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Implementing welfare science: case studies in evidence-based zoo management

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Implementing welfare science: case studies in evidence-based zoo management

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CERTIFICATE OF APPROVAL

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Abstract:

Animal welfare science is a field that focuses on how we can improve the lives and well-being of animals in human care. Modern welfare science has moved away from simply preventing suffering and on to promoting positive welfare states, a concept that has been coined animal “wellness”. A process called evidence-based zoo management has been implemented in many zoos as a way to promote and ensure wellness. This is the idea that husbandry and housing standards should be evaluated and tested for their efficacy using data rather than relying on traditional best practices. In this manuscript we discuss an informal series of steps that can be followed in two case studies of evidence-based management at the Jacksonville Zoo and Gardens. The first explores the process of evaluating a new feeding enrichment for the zoo’s five adult alligators. Outcomes of the enrichment with regards to behavioral goals are discussed as well as shortcomings of the method, including ease of implementation and issues with group social dynamics. The second case study follows the Animal Wellness research team as we evaluated an existing welfare concern with the zoo’s two male coyotes. In particular, pacing and resting behaviors were compared before and after a reduction of staff activity in their immediate environment. These studies aim to provide examples of how an evidence-based method can be successful in informing management decisions and interventions in the zoo environment and how we can use this method to ensure wellness/optimal welfare for zoo animals.

Introduction:

Animal Welfare Science

Animal welfare science, a field of study that emerged in the latter half of the 20th century, focuses on how we can improve the lives and well-being of animals in human care. The field initially emerged as a way to evaluate affective states of farm animals used for food production (Brambell, 1965) and focused on methods to identify and alleviate suffering. A method often used to conceptualize and evaluate welfare in these beginning stages of the discipline was the "Five Domains" model. The model stated that in order to ensure good welfare, an animal must be housed in an environment that provides 1) freedom from thirst, hunger, and malnutrition, 2) freedom from discomfort, 3) freedom from pain, injury, and disease, 4) freedom to express natural behaviors, and 5) freedom from fear and distress (Webster, 2001). This model was sufficient to evaluate the presence of suffering.

Modern welfare science, however, acknowledges that welfare occurs on a continuum (Fig. 1) and has moved away from simply preventing suffering to promoting positive welfare states. This concept has been coined animal "wellness" (Maple & Perdue, 2013) and is distinct from welfare in that the framework does not just aim to prevent suffering, but also promote environments in which animals can thrive rather than just survive. The terms "wellness" and "good/great" or "optimal" welfare will be used interchangeably throughout this manuscript as these are all terms that refer to an animal experiencing well-being towards the positive end of the welfare continuum.

Simply relieving suffering for animals is no longer enough and animal care and management professionals are developing methods to promote and document the presence of positive

indicators of welfare in addition to the absence of negative indicators. The Five Domains model has been useful in this process of ensuring more optimal welfare and encouraging the concept of wellness for captive animals in the domains of nutrition, physical health, environment, behavior, and mental health (Mellor & Beausoleil, 2015). The first four domains have measurable inputs and outputs that can give insight into the quality of the last. Because animals cannot tell us directly what they are thinking or feeling, tracking inputs and measuring outputs from nutrition, physical health, environment, and behavior can help us determine what the affective state of an animal might be and give insight into the mental or psychological domain. Evaluating these five domains for individual animals can give us more holistic insight into their experience and allow us to make informed modifications to husbandry to ameliorate the cause of their distress and to promote positive behaviors that lead to thriving and wellness.

Implementing Evidence-Based Management in the Zoo

Historically, best practice animal management in zoos has been based off "current" practices rather than objectively gathered evidence. Using tradition as best practice has been cited as a barrier to achieving wellness and optimal animal welfare (Melfie et al., 2007; Melfie, 2009). The principle of evidence-based zoo management is the idea that husbandry and housing standards should be evaluated and tested for their efficacy using data rather than relying on methods that have simply "worked in the past" (Maple, 2007; Melfie, 2009). With this approach to monitoring the impact of management decisions, we can ensure that the desired outcomes are achieved, whether that be an improvement in health, behavior, or some other measure of well-being. This method helps us track progress towards long term welfare goals and allows us to move towards providing more optimal welfare for the animals in our care.

Effectively implementing an evidence-based approach in the zoo environment requires a general protocol. In these case studies, the following are the general steps that my research took that I feel would be a good framework for gathering evidence and making management decisions based on that evidence.

1) Identify a problem or area to improve.
    a. This may be an existing welfare concern (problem) or an aspect of husbandry or housing that could be adjusted to promote more positive welfare (area to improve).
2) Gather and evaluate preliminary evidence
    a. This is a step may look different depending on the situation or available resources. It could include simply gathering information from the literature to help inform a solution to the identified problem/area to improve, or it could be as in depth as developing a behavioral observation protocol and collecting in-house data over a period of time.
3) Implement a solution based on evidence
    a. After preliminary evidence has been gathered and evaluated, this information can be used to implement a solution to the problem/area to improve identified in Step 1.
4) Evaluate outcomes of the solution
    a. It is important that, throughout the process of implementing a solution, data continues to be gathered. In this manuscript, the data gathered is on behavior, but this can take a multitude of forms as long as the data gathered is relevant to evaluating the outcomes of the solution that was implemented.

5) Modify and improve the solution if necessary

    a. If the outcomes of the initial solution are satisfactory, this process can stop after Step 4. However, a benefit of this method is that we can continue to work towards optimal welfare. If resources are available, modification and improvements can be made to the original solution.

6) Repeat Steps 4-5 until welfare goals are achieved

    a. It is important to continue to evaluate the outcomes of any solution or modified solution using objectively gathered evidence. This process of evaluating and modifying can continue until welfare goals are achieved.

In this thesis I will explore two case studies in which these steps were followed in order to evaluate solutions that were implemented for two separate species at Jacksonville Zoo and Gardens: alligators and coyotes. Each of these solutions was used to either promote indicators of positive welfare (alligators, Chapter 1) or to eliminate indicators of negative welfare (coyotes, Chapter 2).

Chapter 1: Enrichment for alligators: evaluating efficacy of a novel feeding enrichment with considerations for group social structure and spatial distribution

Introduction

Providing enrichment is an important component of caring for zoo animals and is defined by the Association of Zoos & Aquariums (AZA) as "a process to ensure that the behavioral and physical needs of an animal are being met by providing opportunities for species-appropriate behaviors and choices" (AZA, 2020). Enrichment provides stimulation and opportunity for problem solving that may not otherwise be offered by the captive environment. Enrichment can take several forms and have different goals, but its main purpose is to physically and mentally stimulate animals and provide them with the opportunity to exercise choice and experience novelty. Although regular enrichment is a requirement for all animals in AZA-accredited institutions (AZA, 2020), enrichment for reptiles is rarely evaluated and reported like it often is for mammal and bird species (Eagan, 2018). The few studies that have reported enrichment programs for reptiles find that it is just as stimulating and necessary for this group as it is for others (Bashaw, 2016; Case, Lewbart, & Doerr, 2005; Rose et al., 2014; Tetzlaff, Sperry, & DeGregorio, 2018).

Effective enrichment strategies will target and promote the expression of "highly motivated" behaviors. These behaviors are those that result in a survival benefit in the wild, such as food acquisition behaviors and environmental exploration that leads to finding shelter and mates (Bracke & Hopster, 2006). These behaviors are hypothesized to be intrinsically pleasing to perform and therefore are the logical target of many enrichment programs. Large predators can be difficult to enrich because their (highly motivated) food acquisition behaviors often include stalking, chasing, and capture of living prey. In most cases, feeding live prey to captive

carnivores would be difficult, unethical, and expensive, so zoos must get creative with strategies to present enrichment that provides predators the opportunity to express these behaviors, but without the complications of offering live prey. For example, Metter, Harriger, & Bolen (2008) presented lions (*Panthera leo*) and tigers (*Panthera tigris sumatrae*) with stimulus-based enrichment (including frozen blood balls, fresh zebra dung, scented squash, and cardboard boxes) aimed at increasing behavioral diversity. Carcass feeding has also been used as an enrichment option to stimulate predatory behavior in large carnivores (McPhee, 2002; Stark, 2005).

Another way to potentially enrich captive carnivores is to simulate live prey. This could promote stalking, chase, and capture behaviors that may not be stimulated via other methods of carnivore enrichment. American alligators (*Alligator mississippiensis*) are large, reptilian carnivores native to the southeastern United States that are also abundant in zoos and aquaria. Enrichment for alligators has not been studied extensively, though it does already occur in some locations. My goal was to evaluate a novel feeding enrichment strategy and determine its effects on alligator behavior. I used an evidence-based framework to assess the enrichment for efficacy and to determine whether it would be a suitable option for routine enrichment. Specifically, I evaluated a feeding enrichment designed to simulate live prey and target predatory behaviors in five adult alligators living in a shared outdoor exhibit at the Jacksonville Zoo and Gardens. I hypothesized that simulating live prey organisms within the alligator habitat space would promote chasing, lunging, and capture behaviors. I also hypothesized that activity would increase outside of enrichment offering sessions as well.

Methods:

Study subjects and enclosure setup

The study subjects included five adult alligators, one male and four females, living together in an outdoor habitat at the Jacksonville Zoo and Gardens. During the warmer months (April-October) there is a clear dominance hierarchy within this group of alligators. The male and dominant female are a breeding pair that are dominant over the three subordinate females. The dominant breeding pair maintain full control of the water feature of the exhibit space during the breeding season (April-June) and the duration of the hot summer months. Attempts by subordinate females to access the water feature during this time are often met with aggression from the dominant female. This results in the subordinate females remaining mostly on land for the duration of breeding/nesting season.

The alligators' exhibit includes a large land area and a large water feature. The visitor viewing walkway is on the front half of the space lining the edge of the water and there is a privacy fence surrounding the land to the back of the exhibit. There are three main entry/exit points for the alligators along the land/water border within the exhibit, indicated with circles in Figure 2.

Following the Evidence-Based Framework

This study followed an evidence-based method of zoo management. The details of how this study adhered to these steps is as follows:

1) Identify a problem or area to improve
    a. In this case, there was not an existing and pressing welfare concern, such as the presence of abnormal behaviors, that needed to be addressed for these animals.

Rather, the goal of this enrichment was to promote positive behaviors through enrichment and work towards optimal welfare and wellness.

2) Gather preliminary evidence
    a. In this case, preliminary evidence about natural history, diet, and predatory style was gathered from the literature.
3) Implement solution based on evidence
    a. This information from the literature was used to develop a feeding enrichment with the goal of simulating live prey, promoting predatory behaviors, and increasing general activity levels.
4) Evaluate the outcomes of the solution
    a. Behavioral data were collected before, during, and after implementing the enrichment in order to be able to analyze what, if any, effect the enrichment had on target behaviors (activity and predatory behaviors).
5) Modify and improve the solution if necessary
    a. Based on the outcomes of the enrichment and whether or not it achieved behavioral goals, the method should be modified, improved, and evaluated again until welfare goals are achieved.

Enrichment Description

The goal of this enrichment program was to simulate live prey organisms within the exhibit space in order to provide the alligators with opportunities to express predatory behaviors such as chasing, lunging, and prey capture. To simulate the prey, we attached the alligators' daily diet of frozen/thawed rats one at a time to a line at the end of a long pole. As a precaution, we used catgut (a type of edible fishing line) to secure the rats to the line in the event that the

alligators ingested any of it. Once secured to the line, the rat was cast from the visitor walkway into the water feature of the exhibit and pulled across the surface until it was captured by an alligator. This process was repeated with a new rat until the pre-portioned amount of rats was consumed.

Behavioral Observation and Study Timeline

Behavior data were collected using ZooMonitor (Ross et al., 2016; Wark et al., 2019) during 30-minute simultaneous focal follows of all five individuals for five weeks during July and August of 2019. Information about behavior budget and proximity to other individuals was collected using point sampling at 2-min intervals. Space use was also recorded every two minutes and encoded as X,Y pixel coordinates on a the habitat map image (Fig. 2) using ZooMonitor's space use tool. Interactions with enrichment were recorded only during enrichment offering sessions using an all occurrence sampling method. Behaviors were identified using an ethogram developed during one week of pilot observations in June 2019 (Table 1). The study followed an ABA design, with two weeks of baseline observations with no manipulations preceding two weeks of enrichment offerings. Enrichment was offered twice a week in the mornings between 9:00-10:30am during these two weeks of the study. One week of post-baseline data was collected after the two enrichment weeks. A detailed timeline of the study is presented as Figure 3. A total of 58 hours of data were collected at randomly selected times of day spread evenly over the five weeks of observations. A total of seven people collected all data with an interobserver reliability percentage of >85%.

An additional two weeks (12 hours) of observations were conducted in June-July 2020 in an attempt at a second phase of enrichment testing. This phase was, unfortunately, discontinued due

to the coronavirus pandemic. Data from this period, however, were still used in analyses of substrate use and spatial distribution.

Data Analysis

Activity budget data were first compared between conditions using SPSS v. 26 (IBM Corporation, Armonk, NY) to determine the effects of enrichment on behaviors like swimming and locomotion. The data were found to be non-normal using Kolmogorov-Smirnov tests, a common occurrence in zoo studies with small population sizes (in this case, n=5). Because the data were non-normal, non-parametric Wilcoxon sign rank tests were used to determine the presence of statistical differences. Data collected during enrichment weeks (B) were separated into enrichment offerings and non-enrichment offering sessions. This was done in order to determine if activity increased during enrichment offerings as compared to non-enrichment offering sessions within the same time period. Non-enrichment offering sessions were also compared to data collected during weeks where enrichment was not given (A) in order to determine if enrichment was having any effect on behavior as compared to baseline. All occurrence enrichment interaction behaviors were evaluated quantitatively but no statistical analyses were run.

Engagement (or not) with enrichment was then linked back to social dynamics and use of space. In order to run analyses on this component, the exhibit image used to collect spatial distribution information was first divided into 100x100 pixel “zones” (Fig. 4). Space use XY coordinate information was then sorted into matrices representative of these zones for each individual. One female, Colonel, appeared to be dominant and to patrol the water/land border entry/exit points preventing other females from coming into the water. To test the hypothesis that she preferred these particular zones, we used custom code and RStudio v. 1.3.1073 to run a

permutation analysis that compared Colonel's space use matrix to 10,000 randomly generated matrices. This analysis was run on the other four individuals to determine if there were locations within the exhibit that they preferred as well. The return of this analysis identified zones in which an individual spent significantly more time and therefore preferred. We also hypothesized that during the breeding season the females (including subordinates) had become territorial and carved out particular areas of the exhibit for themselves. To test this hypothesis and determine if there was significant overlap (or not) of spatial distribution between females, we compared each female's space use matrix to the other three using mrqap.dsp analysis with 1000 randomizations. These analyses were run for spatial distribution data from both 2019 and 2020. Detailed R code for both of these analyses can be found in Appendix 1.

This research was conducted at the Jacksonville Zoo and Gardens under permit number 19-004 issued by the UNF Institutional Animal Care and Use Committee.

Results:

Goal 1: Increase general activity levels outside of enrichment offering sessions.

The enrichment was not successful in achieving this goal. In fact, the three subordinate females, Bulldog, Snaggle, and Patty, were nearly 100% inactive for the entire five weeks of study (Fig. 5). The dominant pair, Colonel and Cipowitz, were more active than the subordinate females overall and, although enrichment did increase activity during enrichment offering sessions for Colonel, this increase was not significant ($p=0.068$, $Z=-1.826$) and was not sustained in non-enrichment offering sessions (Fig. 5). It is possible that significance was not found in this analysis because there were very few enrichment sessions ($n=4$) compared to baseline+postbaseline ($n=54$) and non-enrichment ($n=64$) sessions.

Goal 2: promote predatory behaviors like chase, lunging, and prey acquisition.

The enrichment was both a success and a failure in achieving this goal. It was successful in promoting these predatory behaviors in the dominant breeding pair, Colonel and Cipowitz, as they consistently interacted with the enrichment, but the subordinate females did not interact with the enrichment at all. Colonel, the dominant female, interacted with the enrichment an average of 1.83 times per offering (with a range of 1 to 5 interactions) and Cipowtiz, the dominant male, an average of 4.3 times per offering (with a range of 1 to 7 interactions). We define "interaction" with the enrichment as any lunge, snap, or contact related to the rat on the line.

Social Organization and Space Use

There was differential use of substrate (land/water) observed among the five individuals in the group. Subordinate females (Bulldog, Snaggle, and Patty) were rarely observed in the water during the five weeks of study in 2019 (Fig. 6). However, subordinates Bulldog and Patty were observed in the water considerably more in 2020.

Differences in spatial distribution of individuals throughout the exhibit were also observed. Based on the visuals presented in Figure 7, we hypothesized that Colonel was patrolling the land/water barrier and preventing the other three females from entering the water. Analysis on Colonel's space use matrix revealed that she preferred to spend more time in front of the main entry/exit point (zone 14) than anywhere else in the exhibit. The same analysis on the other four individuals found that they, too, each prefer a certain zone over all others (Table 2). The land-based females also appeared to have carved out territories in which their spatial distribution did not overlap with other females. Analyses comparing each female's distribution matrix to the

others revealed that only Bulldog and Snaggle had significantly overlapping spatial distribution (regression coefficient: 1.45, $p<0.0001$). The only other two individuals with overlapping distribution were Colonel and Cipowitz, a breeding pair (regression coefficient: 1.05, $p<0.0001$).

A second phase of the study was attempted in June-July 2020 but had to be cut short due to the coronavirus pandemic. During this time, we were able to gather 12 hours of spatial distribution data that indicate that preferred zones (Table 2) and distribution overlap were not consistent between the two summers. In 2020, Colonel did not prefer zone 14, but rather zone 7 which does not correspond with an entry/exit area along the land/water border. Instead, Bulldog spent most of her time in zone 14 rather than zone 1. Snaggle and Patty maintained their preferred zones from the previous year. Similar to 2019, in 2020 Colonel and Cipowitz had significantly overlapping spatial distributions (regression coefficient: 1.5, $p=0.017$). Unlike 2019, in 2020 Bulldog and Snaggle did not overlap distributions, but rather Bulldog and Colonel significantly shared space (regression coefficient: 0.86, $p=0.011$). Spatial distribution heat maps for 2020 can be found in Figure 8.

Ten total instances of aggression were observed during the five weeks of study in 2019 (Table 3). Two of these interactions occurred during the final enrichment offering session in which Colonel was the aggressor.

Discussion:

Although this enrichment was successful in promoting predatory behaviors in the two dominant individuals, it had several drawbacks that made its effectiveness as a long-term enrichment option questionable. First, there were no long-lasting behavioral effects for the two individuals that did participate. The goal of this enrichment was not only to provide opportunity

to express predatory behaviors, but also to promote an increase in daily activity levels as a result of the stimulation from the enrichment. Excessive inactivity can be a problem for captive animals, leading to conditions such as obesity and muscle tone loss, and therefore increasing activity levels is a goal of many enrichment efforts (Newberry, 1995; Warwick et al., 2013).

This enrichment was also not effective in engaging all individuals. None of the land-based subordinate females came into the water to participate. This is likely due to an established dominance hierarchy among the females in which Colonel is dominant and monopolizes access to both the male and the water feature of the exhibit during the breeding/nesting season. Although it has never been empirically evaluated, anecdotal reports from crocodilian caregivers at multiple institutions have observed fluctuating dominance hierarchies, with strong hierarchies developing in the warmer spring and summer months that disintegrate in the fall and winter as temperatures drop (J. Darlington, personal communication). In pilot observations during May and June 2019, Colonel was observed being aggressive towards other females fairly frequently. However, over the five weeks of study in July and August, only 10 total aggressive interactions were observed and Colonel was the aggressor in only five of them (Table 3). This suggests that Colonel likely asserted her control over the male and the water feature in the beginning of the breeding season (March-April), resulting in more established boundaries by the time this study officially began and less observed aggressive interactions.

Colonel is more easily able to control the other females' access to the water feature due to the design of this particular exhibit. This exhibit is designed so that there are limited points along the land/water boundary in which the alligators can easily enter or exit the water potentially allowing Colonel to patrol these areas and limit access. In an analysis of her spatial distribution, Colonel was found to prefer zone 14 which corresponds to the main entry/exit point along the land/water

boundary (Fig. 4). Her preference for this spot supports the hypothesis that she is using this element of the exhibit in order to successfully keep other females out of the water. Perhaps a modification to the exhibit in which the entire land/water boundary was available for entry/exit would eliminate Colonel's ability to monopolize any particular feature of the exhibit. In future exhibit designs, perhaps also incorporating more than one water feature would be beneficial to prevent any individual from totally monopolizing a limited resource.

A tendency to keep territories and have preferential use of exhibit space was also found for the three land-based females. Patty maintained a strong preference for zone 3 with no overlap with other individuals. Although Bulldog and Snaggle did share some pattern in spatial distribution, each were found to more strongly prefer a different zone than the other (Bulldog, zone 1; Snaggle, zone 13). It is likely that a combination of a tendency to create boundaries and threat of aggression for breaking those boundaries can explain, at least in part, why the subordinate females did not enter the water to participate in enrichment. In fact, during the last enrichment session, both Snaggle and Patty were the targets of aggression from Colonel when they attempted to enter the water.

Another drawback to this enrichment strategy was the time commitment. Although it was inexpensive and required only materials that zoos are likely to already have on hand, each enrichment offering session took keepers an average of 30-45 minutes to complete. At several sessions each week, this is not a trivial amount of time and not every institution's care staff will have that kind of time to spare on a continuous basis, particularly for an enrichment that does not engage all individuals and does not reach all behavioral goals. Each enrichment session did draw a large crowd, however, and was greatly enjoyed by visitors. Perhaps if this enrichment could be paired with an educational experience for guests, it would be worth the time it takes to offer it.

Future enrichment efforts for this group need to take into account the dominance hierarchy and tendency for females to carve out preferred territories within the exhibit during the breeding season. The presence or absence of a nest might be a good indicator of whether or not these territories will form in a particular year. In summer 2019 when Colonel monopolized the water feature and the other three females were land-based, there was a female-built nest present during all five weeks of study. Interestingly, this pattern of monopolization did not appear the following summer (2020) when there was no nest built early in the season. In this case, subordinate females were found to use the water feature more often (Fig. 6) and also share space with Colonel. It is possible that in a year where this looser social structure is observed and subordinate females have wider use of the exhibit space that a water-based enrichment like the one tested here would engage more individuals.

Future enrichment should also aim to engage individuals for longer periods of time. Rather than trying to simulate prey capture, perhaps predatory behaviors could be promoted in different ways that might have more long-lasting effects and that are less time consuming for caregivers. For example, it is likely that we could learn a lot from evaluating enrichment often used with big cats. Evidence suggests alligators use olfaction to detect chemical cues in both air and water to detect prey (Weldon et al., 1990; Weldon et al., 1992). Perhaps an enrichment like the frozen blood balls used by Metter et al. (2008) that plays to their olfactory abilities could be stimulating for alligators. An enrichment such as this would also introduce novelty, as a floating ball of ice is likely to be an object they have never before encountered. Introducing novelty has been found to be enriching for reptiles (Moszuti et al., 2018) and some species have even been described as exhibiting play behaviors when presented with novel enrichment objects (Burghardt, Ward, & Rosscoe, 1996).

Although anecdotal evidence suggests that there is more enrichment being provided for reptiles in captivity than is being reported, there are few studies evaluating specific enrichment methods for their effectiveness in reptilian groups (Eagan, 2018). This study aimed to add to this small body of knowledge by implementing an evidence-based framework and evaluating a feeding enrichment that simulated live prey for alligators, a large reptilian carnivore. The lessons learned about the logistics of providing this type of enrichment are valuable even if it was not successful in achieving all behavioral goals. If this type of enrichment is attempted in the future, group social dynamics and keeper time/effort should be taken into consideration.

Chapter 2: Effects of human activity on stereotypic pacing behavior in coyotes: implementing an evidence-based framework in real time to make decisions regarding environmental intervention and its impact on welfare

Introduction:

Captive animals may experience stress for a variety of reasons. Stress is a difficult term to define, but I will use the definition from Morgan and Tromborg (2007), "anything that challenges homeostasis." The authors explain that this challenge can be real and physical (e.g., restraint, exposure to extreme temperature or weather without reprieve) or a perceived threat (e.g., a veterinarian approaching with gloves or a display from a more dominant individual). Any exposure to real or perceived threats results in the animal preparing for a “homeostatic challenge” and engaging the fight-or-flight response.

Consistent exposure to human activity can elicit stress and anxiety related behaviors in both wild and captive animals. Brown et al. (2012) evaluated the impact of noise and human activity on vigilance behavior in wild elk and pronghorn, revealing that these behaviors increased in response to pedestrian traffic but not other types of human activity more associated with noise, like freeway traffic. This might suggest that these groups are more sensitive to the visual presence of humans rather than disturbed by the noise associated with human activities. Jayakody et al. (2008) found similar results in wild red deer (*Cervus elaphus*) where deer in areas more highly disturbed by tourist activity exhibited much more vigilance behavior than deer in less disturbed areas. Human activities like hunting have also been found to increase vigilance behaviors, flying, and fecal glucocorticoid metabolite concentrations in free ranging little bustards (*Tetrax tetrax*; Tarjuelo et al., 2015).

Due to the nature of the zoo environment, animals come into proximity with people multiple times throughout every day. Zoo visitors are an unavoidable component of a zoo animal's environment and exposure to guests has been shown to have negative impacts on behavior and other indicators of well-being in a variety of species. Increased aggression, vigilance, and hiding behaviors have been observed in little penguins (*Eudyptula minor*) in the presence of zoo visitors compared to when their exhibit was closed to the public (Sherwen et al., 2015). Koalas (*Phascolarctos* cinereus) also respond with increased vigilance behaviors as number and noise levels of visitors increase (Larsen et al., 2014). Greater rheas (*Rhea americana*) have been shown to exhibit alert behavior more in the presence of visitors than in their absence (Azevedo et al., 2011). Pere David's deer (*Elaphurus davidianus*) show more vigilance behavior when kept in an enclosed pen area than when able to free range and distance from tourists (Li et al., 2007). Schell et al. (2013) evaluated the effects of proximity to the park entrance on stress levels in coyotes (*Canis latrans*) and found that individuals housed in pens closer to the entrance had higher peaks in stress hormones than those housed in more remote locations of the park. The authors hypothesized that being in the consistent sightline of unpredictable human activity decreased the coyotes' sense of being able to cover or conceal themselves and led to the increase in stress.

Staff activities are also a component of the captive environment that bring animals within proximity of humans on a daily basis. The act of keepers entering enclosures for routine husbandry tasks has been discussed as a component in stress and anxiety related behaviors in a variety of captive species. In captive ungulates, keeper presence within the enclosure increases visual orientation behaviors (Thompson, 1989). Capuchin monkeys (*Sapajus apella*) and macaques (*Macaca* mulatta) show increased fear and stress-related behaviors when keepers enter their enclosures (Rimpley & Buchanan-Smith, 2013; Gottlieb, Coleman, & McCowan, 2014).

Even just the presence of keepers near an animal's area can trigger these behaviors related to stress and anxiety. A study on coyotes showed a two-fold increase in vigilance behavior during husbandry related activity, even if care takers were not directly attending to the focal individual's enclosure (Schultz & Young, 2018).

The ability for a captive animal to conceal themselves or to retreat from human activity is an important factor in decreasing the prevalence of these anxiety related behaviors. The majority of these studies have evaluated the value of retreat space and visual barriers with regards to visitors, but these concepts may be extended to the ability of animals to distance themselves from all human activity associated with the captive environment, including zoo staff and keeper activities. Anderson et al. (2002) found that restricting retreat space for petting zoo animals (African pygmy goats, *Capra hircus*, and Romanov sheep, *Ovis aries*) had negative consequences on their behavior and that undesirable behaviors were exhibited at lowest frequencies when the animals were offered a full retreat space. When given access to visual barriers, a variety of species will use them to conceal from visitors (Gorillas, *Gorilla gorilla*, Blaney & Wells, 2004) and approaching humans (Blue foxes, *Vulpes lagopus*, Mononen et al, 2001). Blaney and Wells (2004) found that gorillas exhibit fewer abnormal behaviors and are less aggressive towards enclosure companions when visual barriers are placed in the guest viewing area.

Chronic exposure to human activity that is perceived negatively by an animal can lead to the development of maladaptive and abnormal behaviors called stereotypies. The presence of stereotypies is generally interpreted an indicator that an animal is experiencing a negative affective state and suggests suboptimal welfare (Mason, 1991). Stereotypies have been hypothesized to manifest as a sort of "do-it-yourself" enrichment in animals that are experiencing boredom due to under-stimulating environments (Mason & Latham, 2004). The

most commonly assessed stereotypy is pacing, a repetitive locomotion pattern associated with stress, anxiety, and inadequate captive environments. One of the many hypotheses addressing why pacing might manifest suggests that it emerges as an outlet for species with naturally large home range sizes to replace natural locomotion patterns. This is cited as a common reason for pacing behaviors in carnivore species (Clubb & Vickery, 2006). In fact, pacing has been found to positively correlate with home range size in an analysis of 33 species of carnivores (Clubb & Mason, 2007). This analysis found that median distance traveled by a species in the wild was the best predictor of whether an individual of that species would develop pacing behaviors in captivity.

The current study will evaluate the effects of human activity on pacing behavior as an indicator of stress in a zoo-housed, naturally wide-ranging species, the coyote (*Canis latrans*). This project aimed to implement an evidence-based framework while determining a solution to existing welfare concerns in two adult male coyotes at the Jacksonville Zoo and Gardens. These brothers, Watson and Sherlock, reside in an enclosure that is open to all four sides with chain link fencing. This project was initiated by care staff who were concerned that excessive human activity, namely the proximity of the zoo train tracks and a staff-only path running directly adjacent to the back fence of the exhibit, was a source of stress and welfare compromise for these individuals. They brought this concern to the attention of Animal Wellness research staff with the hopes of collecting more information about the coyotes' behavior budgets and reactions to traffic and human activity behind their exhibit. Research staff developed an ethogram and began to investigate the issue. The following chapter explores the timeline of this project and how evidence was used to help inform a decision about a change to the coyotes' environment that ultimately led to an improvement in welfare.

Methods

Study Subjects

The subjects of this study were two adult male coyotes, Watson and Sherlock, residing in the Wild Florida loop at the Jacksonville Zoo and Gardens. These coyote brothers were wild born but eventually found their way to a wildlife rehabilitator as pups. Unfortunately, they became too habituated to humans to be released and the Jacksonville Zoo and Gardens offered to serve as their permanent home. They have been residents of the zoo since 2016. Watson appears to be the more dominant individual, becoming aggressive and possessive over resources such as keepers and food. He is also much more likely to approach people and appears to be the more "confident" of the two. Sherlock appears to be the more submissive individual, often on the receiving end of aggression from his brother, seeming nervous and avoiding approach from keepers and other humans.

Enclosure Description

The coyotes' exhibit is a long rectangle enclosed with a chain link fence on all sides. To either side of the enclosure are adjacent animal exhibits separated by chain link fences and natural vegetation. To the front, the guest walkway is separated by an additional wooden fence and about three feet of space. Guest traffic in the Wild Florida area is generally low, with guests present in only 23% of observation intervals (7 min per 30 min session). To the back of the exhibit, there is a staff-only path running directly adjacent to the exhibit fence. Staff traffic along this path includes golf carts, bikes, and foot traffic throughout the day. 15.5 feet removed from the staff path are the zoo train tracks. The train runs about every 30-45 minutes during operational hours all days of the week. 9 feet beyond the train tracks is another chain link fence

lined with natural vegetation directly adjacent to the parking lot. There is a seven-foot-wide sidewalk separating this fence from parking cars. The staff path, train tracks, and parking lot are all visible from the guest walkway and from inside the coyote exhibit.

Following the Evidence-based Framework

This study followed an evidence-based method of zoo management. The details of how this study adhered to these steps is as follows:

1) Identify a problem or area to improve
    a. Care staff identified an existing welfare concern for our coyotes and brought it to the Animal Wellness research team for further investigation. Their main concerns were that the pair seemed to be vocalizing to the zoo train every time it passed and that they generally exhibited high rates of stereotypic pacing behaviors.
2) Gather preliminary evidence
    a. The JZG Animal Wellness research team worked with care staff to set down goals for the project, namely identifying sources of stress that might be causing pacing and to gather evidence to confirm or refute the claim about the train's impacts. Wellness developed an ethogram and began gathering behavior budget data as well as reactions to staff traffic along the path behind the exhibit.
3) Implement a solution based on evidence
    a. During step 2, enough evidence was gathered to support the case that the coyotes' welfare would be improved by restricting access to staff traffic along the path behind the exhibit.
4) Evaluate the solution

    a. Data continued to be gathered after implementing the solution in order to determine if the desired effects were being maintained.

5) Modify and improve

    a. Even after implementing this solution, some concerns about this pair's welfare remain. Data collection continues so that we might monitor the situation and continue to make improvements to their environment that are beneficial for behavior and welfare.

Study Timeline

This study began as purely observational as a way to gather evidence about how the zoo train and traffic along the staff-only path behind the exhibit were influencing coyote behaviors. About six months into the study, as solutions to behavioral issues were being discussed, a natural experiment presented itself when the zoo closed to guests for eight weeks due to concerns surrounding the covid-19 pandemic. During the shutdown, the zoo maintained a reduced staff and this decrease in staff capacity led to reduced cart, foot, and bike traffic along the path behind the exhibit. Wellness staff continued taking observational data during the closure and a preliminary analysis showed that the decrease in staff-only path traffic was effective in reducing pacing and increasing resting behaviors. Upon reopening to guests and regaining full staff capacity, Wellness staff and management worked together and used these preliminary results to come to the decision to close the staff-only path indefinitely to most traffic (some foot traffic is still allowed, although staff are encouraged to take a different route if able). Data collection continued for four additional months after the zoo reopened to guests, regained full staff capacity, and official staff path restrictions were implemented. A detailed timeline of the study can be found in Figure 9.

Data Collection

Data collection began on September 1, 2019 and ran through August 22, 2020, encapsulating a full year with a total of 91.5 hours of in-person observations (50 hours before traffic restrictions and 41.5 hours after). Focal follow observation sessions lasted 30 minutes and data were collected using the ZooMonitor application (Ross et al., 2016; Wark et al., 2019). Behavior budget data were collected at 1-min intervals as well as all occurrences of reactions to staff path traffic and the zoo train. A full description of behaviors and traffic types can be found in Table 4. An interobserver reliability of > 85% was obtained between all four observers.

Statistical Analyses

Kolmogorov-Smirnov tests were run to determine the normality of the data. Significance of these tests ($p<0.05$) indicated a non-normal data set, which is to be expected when using such a small sample size ($n=2$) and standard in many zoo-based case studies. Due to failure to meet standards of normality, the non-parametric Wilcoxon signed rank test was used to compare activity budget data between conditions (before, during, and after shutdown) for both individuals. Since multiple comparisons were run on each dependent variable (activity budget behaviors) a Bonferroni’s correction was conducted. This correction did not drastically change the critical alpha value (from 0.05 to 0.049) and did not influence the significance of any comparisons.

Results

Initial Observations

Initial observations of behaviors were conducted from September 1, 2019-March 12, 2020 without any alterations to the amount of traffic present along the staff-only path behind the

exhibit. During this time, point samples of activity budget behaviors were recorded at 1-min intervals and reactions to staff path traffic, including golf carts, foot traffic, and bikes, as well as reactions to the zoo train were recorded as all occurrences. Each focal follow session lasted 30-min and behaviors of the two individuals were recorded simultaneously, though separately.

The first result to come out of this six-month preliminary period was that the zoo train was only eliciting a vocalization response from the pair about 8% of the times that it passed the exhibit (Fig. 10). This was fortunate news as this was the primary concern of care staff who initiated the project. Reactions to other kinds of traffic along the staff-only path were also recorded during this period. The most common reaction to staff traffic along the path was "none", however, "approach", "startle", and "avoid" reactions were also common (Fig. 10).

Examination of behavior budget data revealed that both coyotes exhibited relatively high rates of pacing behavior, on average 9.6% for Watson and 18.8% for Sherlock (Figure 11a&b). Sherlock also exhibited low rates of resting, in fact pacing more on average than he rested (Figure 11b).

Covid-19 Closure: A Natural Experiment

On March 13, 2020, a natural experiment presented itself when the zoo closed for eight weeks due to concerns surrounding the covid-19 pandemic. Along with closing its doors to guests, the zoo's response included temporarily reducing its staff. This limit on staff capacity led to reduced traffic along the path behind the coyote exhibit (Fig. 12). Wellness staff continued to observe the coyotes during this time and noticeable reductions in pacing and increases in resting behavior were observed (Fig. 13). This lead Wellness staff to hypothesize that perhaps the observed behavioral changes were due to the reduction in staff path traffic. This evidence helped

management and Wellness staff work together to make the decision to permanently restrict traffic along the staff-only path after reopening the zoo to guests and regaining full staff capacity.

Final Results After Reopening to Guests and Implementing Official Traffic Restrictions

The reductions in pacing and increases in resting behaviors observed during shutdown were maintained after the zoo reopened to guests and regained full staff capacity. Sherlock maintained a significant decrease in pacing and increase in resting after official path traffic restrictions were implemented (May-August, 2020; $p<0.001$; Fig. 13). Watson experienced only a significant increase in resting during this same time period ($p<0.05$) while his pacing levels returned to those similar to pre-restriction levels (September 2019-March 2020; Fig. 13). A full comparison of statistical analyses for pacing behavior between each condition can be found in Table 5. Percent change in pacing and resting behaviors before (Sept 2019-March 2020) and after (May-Aug 2020) official path restrictions can be found in Figure 14.

Discussion:

Overall, the results from this intervention were positive and resulted in more balanced behavior budgets for both individuals. During closure, both individuals experienced an increase in time spent resting and this increase was sustained after reopening to guests and implementing permanent restrictions to staff path traffic. Ideally, if the information is available, activity budgets of species living in the wild are good benchmarks when evaluating what is and is not an appropriate spread of behaviors for an individual in captivity. Fortunately, since coyotes are such a widespread species, there is lots of information about their behavior in the wild (Bekoff & Gese, 2003), a luxury not often afforded to many species kept in zoos. Wild coyotes have been observed to spend an average of 58% of their activity budget resting, so this is the proportion that

would be ideal for captive coyotes as well (Shivik et al., 2009). Before path traffic restrictions, neither Watson or Sherlock were even approaching this rate of resting, with Sherlock resting only an average of about 13% and Watson an average of 38%. However, after traffic restrictions, each individual is dedicating between 55-60% of their activity budget to resting. Additionally, studies have shown that an increase in resting behavior from a state of hyperactivity can be an indicator of positive welfare and increased feelings of comfort. Dairy cows that have been provided larger stalls and mattresses to lie down on have been observed to rest and lay down more than cows provided with small concrete stalls (Haley, Rushen, & Passille, 2000). We can interpret the observed increase in resting for the coyotes similarly and conclude that the solution we implemented was successful in relieving stress and promoting a calmer environment that increases feelings of comfort.

There were also changes in pacing behavior for both individuals throughout the study. Sherlock's decrease in pacing from an average of 19% of his budget to 6% is an overwhelming signal of improved welfare. Since pacing is often cited as a sign of stress and indicator of poor welfare, any decrease in this behavior is likely to suggest improved welfare and affective state (Mason, 1991). The observed decrease in pacing behavior for Sherlock and the maintenance of this decrease after reopening the zoo to guests suggests that it was likely the traffic along the path that was the main source of stress for him and that removing this stressor was a net positive for his well-being.

Watson, however, experienced a return to pacing levels similar to those observed before traffic restrictions were enforced. This demonstrates an important principle in modern welfare science that every animal is an individual and may react differently to certain environmental components than even members of its own species (and in this case even a related individual!). Studies

evaluating how individual differences can influence welfare are common in the literature. Barlow et al. (2007) examined how Diana monkeys (*Ceropithecus diana diana*) reacted differently to visitor presence depending on individual personality factors. Consistent individual differences have also been found to influence behavioral responses to enrichment in squirrel monkeys (*Saimiri sciureus*; Izzo, Bashaw, & Campbell, 2011) and bottlenose dolphins (*Tursiops truncatus*, Eskelinen, Winship, & Borger-Turner, 2015). This study is yet another example of how individual differences need to be considered when evaluating welfare for zoo animals.

The end result levels of pacing, 6% of budget for Sherlock and 11% of budget for Watson, however, are still not ideal. On a management level, this indicates that there are still components of the environment that are not compatible with these individuals. There may be certain biological factors that would predispose coyotes to developing stereotypies like pacing in captivity. This species has naturally large home ranges which require them to travel long distances in the search and pursuit of prey. Evidence has shown a correlation between home range size (Clubb & Mason, 2007) and hunting style (Clubb & Vickery, 2006) as components of natural behavioral biology that might predispose an animal to developing stereotypies. A study that evaluated the impacts of different enclosure sizes on stereotypic behavior in coyotes found that these undesirable behaviors decreased with increased enclosure size (Brummer, Gese, & Shivik, 2010). In this case, increasing the enclosure size may be a suitable future option for decreasing remaining pacing behaviors in these individuals.

On the other side of this hypothesis, Mason and Latham (2004) hypothesize that pacing in wide ranging species can manifest as a sort of "do-it-yourself" enrichment in order to replace natural locomotion patterns and mimick natural activity levels. In this case, the presence of pacing would be a sign that the animal is coping effectively within its given environment.

Another reason for pacing that might not necessarily indicate a poor environment is anticipatory pacing. This is pacing behavior that occurs surrounding positive events, like feeding or enrichment, that occur around the same time every day. It is possible that some of Watson and Sherlock's remaining pacing behavior is anticipatory response to positive events like feeding. More investigation would be needed to confirm or deny any of these hypotheses. Data collection on this project continues so that we may identify components of positive and negative welfare for these individuals and continue to improve the pair's enclosure in order to move towards more optimal welfare.

Conclusions

In each of these case studies we observed how collecting evidence over time helped to inform management and husbandry decisions. In the first case, we were able to use data gathered about behavior to determine that the novel feeding enrichment should not be continued. This enrichment, in theory, seemed like it would be effective and worth the time and effort it took to implement it on a regular basis moving forward. But by gathering behavioral data over the course of implementing the enrichment, we were able to determine that this wass not the case. We were also able to identify a unique social hierarchy which was not the initial intent. This discovery, however, will likely be helpful in making future decisions about housing, habitat modifications, and enrichment for this group.

In the second case, we were able to use data collected over a long period of time to identify behavioral patterns in response to environmental changes in our coyotes. Had we not been tracking their behaviors, we likely would not have known that a decrease in staff activity resulted in a decrease in pacing and increase in resting. The intervention that led to maintaining these changes in behavior would not have been made without this evidence and welfare would not

have improved as a result. It is my hope that these case studies will contribute to the growing body of literature that advocates for methods of evidence-based zoo management to be used more frequently and promoted as the norm in the modern zoo.

Appendix 1

Intraindividual spatial distribution matrix evaluation

```
"Individual space use matrix"<-as.data.frame(listMarisa[[2]])

"Individual space use matrix"<-"Individual space use matrix"[,2:6]

rownames("Individual space use matrix")<-colnames("Individual space use matrix")

"Individual space use matrix"<-as.matrix("Individual space use matrix")


permMatList <- list()

    for (i in 1:10000){

        mat<- "Individual space use matrix"[sample(nrow("Individual space use
matrix")),sample(nrow("Individual space use matrix"))]

        permMatList[[i]] <- mat

    }


cRWMat <- matrix(nrow = length("Individual space use matrix"[,1]), ncol = length("Individual
space use matrix"[1,]), 0)

dRWMat <- matrix(nrow = length("Individual space use matrix"[,1]), ncol = length("Individual
space use matrix"[1,]), 0)


for(i in 1:10000){

        perm<-permMatList[[i]]

        obser<-"Individual space use matrix"

        for( i in 1:length(perm[1,])){

            for( j in 1:length(perm[1,])){

                if (perm[i,j] >= obser[i, j]){

                cRWMat[i,j] <- cRWMat[i,j] + 1

                }else{

                dRWMat[i,j] <- dRWMat[i,j] + 1

                }

            }
```

```
		}
}


sigMat<- matrix(nrow = length("Individual space use matrix"[,1]), ncol = length("Individual space use matrix"[1,]), 0) # positive signifcance

rownames(sigMat) <- rownames("Individual space use matrix")

colnames(sigMat) <- rownames("Individual space use matrix")


for( i in 1:length(cRWMat[1,])){
	for( j in 1:length(cRWMat[1,])){
		if(cRWMat[i,j] <= 500){
			sigMat[i,j]<-1
		}else{
			sigMat[i,j]<-0
		}
	}
}
```

Interindividual spatial distribution comparison

```
library(readxl)

filename <- list.files(path = "path to file with spatial distribution matrices"*.xlsx", full.names = T)

listfiles<- lapply(filename, read_excel, col_names = T)

listfiles2 <-lapply(listfiles, as.matrix)

listfiles3 <-lapply(listfiles2, x[,2:6])

library(asnipe

mrqap.dsp()
```

```
“Individual 1”<-listfiles2[[“corresponding list number”]]

“Individual 1”<-“Individual 1”[,2:6]

“Individual 1”<-as.data.frame(“Individual 1”)

rownames(“Individual 1”)<-names(“Individual 1”)


mrqap.dsp(as.matrix(“individual 1”) ~ as.matrix(“Individual 2”) + as.matrix(“Inidividual 3”) +
as.matrix(“Individual 4”), directed = T, test.statistic = "t-value",

        tol = 1e-07, randomisations = 1000)
```

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Table 1. Ethogram of alligator behaviors developed during one week of round-the-clock observations in early June 2019.

Type	State	Sub-state	Event	Description
All Occurrence	Social	Aggression	Contact	Focal animal directs agonistic social behavior towards conspecific and makes contact. Includes biting and direct contact resulting from a chase. Identify other individual involved.
			Non-contact	Focal animal directs agonistic social behaviors towards conspecific but does not make contact. Can include chasing and actively displacing another individual. Identify other individual.
			Intimidation	Dominant female sits perpenicular to shore while vigorously waiving tail back and forth.
			Passive Displacement	Dominant female waits below a subordinate female on land, preventing her from entering the water for 3 seconds or more. Dominant female must be perpendicular to land, at least 3' from water's edge. Subordinate female must be at least 3' from water's edge.
		Courtship	Headslapping	Focal animal engages in any stage of the fixed action pattern head slapping display.
			Bellowing	Focal animal assumes arched back posture and emits low frequency, growl type sounds from the mouth. Bouts of bellowing are separated by at least one minute..
			Copulation	Focal female engages in copulation with the male, or focal male engages in copulation with a female.
			Mount	Focal female mounts another individual, either the male or another female, so that her body is directly on top of the other's for 3 seconds or more. Indicate other individual.
	Object		Investigate	Focal animal exhibits open-mouthed snout swaying investigation behavior
			Snap	Focal animal quickly open and closes mouth in relation to feeding; often occurs during bouts of investigation. If snapping at wildlife, please note the kind of animal.
			Contact with Enrichment	Focal animal makes contact with an enrichment device (rat)
Point Samples	Active	Locomotion	Terrestrial	Focal animal changes location on land by at least three steps in a single bout, propelled by hind and front legs.
			Swimming	Focal animal changes location in the water by at least 6', propelled by leg and tail movements. Bouts of swimming are separated by at least 3 seconds.
	Other		Out of View	Focal animal is out of view or behavior cannot be confidently determined.
			Inactive	Focal animal is immobile and not displaying any other notable behavior.
			Other	Focal animal is displaying a behavior not already described. Please describe in notes section.
	Space Use	Proximity	Distant	Focal animal's head is greater than 6' away from any part of another animal's body
			Proximate	Focal animal's head is less than 6' away from any part of another animal's body; indicate which individual is within distance.
			Contact	Any part of focal animal's body is visibly touching part of another alligator. Do not assume contact underwater. However, if an animal is obviously on top of another (such as during mounting) it is okay to code contact even if you can't directly see where they touch. If unsure, code proximate.
			Unknown	Focal animal's proximity to other alligators is unknown. Includes when the focal animal is out of view or submerged.
		Substrate	Land	Focal animal's entire body is on dry land.
			Water	Focal animal's entire body is in water.
			Edge	Focal animal's body is in contact with both water and land substrates.

Table 2. Results of individuals' preferred zones in the exhibit based on space use matrix analyses.

Individual	Preferred Zone 2019	Preferred Zone 2020
Bulldog	1	14
Snaggle	13	13
Patty	3	3
Colonel	14	7
Cipowitz	14	7

Table 3. List of observed aggressive interactions in the presence/absence of feeding enrichment.

Enrichment present?	Initiator	Target
None	Colonel	Snaggle
None	Bulldog	Snaggle
Present	Colonel	Snaggle
Present	Colonel	Patty
None	Cipowitz	Bulldog
None	Cipowitz	Bulldog
None	Bulldog	Snaggle
None	Bulldog	Snaggle
None	Colonel	Patty
None	Colonel	Patty

Table 4. Ethogram of behaviors and descriptions of traffic types.

Channel Type	Behavior Category	Behavior	Definition
All Occurrence	Reaction to Traffic	Startle	Focal animal suddenly changes behavior in the presence of stimuli (back path traffic, visitors, train, etc.). Includes rising from a resting position and sudden, drastic changes in body posture.
		Avoid	Focal animal moves in the opposite direction of a stimulus. May or may not occur after a startle.
		Approach	Focal animal moves towards a stimulus (ex. Individuals greet keepers as they walk past on the back path).
		None	Focal animal does not break from behavior and has no noteable reaction to the presence of a stimulus.
	Traffic Type	Train	Zoo train passes behind the exhibit. Wait to record individuals' reactions until after the train has passed.
		Foot Traffic	Staff member(s) pass behind the exhibit on foot. Wait to record individuals' reactions until after staff has passed.
		Bike	Staff member(s) pass behind the exhibit on bicycles. Wait to record individuals' reactions until after staff has passed.
		Golf Cart	Staff member(s) pass behind the exhibit in a golf cart. Wait to record individuals' reactions until after staff has passed.
Interval	Aberrant	Pace	Translocation from point A, to B, back to A (or a series of locations). Bouts of pacing end after the animal is engaged in another behavior for more than 3 seconds or breaks the locomotion pattern.
	Locomotion	Walk	Coyote moves more than one body length in a calm smooth gate.
		Trot	Coyote moves more than one body length in a slightly faster, bouncy gate.
		Run	Coyote moves more than one body length in a very fast, canter or gallop-like gate.
	Other	Stationary	Focal animal is not moving or engaging in any other noteable behavior, but is still alert (not resting). Eyes must be open and head picked up off the ground. May be sitting or standing.
		Rest	Focal animal is not moving or engaging in any other noteable behavior, but is resting. Must be laying down with eyes open or closed.
		Vocalize	Animal produces audible sound from the mouth.
		Other	Any behavior that does not fall under any other description. Make sure to note what occurred in the all occurrence section.
		Out of View	Not able to be seen, inside of night house, off exhibit, behind shrubs.
	Visitor Denisty	Present	Guests are present within the range of the exhibit fence.
		Not Present	Guests are *not* present within the range of the exhibit fence.
	Proximity	Contact	Individuals are touching.
		Proximate	Individuals are within one coyote body length of one another.
		Distant	Individuals are farther than one coyote body length from one another.
		Unknown	One or both individuals cannot be seen and proximity cannot be determined.

Table 5. Full statistical analysis of pacing and resting before, during, and after the zoo's closure due to covid-19. During and after conditions reflect decreased and restricted traffic along the staff-only path behind the exhibit. Before = Sept. 1, 2019-March 12, 2020, During = March 13-May 9, 2020, After = May 10-August 22, 2020. *= $p<0.05$

Focal Animal	Behavior	Comparison	P value
Sherlock	Pace	Before-During	0.833
		Before-After*	<0.001
		During-After	0.799
	Rest	Before-During*	0.01
		Before-After*	<0.001
		During-After	0.373
Watson	Pace	Before-During	0.201
		Before-After	0.493
		During-After	0.293
	Rest	Before-During*	0.029
		Before-After*	0.007
		During-After	0.789

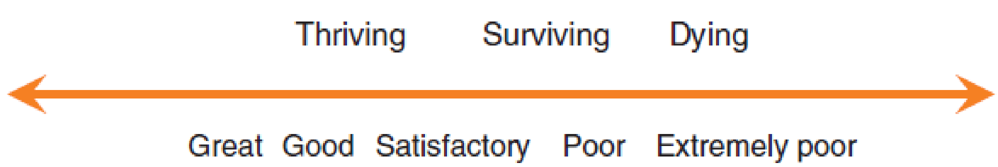

Figure 1. Continuum of animal welfare (from Melfie, 2009).

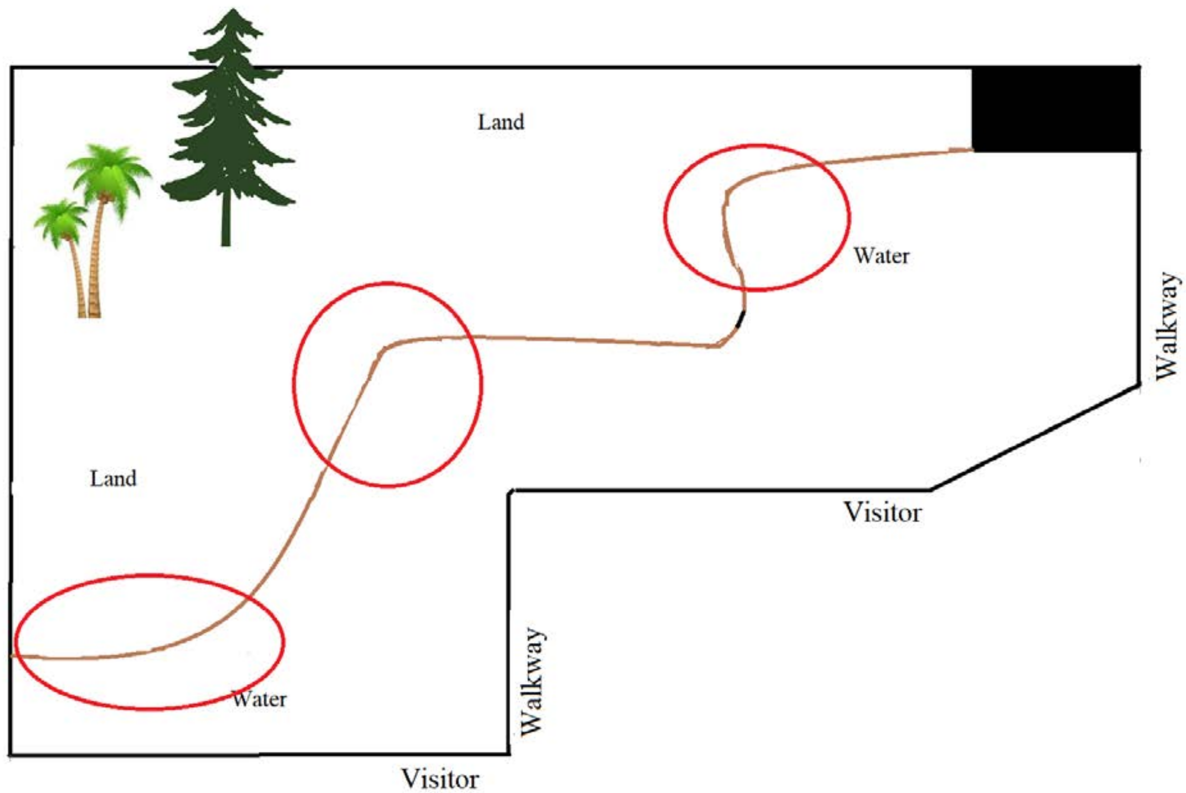

Figure 2. A top-down diagram of the alligators' outdoor exhibit. This map was used during observations to record space use information. Circles indicate water entry/exit points.

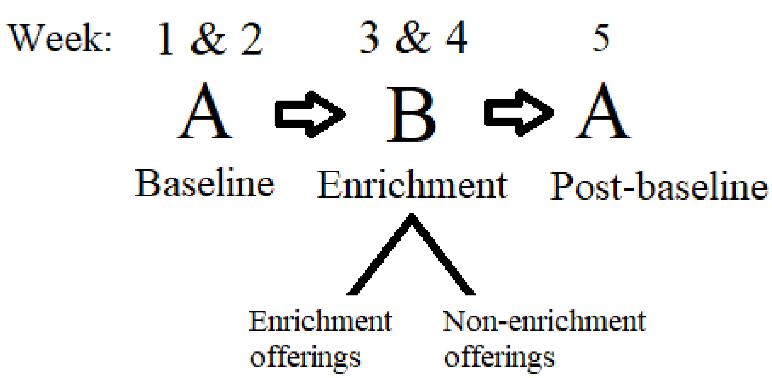

Figure 3. Detailed timeline of the study.

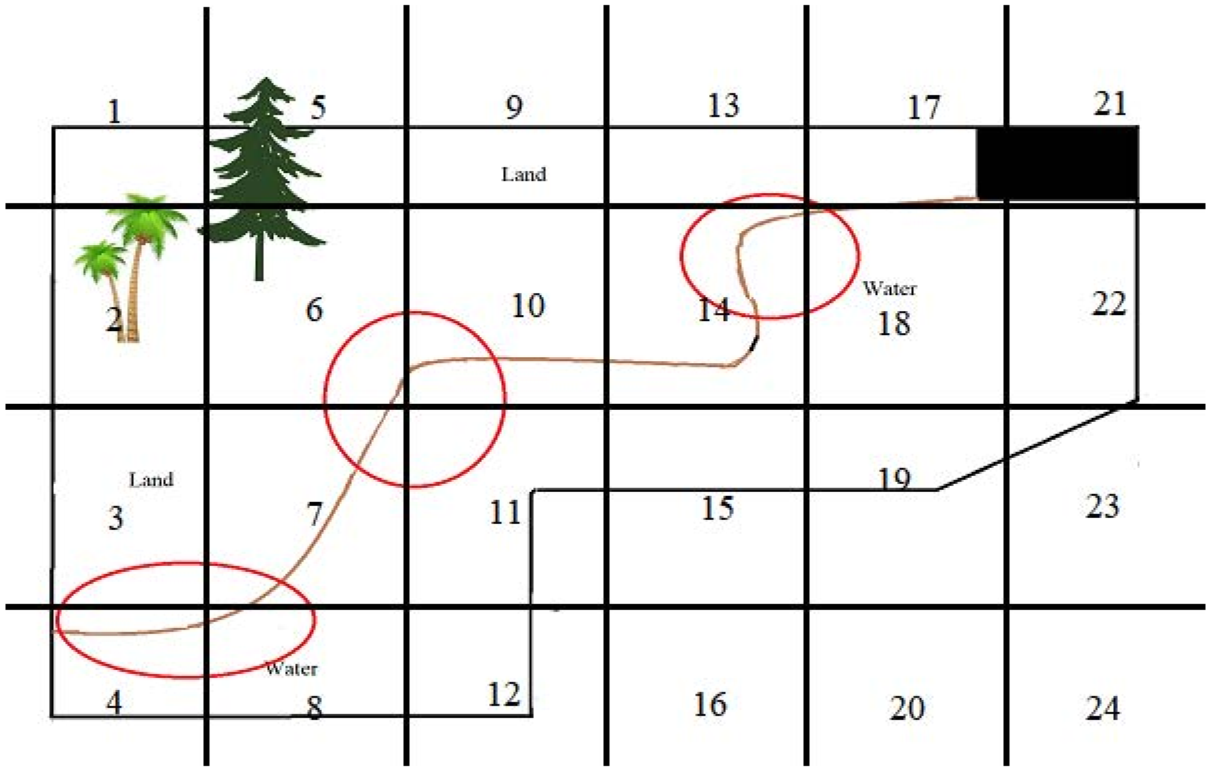

Figure 4. Habitat map used to collect space use data divided into 100x100 pixel zones.

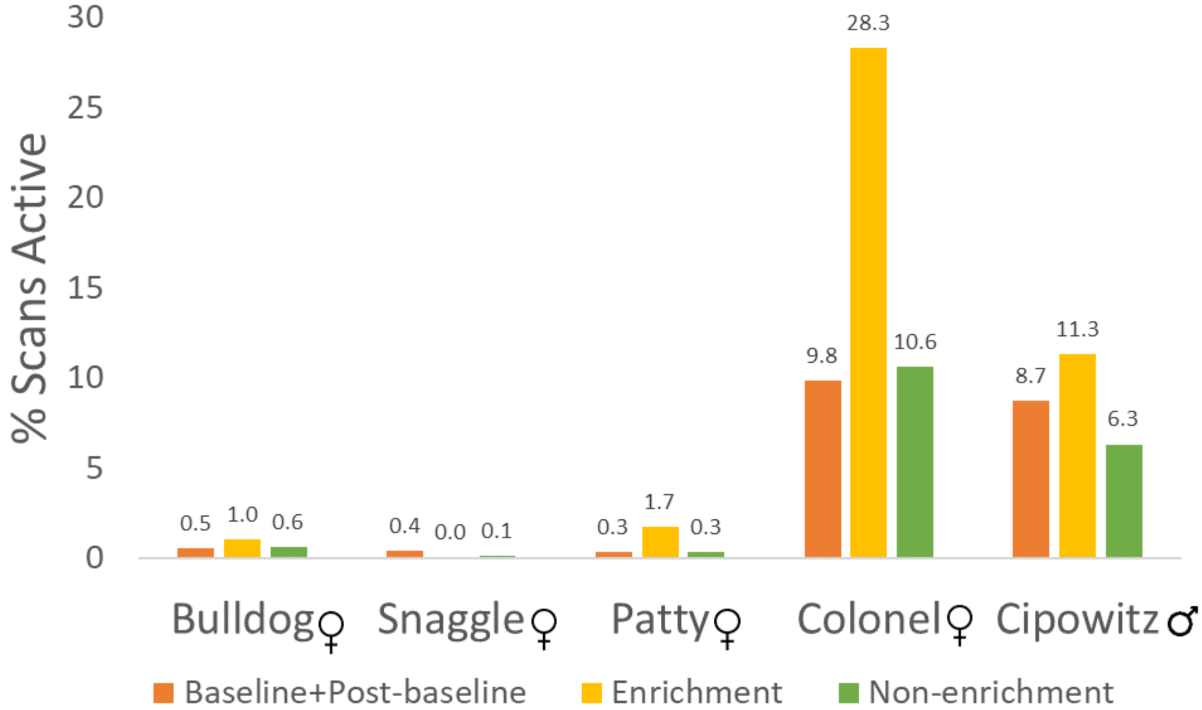

Figure 5. Percent of 2-min interval scans individuals spent active (locomotion and swimming). Baseline + Post-baseline bars represent A weeks, Enrichment and Non-enrichment bars represent B weeks.

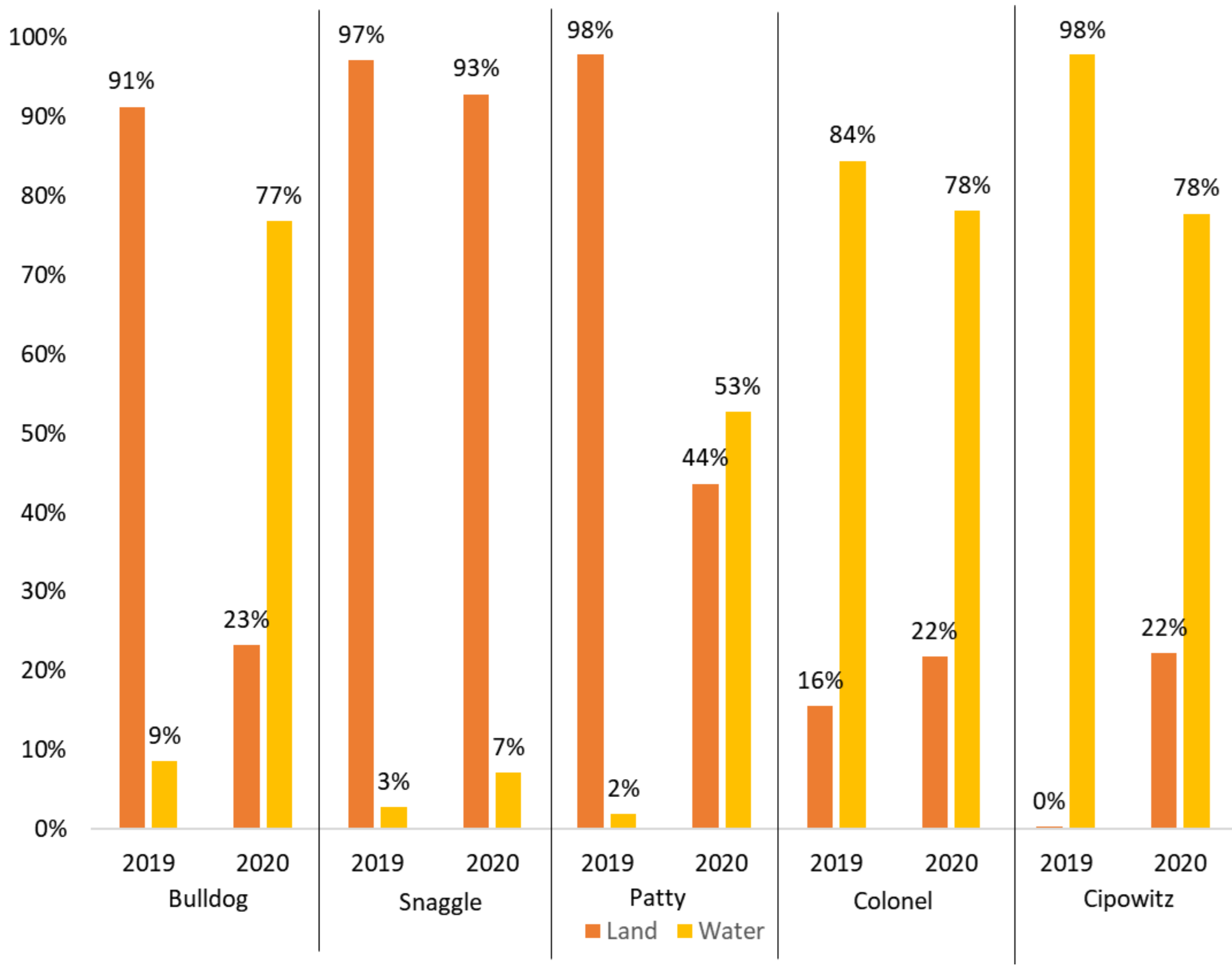

Figure 6. Individual land and water usage over five weeks (58 hours) of study from July-August 2019 and two weeks (12 hours) in June-July 2020.

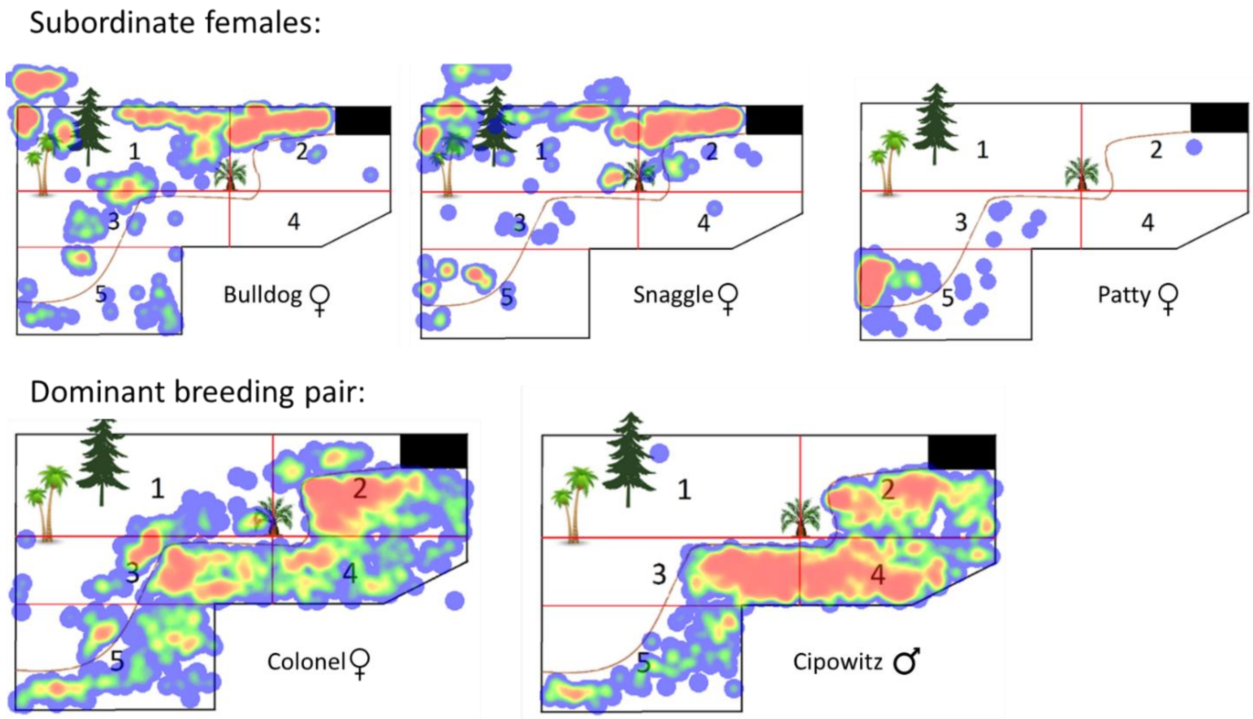

Figure 7. Space use maps of subordinate (Bulldog, Snaggle, and Patty) females and the dominant breeding pair (Colonel, F; Cipowitz, M) for July-August 2019. Red areas indicate places that the animal spent more time. Numbers 1-5 do not correspond to spatial distribution zones but are an artifact from a previous analysis.

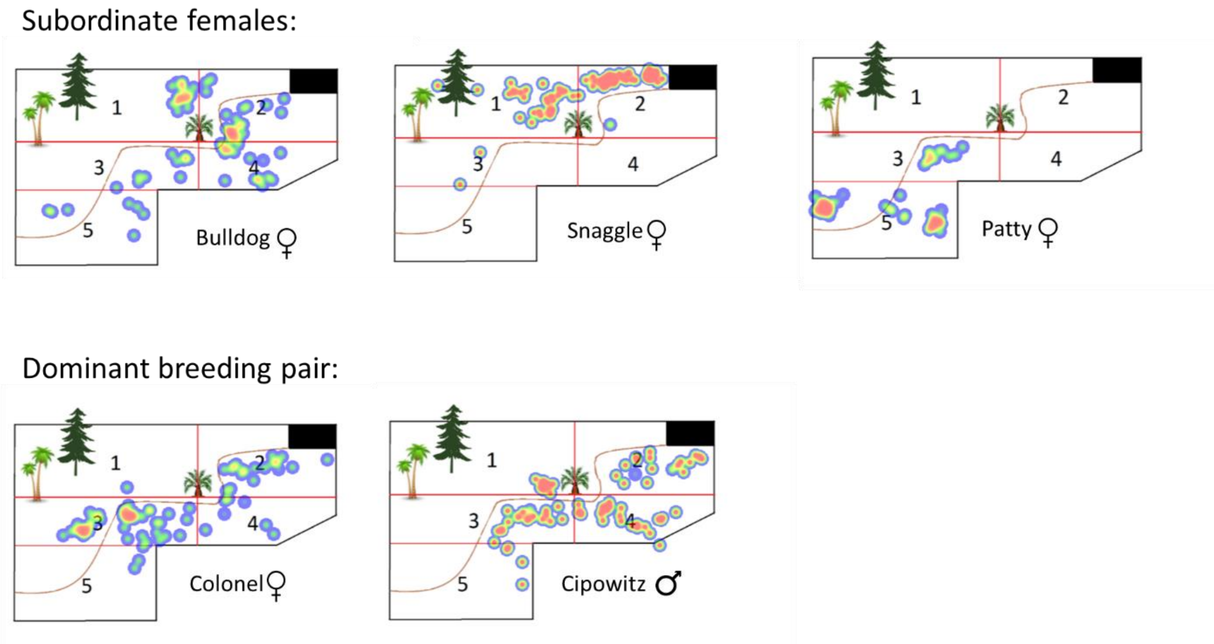

Figure 8. Space use maps of subordinate (Bulldog, Snaggle, and Patty) females and the dominant breeding pair (Colonel, F; Cipowitz, M) for June-July 2020. Red areas indicate places that the animal spent more time. Numbers 1-5 do not correspond to spatial distribution zones but are an artifact from a previous analysis.

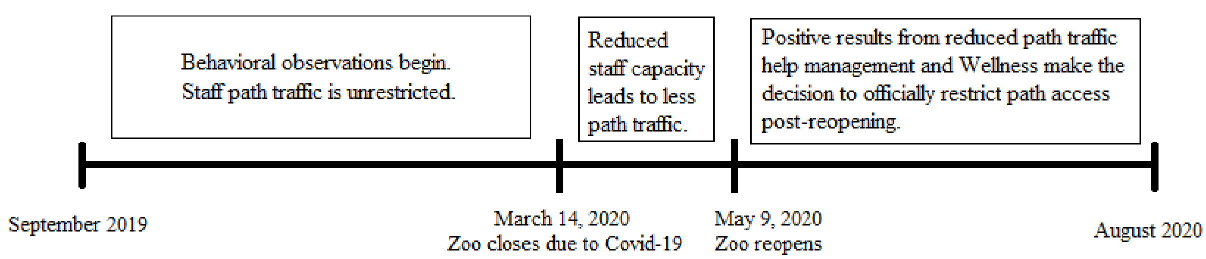

Figure 9. Detailed study timeline from September 2019-August 2020.

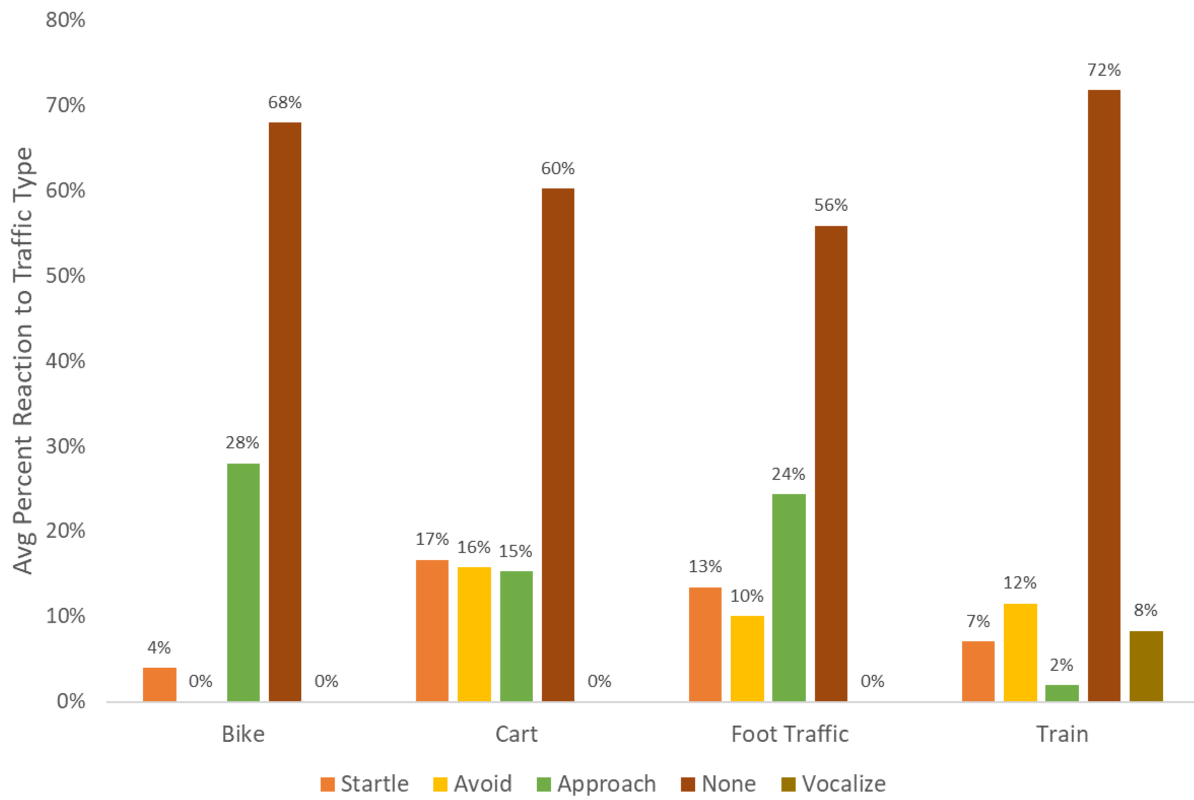

Figure 10. Reactions to staff path traffic by percentage of each traffic type before covid-19 closure and reduction of staff-only path traffic (Sept 2019-March 2020).

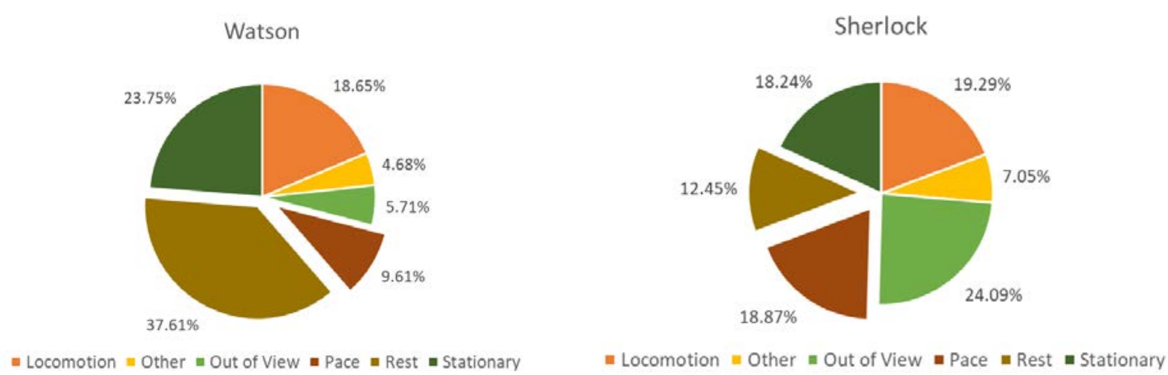

Figure 11a & 11b. Activity budgets for Watson and Sherlock before covid-19 shutdown and reduced staff-only path traffic (Sept 2019-March2020).

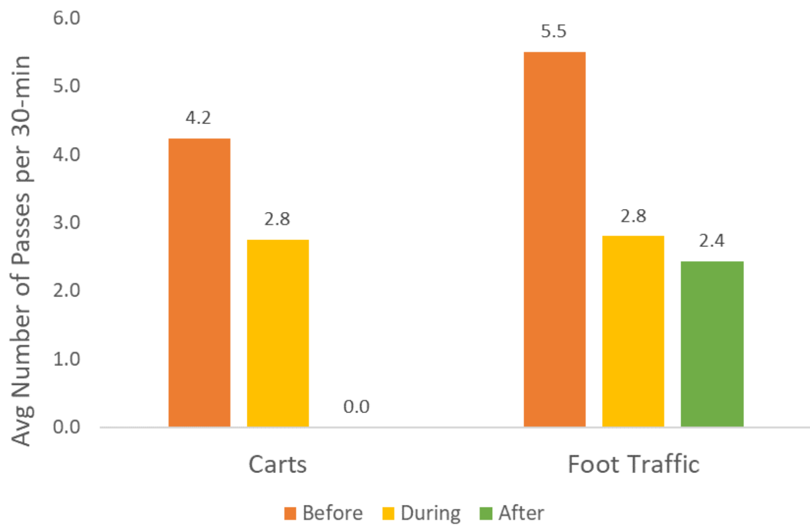

Figure 12. Average number of passes of carts and foot traffic along the staff-only path behind the coyote exhibit per 30-min observation session before, during, and after covid-19 closure. Reduced staff capacity was present under the "during" condition and official staff path restrictions were present during the "after" condition. No special regulations on staff path traffic were present during the "before" condition.

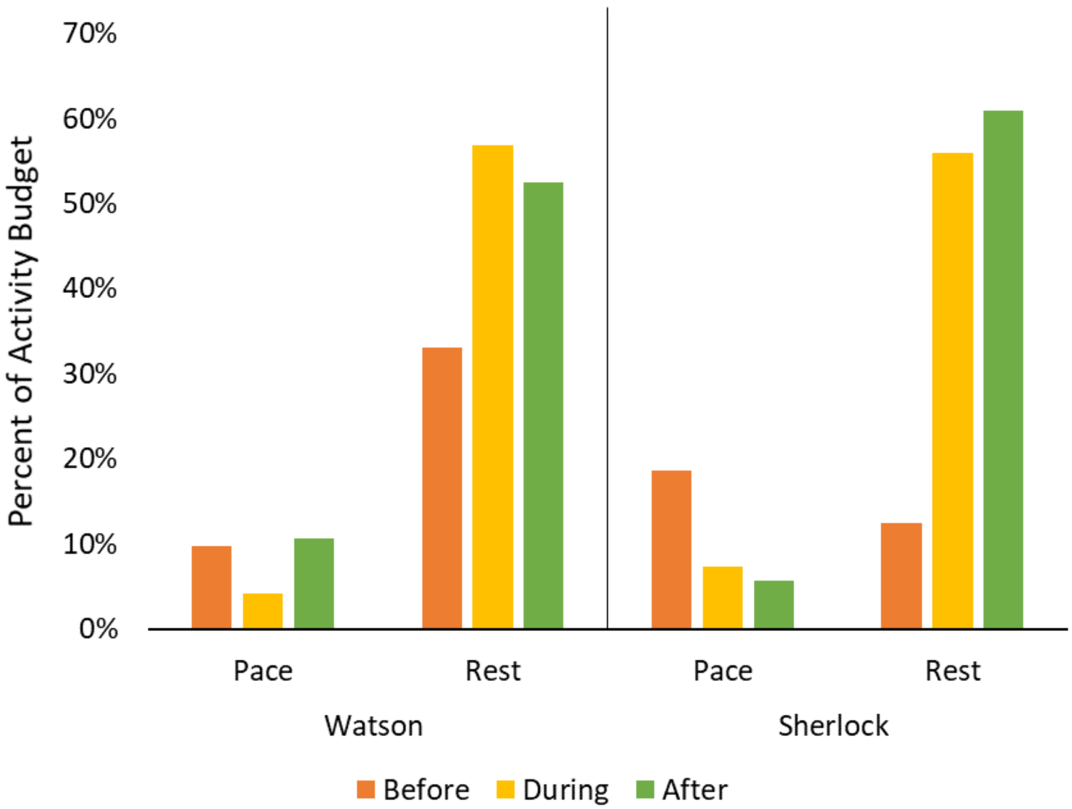

Figure 13. Average percent of activity budget spent pacing and resting before path restrictions, during covid-19 closure with reduced path traffic, and after reopening and official path restrictions.

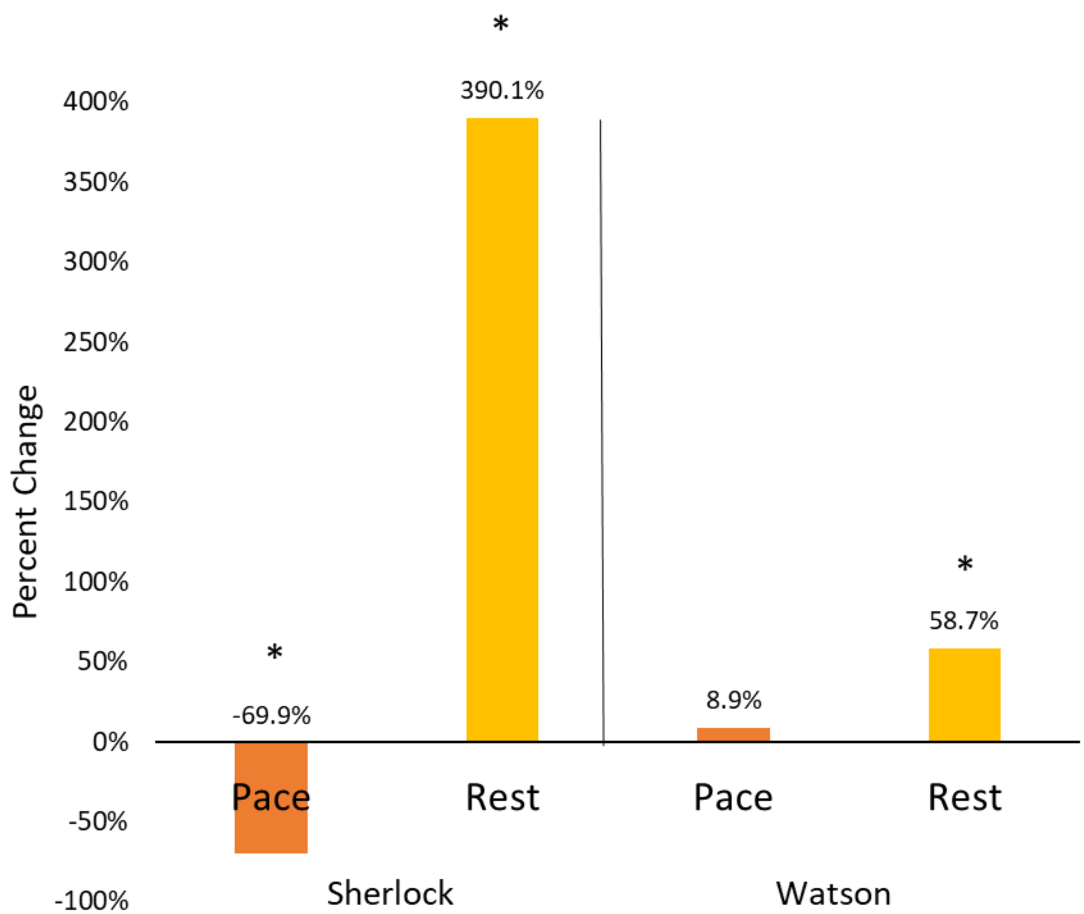

Figure 14. Percent change in pace and rest behaviors for Watson and Sherlock. Change was calculated for behaviors before (Sept 2019-March 2020) and after (May-Aug 2020) official staff path restrictions, excluding the eight weeks of shutdown between March-May 2020 when guests were not present. *=p<0.05.

Vitae

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Education

2019-2020	Master of Science in Biology University of North Florida, Jacksonville, FL
2014-2016	Bachelor of Science in Zoology Michigan State University, East Lansing, MI

Positions and Fellowship

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Publications and Presentations

Spain, M.S., Fuller, G., & Allard, S.M. (2020). Effects of habitat modifications on behavioral indicators of welfare for Madagascar giant hognose snakes (*Leioheterodon madagascariensis*). *Animal Behavior and Cognition*, 7(1), 70-81.

AZA Annual Conference Poster Presentation, "Wellness for coyotes: a case for evidence-based zoo management" (2020).