-show setup. Whether this was because of the speaker noise or anticipation of large crowds forming remains unknown, but could serve as an interesting hypothesis to test.

The effect of noise, both urban and crowd-generated, has been studied as a common stressor in captive animals, and one that can increase the frequencies of stereotypies (Mason 1991, Fentress 1976). In giant pandas (*Ailuropoda melanoleuca*), days with louder levels of noise not only increased the frequency of stereotypical behaviors but increased stress responses had long-lasting physiological effects. Low frequency sounds had the greatest effect on the pandas, but reactivity to frequency may be species-specific based on the range of auditory perception (Owen 2004). Mason's review of stereotypies (1991) finds that intense sounds can interrupt stereotypy performance to trigger a fight-or-flight response, but typically the stereotypical behavior will resume later at a greater intensity. For the wolves, the noise level may be why more pacing occurred when people were present and would explain why they became extremely agitated during shows where the speakers and microphone were used. Further testing with a decibel meter to compare ambient noise levels to incidences of pacing could be used to determine if this is the disturbing factor about crowds in which wolves are reacting.

We found that while there was a significant difference in pacing totals over keeper presence, there was significantly more pacing when keepers were not present. Although not a named phenomenon, keeper interaction may also increase stress levels in captive animals, potentially leading to stereotypy development and implementation (Wielebnowski 2002, Carlstead 2009). Though no significant difference was found in this study, markedly different

patterns of behavior were observed when keepers were near or in the wolves' exhibit. This finding may be due to a drastically lower frequency of keeper presence compared to the total amount of data points. Keepers with food have been shown to induce stereotypies with other canids, whether or not this is born out of anxiety or anticipation is unknown (Fentress 1976). Some keepers seemed to elicit stronger responses than others, though the presence of any keeper walking past the enclosure made the wolves more alert in general. Keepers with buckets would excite the wolves greatly because during feeding times their diets are carried in distinct red buckets past their enclosure. Other animals also received food carried in red buckets, so this response would be repeated in anticipation of potentially receiving food while other animals were being fed. On these occasions, all three wolves would trot or run the perimeter of their enclosure until food was delivered or enough time passed for the wolves to realize food would not be delivered.

Another factor at play may be the exhibit cleaning protocol wherein keepers clean the exhibit while the wolves are in the same area with the off-exhibit areas open. This unique procedure is due to the fact that the Species Survival Plan and Husbandry Manual for the Mexican Gray Wolf requires that no behavior modification be performed as the wolves can, at any time, be reintroduced to their native range (USFW 1982, 2009). This means that keepers cannot train the wolves to go off exhibit for cleaning, like most other exhibit animals, and can only offer food in the off-exhibit areas in hopes of enticing them in and closing the gate. One or two keepers would then clean while another stood guard with a rake or shovel in a non-threatening, defensive manner to ward off any wolf that came too close. As observed personally on multiple occasions, Chico and Diego would become very agitated and start to pace in areas opposite the keepers, and often wouldn't stop until some time after the keepers left. Durango

didn't seem perturbed and would often approach the keepers in a non-threatening manner, but close enough to warrant being shooed away.

The location of the wolves' enclosure might also have an effect on their pacing. Being located on a main thoroughfare to access the Africa section at the other end of the zoo certainly increases the volume of visitors that pass by at any given time. As well, they are located across from an exhibit containing two Amur tigers (*Panthera tigris altaica*). One of the carnivore pacing stereotypy theories offered by Clubb (2006) is that pacing in born out of a drive to escape aversive stimulus in the environment, but is blocked by their fenced-in enclosure. Not only are large congregations of visitors and loud noises aversive stimuli to escape from, but so is the presence of potential predators. It is possible that the presence of the much larger Amur tigers could be prompting a frustrated desire to escape the enclosure that manifests as stereotypic pacing. Considering the canid capacity for olfaction, the wolves could also be responding to the frequent scent marking performed by the tigers, as well as the presence of the neighboring spotted hyena (*Crocuta crocuta*). Only by moving the wolves' exhibit location and measuring the incidence of stereotypies could this be tested.

The presence of elevated levels of pacing should be a concern for this species as the possibility of it being stress-induced may affect their reproductive successes in later breeding programs. As the Mexican gray wolf has a wild population of roughly 100, according to the 2014 survey by the U.S. Fish and Wildlife Service, breeding in order to establish a stable gene pool and increase the population is imperative to the recovery program (USFW 2015). Those in captivity are still owned by USFW and can be utilized for breeding purposes if they possess desirable genetics, as was the case with Diego, or be slated for reintroduction. As stereotypies can be induced by stress, their frequency can be used as an indicator of stress levels in an animal,

and thus their capacity for reproductive success and even reproductive capability. Prolonged stress can have severe physiological impacts ranging from self-harm, immune system suppression, to impaired reproductive function or epigenetic damage to resulting offspring (Clubb 2006, Mason 1991).

By identifying the source of the problem, steps can be taken to address it and ensure the physical and mental wellbeing of captive animals for the duration of their time in zoos. We must first establish that stereotypic behaviors are occurring in abnormal frequencies, we can then discover the prompt of these behaviors, whether it is their enclosure, location, or the presence of patrons or keepers. Often, enrichment programs are used to reduce stereotypies, but this is only effective if the enrichment changes often enough in sensory modality and implementation as to not get "stale" and expected (Skibel 2007, Mason 2007). Based on the results herein, a few proposals are offered to reduce the stereotypic behavior of these wolves. A crowd control protocol could be implemented to attempt to reduce ambient noise through educating patrons about the wolves' sensitivity to human presence. The wolves could also be moved to a section of the zoo where there is less foot traffic and thus less noise, but also removed from other animals that may be perceived as threatening. Finally, the wolves could be moved to a facility with limited human interaction and more space as to satisfy their environmental needs. The latter is currently the case for Diego who has been moved to a breeding facility; however Chico and Durango have been relocated to another zoo. The Living Desert Zoo in southern California may provide a more natural habitat than western New York and they serve as the coordinator of the Mexican gray wolf captive program, but the visitor density, exhibit location, and enclosure size are unknown. They may be liable to fall back on stereotypical pacing if the stimulus to trigger it is still present. Ultimately, the mental and physical wellbeing of these animals is of the utmost

importance, especially for a species with a critical need for breeding and reintroduction individuals.

Appendix A: Volunteer training protocol

WILL NEED: paper data collection sheets, 2 each (1 for real, 1 for practice) pencils, app protocol and ethogram packets (1 for each)

- Each Teen Signs in, picks 4 digit code
- Introduction into the what and why of the project
    - What? Tracking each wolf's behavior, number of people in crowd, and keeper presence
    - Why? See if the wolves are pacing and what may cause that
- Why the teens are important
    - I can't be here forever, need people to take my place for the summer to improve study, add more data

Step one: ID wolves

- Explain markings/bodily differences between Chico, Diego, and Durango, some personality differences, show pictures
- Point out usual resting niches, pacing routs, places that they hide, where they can be seen from
- Play "where's Chico/Diego/Durango" Game
- Questions?

Step Two: ID Behaviors

- Go over each behavior in ethogram, with some acting out and explanation
    - ID pacing as a rout repeated at least 3 times! Show video on iPad
    - Define each gait as different
    - Define interaction w/keepers as play bows, jumping at fence
    - Explain which behaviors they'll see the most of from each wolf
    - If not listed, go with what behavior fits best. (if grooming (not on list), indicate if resting or standing) only interested in certain behaviors to keep it simple.
- Look at wolves and ID current behavior
- Explain how stimulus changes their behavior, what to look for (i.e. Keepers make them alert, stand up, and become animated. Food time makes them run in circles, etc.)
- Play wolf charades
- Questions?

15 minute break if needed

Step three: Data collection process

- Explain scan sampling of three individuals, one per minute for one hour; seems like a lot, but passes quickly.
- Show them how to work iPad app, from start to finish (launching app, to email)
    - Explain they are responsible for timer, no noises, limit distractions
    - Explain importance of ID code, saving log files and emailing them
    - Explain that if mistakes are made, note in notebook when/what/who
    - Log any unusual behaviors in notebook (vomiting, eating grass, butt-scoots)
- Show them notebook, how to record crowd count/keeper presence, do AFTER behavior
    - Keep track of how many crowd figures, should have 60 by end.
    - Crowd counts are people on the fence line or close by, rough estimate
    - Keepers are in visual or auditory range to be present. Wolves will spot them before you do.
    - What to do if patrons are in way/ask questions
- Do a mockup demo, explain importance of keeping track of where wolves are in enclosure, if you can't see a wolf where stationed, get up and move until visible. Keep track of area in case he moves
- Quickly go over paper reliability assay, explain why it's used
    - 85%reliability ensures that data is consistent for analysis. Nothing personal, it's science
    - If not 85%, come in and retake until 85% is achieved with practice
- Practice with iPad, Others use paper trackers
- Discuss before and after routines. Bring sunscreen/umbrellas, lunch, drink with them, pick up iPad and binder from Anneke's office, set up space. After: save logs, email logs in ZOT zone, double check they've sent in email app, return all materials to Anneke, make sure charged for next use
- Show them my email address in case they have questions/problems/issues
- Questions?

10 min break before assay starts

Step four: reliability assay (start at 11:30 at Latest!)

- Review how paper sheet works, behavior and crowd count, set up stopwatch/timer
- Set up along fence, allow them to move if needed
- Start reliability assay (30 minutes)
- Collect sheets
- Questions?
- Allow to practice more while I collect data at 12PM-2PM, can do additional reliabilities if they want to
- I will process reliability, let Anneke know of results

Appendix B: Animal Behavior Pro Protocol

BEFORE Shift (10-20 minutes prior)

- Make sure iPad is charged enough for an hour of use
    - If not, please plug it in for charging at somebody's office
    - Ask for/ obtain a paper data collection sheet, working pen, stopwatch (or use phone), and the observation notebook for your observation hour
- Get the observation notebook, write the date, time, your initials, and a brief weather report as the header
- Grab anything you might need, like a water bottle, snack, or book before heading out. If raining, bring an umbrella
- Hit power button on the iPad, located above the rear camera, swipe to unlock and enter the passcode : 4567
- Touch the animal behavior pro app in the top left-hand corner. You will be prompted to enter your 4 digit pin number, and then hit OK. This helps me know who recorded what data. You can create any combination of digits, as long as I know who pick what ID number so there are no overlaps.
- On the Configuration screen, press "Scan" in the white box, then coding, and select wolf. Move the time dial at the bottom to one minute intervals, then hit Save at the top right corner. The scan option will now say "ready. All behavior, 01 min interval"
- Prepare for data collection!

DURING Shift

- Once scan is set up, or if it has already been set up prior, the "Scan" tab at the bottom (the little binoculars icon) will now be highlighted and ready for use.
- Scan the enclosure for the wolves. Chico has the lightest coat, Diego has the darkest colorations (and is slightly heftier than Chico), while Durango is a medium dark color with black vertical splotches on his forelegs and a brown face (and the biggest of the three)
- Select a name, then accompanying behavior as observed <u>in that moment</u>, then hit the green OK button when you are sure it is correct.
    - As soon as you hit one of the names, the timer will count down. Don't worry about it, just put in entries for each.
    - Once you hit OK, you can't change the entry. If you need to change your entry, select the "Actor" or "Behav" options to change your selection. When it is correct, hit the green OK button. If you entered in something by accident, make a note for me in the observation notebook so I can change it.
- Make note in the observation notebook if there are any new enrichment items visible, or the time enrichment is administered. <u>After</u> an entry, jot down how many people are in front of the exhibit at that time. These should be written as estimates of "5 or less, >5, >10, >20" etc. It is vital to the study that we know when large crowds are in front of the exhibit.

- Make notes on any unusual or interesting behavior and the time it took place, such as howling, play bows to the crowd, or other rare behaviors (i.e., when Durango eats grass, then throws it up later, or little micro aggressions)
- Continue for an hour's worth of time, or 60 entries (counting the crowd figures on paper). Please pay attention to the iPad since the app will re-highlight the names, or if outside the app it will give a pop-up notification, but **no sound**. It is your responsibility to keep track of the timer and record data every minute.

AFTER shift

- Once the last recording has been taken, hit the red "End" button on the top left, and select yes
- Hit "Logs" in the top right corner, and select the entry you just finished taking. Tap the most recent data log, the date and time of your shift. A menu should pop up. Select "export to library", (This saves it to the device, which is *critical*, since the app won't store data if the app is closed out or the iPad is powered down)
- Repeat, and select "export to Email". This should bring up an email composing screen.
- In the "To:" field of the email screen, type in "wolf". An email suggestion should appear that says spzwolfproject@gmail.com. Select that address and tap send in the top right corner.
- Head towards the ZOT Zone, where it should connect to Wi-Fi and send the email automatically. Check and see in the email app that there's nothing in the outbox to be delivered.
- Return iPad to Anneke's office, you're done!
    - If you notice the iPad is low on charge PLEASE PLUG IT IN, especially after the AM shift since there will usually be enough time to charge at least half way before the next shift.

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