Title
Testing Species Independent Concepts of Zoo Animal Behavior: Effects of Keeper Presence & Enrichment Type on Indicators of Individual Animal Welfare

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Testing Species Independent Concepts of Zoo Animal Behavior: Effects of Keeper Presence & Enrichment Type on Indicators of Individual Animal Welfare

By

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DISSERTATION

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Abstract

Animal behavior is the primary source of information that zoo professionals use to infer animals' psychological state and welfare. Chapter 1 is a general introduction for the dissertation and a background on the topics covered. In Chapter 2, I introduce a statistical analysis approach to evaluate general behavioral theories by observing individuals of many species, called Species-Independent Combined-Individual Mixed-Model Analysis (SICIMMA). This chapter guides on the importance of zoo researchers testing general behaviors at the individual level as they house hundreds of different species and many behavior theories are general, not species-specific. I applied this approach to assess if the presence of the primary keeper impacts the behavior of 15 individuals (Chapter 3). I hypothesized that animals behave differently in the keepers' presence than in the keeper's absence because caretakers are associated with primary reinforcers. Overall, I observed animals being more active in their keepers' presence than when the keeper was not present. We demonstrated that keeper presence is an environmental context in which animals behave differently than in keeper absence. In Chapter 4, I applied the statistical approach SICIMMA to investigate how two different categories of environmental enrichment influence the behavior and welfare indicators of 8 individuals. I hypothesized that compared to manipulable objects, problem-solving opportunities would result in more activity and exploration throughout the day and would have greater impact on anticipatory behavior that occurs at the end of the day prior to a reliable reward. I observed that puzzle feeders decrease anticipatory and anxiety-like behavior and increase social behavior more than object enrichment. Overall, this research offers guidance on testing general behavioral concepts on multi-species individuals and demonstrates the influence of two zoo environmental contexts on animal behavior and welfare indicators.

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Chapter 1:
General Introduction

Animal welfare is the cumulative balance of emotional experiences, which is considered an animal's affective state over time (Watters et al., 2019). Animal behavior is the first response to environmental changes and is used to measure affective states. Thus, it is vital to understand the changes in the behavior of captive animals regarding environmental factors or contexts they experience daily, as these changes can influence animals' emotional experiences.

Objective

My dissertation aims to test general behavioral theories by observing individuals of many species. I introduce a statistical approach, Species-Independent Combined-Individual Mixed-Model Analysis, and provide the rationale for applying this analysis and suggest that zoological institutions are ideal to test it (Chapter 2). I test the statistical approach by evaluating animals' behavioral changes in the presence of their keeper (Chapter 3) and under two types of environmental enrichment (Chapter 4).

Background

Zoo Welfare

The Five Domains model facilitates animal welfare via four physical/functional domains: nutrition, environment, physical health, behavior, and the fifth domain of the animal's mental state (Mellor et al., 2015; Figure 1). This model identifies two primary sources of what an animal can experience, which will influence its mental state. The first is the effects that motivate animals to perform behaviors essential for survival (e.g., drinking, eating, retreating) typically covered by the first three domains of the model. The second source captures subjective experiences through the behavior domain, which can be positive or negative experiences. The four physical domains can provide a range of conditions that result in subjective experiences within the mental domain. The overall net impact of all the experiences represents the animal's welfare status. The goal of animal welfare assessments and management aims to monitor, detect and correct poor welfare and maintain good to very good welfare (Mellor et al., 2015). Most zoos use animal behavior as a primary source of information to infer the mental domain and assess animal welfare (Mellor et al., 2015). Thus, behavioral monitoring programs are important in revealing whether animals are in an appropriate environment and provide information on other health or husbandry-related problems (Crockett, 1996; Watters et al., 2009). They provide baseline information that helps identify what is "normal" time budgets and can track changes. However, behavioral monitoring programs require objective data collection to represent the animal's repertoire thoroughly. Captive animals are on a predictable schedule daily, which can largely entail changes in the environment and behavior domain. Potential factors influencing the animal's behavior, including human-animal relationships and the enrichment categories provided, may be overlooked.

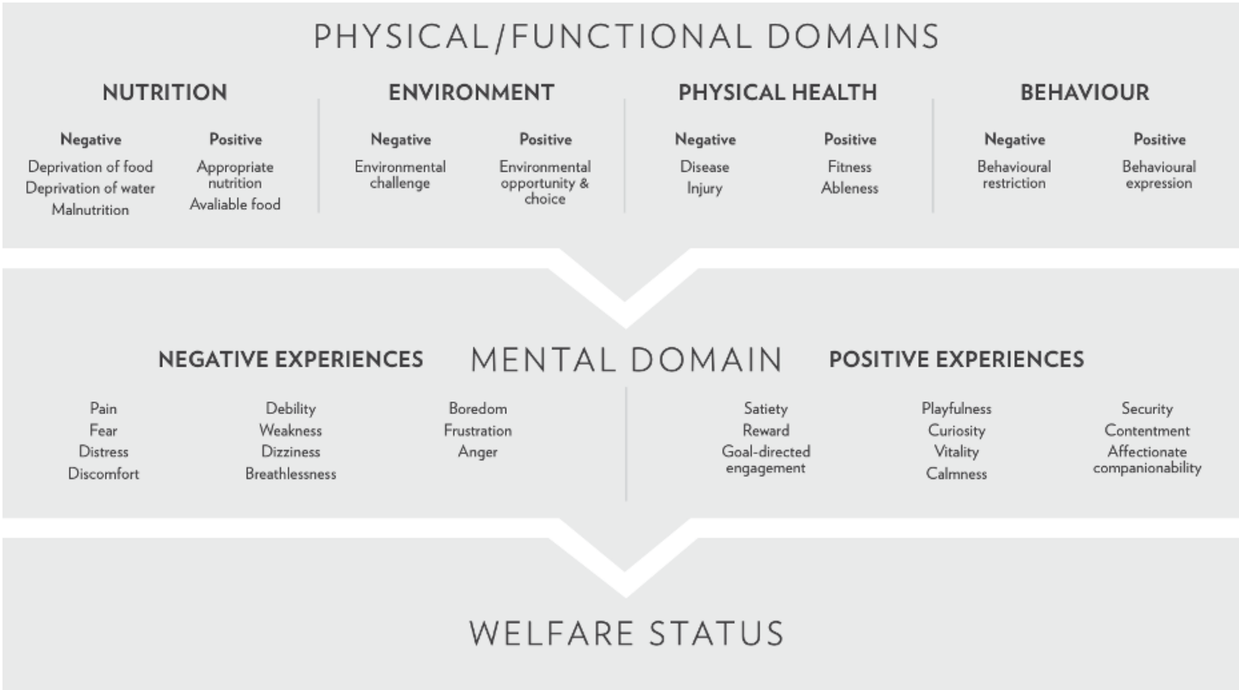

Figure 1. The Five Domains model for animal welfare from WAZA Animal Welfare Strategies (Mellor et al., 2015; originally modified from Mellor & Beausoleil, 2015).

Human-Animal Relationship

Captive animals are cared for by humans in settings that include homes, laboratories, farms, zoos, aquariums, and sanctuaries. Animals in captivity rely on humans for daily care through human-animal interactions (HAI). An HAI is a sequence of behaviors between a human and an animal where individual A exhibits a behavior directed towards individual B and individual B may respond or vice versa (Hinde, 1976; Hosey, 2008, 2013). Based on this definition, HAI is a dyadic event between one human and one animal. This can include interactions such as visitors touching goats and sheep in a contact yard (Anderson et al., 2004) or keepers having a non-contact play session with apes across a protective barrier (Chelluri et al., 2013). HAIs have been studied extensively in agricultural animals, resulting in behavior and physiology changes impacting health, productivity, and welfare (as reviewed in Hemsworth &

Coleman, 2010). The nature of the interaction between the animal and human influences the specific changes in behavioral and physiological measures the animal experiences. They can be labeled as positive (e.g., feeding, gentle handling, resulting in positive experiences), negative (e.g., aggression, rough handling, resulting in negative experiences), or neutral. The type and quantity of HAI can create a human-animal relationship (HAR).

A HAR is a series of interactions between an animal and a human in which they acquire familiarity with each other's behavior. The human and animal in this relationship can make predictions about the behavior of the other (Hinde, 1976; 1987). HARs have been documented between stockpersons and agricultural animals (Waiblinger et al., 2006; Hemsworth & Coleman, 2010) and between zoo animals and visitors (Hosey, 2008, 2013; Sherwen, Hemsworth, Butler et al., 2015). HARs are generally labeled as negative, neutral, or positive, depending on the net quality of interactions between the two individuals. For example, a negative HAR can develop if most interactions are aggressive or involve rough handling. Such a HAR may result in an animal behaving in a highly fearful way, retreating or avoiding contact with a particular human. A neutral HAR may result in low levels of fear but the animal may ignore the human. A positive HAR would develop when an animal associates a specific human with positive outcomes. In this case, the animal is not fearful and shows confidence or interest in the human, or the animal may move toward the human (Waiblinger et al., 2006). A documented example of a positive HAR between rats and humans concluded that rats decreased fear and moved toward humans after being playfully handled (Cloutier et al., 2012).

Studies have shown HARs influence animal behavior whether it is by zookeepers (Wielebnowski et al., 2002; Chelluri et al., 2013) or by zoo visitors (Hosey & Druck, 1987; Quadros et al., 2014; Sherwen, Magrath, Butler et al., 2015). While relationships can be developed with zoo visitors, a zoo animal has the most opportunities to become familiar with and potentially develop a HAR with its keeper. Studies have shown that keeper personalities and management styles may elicit different responses from animals (Anderson et al., 2004; Phillips & Peck, 2007; Carlstead, 2009). Evidence suggests animals can distinguish between familiar and unfamiliar people in zoos and other settings (as reviewed in Hosey & Melfi, 2014). Martin & Melfi (2016) showed a decrease in avoidance behavior towards familiar keepers compared to non-familiar keepers, suggesting that the behavioral changes an animal exhibits can be specific to the human involved. Positive keeper attitudes and interactions exhibited lower mean serum cortisol concentrations and calmer behaviors in animals, which demonstrate HAR in zoos have welfare implications (Carlstead et al., 2019; Patel et al., 2019). Thus, investigating the impacts of keeper presence on animal behavior is vital as keepers can impact animal welfare and they are relied on to provide summaries of animals' health and behavioral repertoire. Keepers assess the welfare of animals in accordance with their experience with a particular species and the amount of time spent around them. Highly experienced keepers often show an ability to observe small changes in animal behavior. Nevertheless, keepers have a time-constrained schedule that may influence the time they have to do a behavioral observation and may only observe their animals in specific contexts.

Environmental Enrichment

Environmental enrichment is a component of animal husbandry that aims to provide a dynamic environment through varied behavioral opportunities for animals (Shepherdson, 1994; Swaisgood & Shepherdson, 2005; Watters et al., 2019). Studies have shown that providing enrichment can lead to an increase in behavioral diversity and species-specific behaviors, a reduction in abnormal behaviors, an expansion of space use, promote more natural time budgets, and provide practice to cope with challenges (Lutz & Novak, 1995; Vick et al., 2000; Kells et al., 2001; Young, 2003; Fay & Miller, 2015: Lauderdale & Miller, 2020; Nagabaskaran et al., 2022). The behavioral changes resulting from environmental enrichment support improvements in animal welfare. The benefits of enrichment have been demonstrated in farm, laboratory, and zoo animals through behavioral, physiological, and neurological evidence (Reviewed in Van Praag et al., 2000; Young, 2003; Coleman & Novak, 2017; Arechavala-Lopez, 2022). As a result, environmental enrichment is widely accepted as a method for adjusting animal behavior. Environmental enrichment is now an industry standard best practice for accredited zoos and aquariums (Mellor et al., 2015; Association of Zoos and Aquariums, 2022). Environmental enrichment can include feeding varied feeding opportunities strategies, enhancements to sensory environments, social opportunities, structural variation, and cognitive challenges (Young, 2003; de Azevedo et al., 2007).

Each enrichment category elicits a specific goal or behavior but is not mutually exclusive (e.g., puzzle feeders are both feeding and cognitive enrichment). The goal or target behavior desired by the enrichment item should also reflect an understanding of that behavior for each species (Young, 2003). Feeding enrichments aim to increase foraging, extraction, and processing time, add variability to feeding times or modify the number of feedings per day (Swaisgood & Shepherdson, 2005). Sensory enrichment stimulates the animal's senses (e.g., the addition of music, mirrors, or scents; Wells, 2009). Social enrichment provides opportunities to engage with conspecifics, caretakers, or visitors (De Rouck et al., 2005; Chelluri et al., 2013). Structural enrichment consists of non-permanent physical features added or moved around the exhibit (e.g., climbing frames, tunnels, platforms) or objects for manipulation. Structural enrichment promotes exploration, manipulation of objects, and play (Swaisgood & Shepherdson, 2005). Cognitive enrichment provides problem-solving and learning opportunities such as mechanical apparatuses, computer tasks, and mazes (Swaisgood & Shepherdson, 2005; Meehan & Mench, 2007; Clark, 2017). Cognitive enrichment is the least used category in captivity, but puzzle feeders are commonly used. Puzzle feeders offer a problem-solving opportunity to retrieve food instead of more traditional methods, such as being fed from a dish or scatter fed throughout the enclosure. Puzzle feeders with food have increased activity, foraging, and interaction with enrichment compared to empty puzzle feeders (Gilloux et al., 1992; Sanders & Fernandez, 2020). Enrichment programs in zoos typically utilize multiple enrichment categories daily; however, some categories are applied more than others due to ease of use, availability, or time constraints on care staff. For example, structural enrichment is the most common form of environmental enrichment across all types of animals, regardless of housing locations (laboratory, farm, and zoo). One study found that zoo settings' most common enrichment categories are food-related and structural (de Azevedo et al., 2007). Another study determined caretakers provided the highest frequency of feeding, human-animal interactions, and tactile (novel objects) enrichment over one week (Hoy et al., 2010). Providing different types of enrichment at variable schedules keeps animals interested and avoids the effects of habituation (Kuczaj et al., 2002). Although using multiple enrichment categories to provide positive behavioral opportunities for animals is the best practice for animal management, it may lead to difficulty in understanding which types of enrichment lead to more robust changes in animal behavior (de Azevedo et al., 2007). Assessing the behavioral impacts of different forms of enrichment individually will help better understand how to support animals' behavioral needs.

Providing a problem-solving opportunity with a reward allows animals to use cognitive skills for food acquisition (Shettleworth, 2001; Meehan & Mench, 2007). Puzzle feeders are more common and feasible at zoos than mechanical apparatuses or computer tasks, nonetheless, they are still cognitively challenging (Swaisgood & Shepherdson, 2005). However, statistical analysis for identifying gaps in the enrichment literature by de Azevedo et al. (2007) discovered that 3.5% of zoo studies utilize cognitive enrichment. Using cognitive skills via tasks or enrichment decreases stress and abnormal behaviors and increases exploration and positive affect (Puppe et al., 2007; Manteuffel et al., 2009). Problem-solving opportunities result in an increase in activity and exploration which have been recognized as important behavioral opportunities for supporting animal well-being (Meehan & Mench, 2007; Clark, 2011; Clark & Smith, 2013; Clark et al., 2013). Providing a cognitive challenge is important as it can increase interest in the wider environment; animals may seek opportunities to explore and problem-solve in their environment (Spinka & Wemelsfelder, 2011). For example, bottlenose dolphins became more interested in their environment when exposed to problem-solving opportunities (Clark et al., 2013). Therefore, cognitive challenges result in behavioral changes throughout the day, but comparisons of different enrichment types have not been studied.

Anticipatory Behavior

Anticipatory behavior has gained interest as a behavioral indicator of welfare over the years. This goal-directed behavior occurs when an animal perceives that a reward is likely to be available and appears to reflect the animal's own perception of how much it needs (or wants) an expected reward, also known as its reward sensitivity (Spruijt et al., 2001; Watters, 2014). Anticipatory behavior is a measure of reward sensitivity, and its intensity is expected to increase when animals have few opportunities to acquire rewards (van der Harst et al., 2003; Watters, 2014). This indicator has gained interest in zoos as most animals live in highly predictable environments where resource availability is generally scheduled, and keepers' presence is predictable or signaled. These conditions can promote the development of anticipatory behavior (Krebs et al., 2017; Krebs & Watters, 2017; Clegg et al., 2018; Ward et al., 2018; Podturkin et al., 2022).

Anticipatory behavior can be expressed differently across species and individuals and may depend on how they acquire rewards (Watters et al., 2019). For example, anticipation prior to scheduled feedings has increased activity in different species, such as lambs (Anderson et al., 2015), hamsters (Dantas-Ferreria et al., 2015), and salmon (Folkedal et al., 2012). Along with increased activity, some animals may also express ‘searching’ behaviors (e.g., sniffing and looking) or focus activity near the location of the expected reward (Krebs et al., 2017). While increased activity is often the expected anticipatory behavior, a more subtle “sit and wait” behavior has also been observed in great apes and dolphins (Bloomsmith & Lambeth, 1995; Jensen et al., 2013; Krebs et al., 2017).

Anticipatory behavior and enrichment has been studied only a handful of times in zoos. For example, anticipatory behavior was generated using reliable cues before a positive human-animal interaction in a western lowland gorilla, red panda (Krebs et al., 2017), and bottlenose dolphins (Clegg et al., 2018). This positive human-animal interaction was anticipated more than toys and showed that higher anticipatory behaviors before each event were correlated to higher levels of participation in the event itself (Clegg et al., 2018). Another study saw a decrease in anticipatory behavior in the afternoon, before the evening meal when a rhino was given a timed puzzle feeder compared to weeks without the feeder (Krebs & Watters, 2017). A recent study also observed decreased anticipatory behavior and overall improvement in longterm welfare with cognitive enrichment than with non-cognitive enrichment items (Clegg et al., 2023). These studies demonstrated that animals in zoos show varying levels of anticipatory behavior for different enrichment and can help animal managers understand the animal's "wants" and adjust accordingly if needed. This limited literature on anticipatory and enrichment reveals a gap in knowledge that requires further investigation on how different enrichment impacts anticipatory behavior throughout the day.

References

Anderson, U. S., Maple, T. L., & Bloomsmith, M. A. (2004). A close keeper–nonhuman animal distance does not reduce undesirable behavior in contact yard goats and sheep. Journal of Applied Animal Welfare Science, 7(1), 59-69.

Anderson, C., Yngvesson, J., Boissy, A., Uvnas-Moberg, K., & Lidfors, L. (2015). Behavioural expression of positive anticipation for food or opportunity to play in lambs. Behavioural Processes, 113, 152–158. doi:10.1016/j.beproc.2015.02.003

Arechavala-Lopez, P., Cabrera-Álvarez, M. J., Maia, C. M., & Saraiva, J. L. (2022). Environmental enrichment in fish aquaculture: A review of fundamental and practical aspects. *Reviews in Aquaculture*, *14*(2), 704-728.

Association of Zoos and Aquariums. (2022). The Accreditation Standards & Related Policies. https://assets.speakcdn.com/assets/2332/aza-accreditation-standards.pdf

Bloomsmith, M. A., & Lambeth, S. P. (1995). Effects of predictable versus unpredictable feeding schedules on chimpanzee behavior. Applied Animal Behaviour Science, 44, 65–74. doi:10.1016/0168-1591(95)00570-I

Carlstead, K. (2009). A comparative approach to the study of keeper–animal relationships in the zoo. Zoo Biology: Published in affiliation with the American Zoo and Aquarium Association, 28(6), 589-608.

Carlstead, K., Paris, S., & Brown, J. L. (2019). Good keeper-elephant relationships in North American zoos are mutually beneficial to welfare. *Applied Animal Behaviour Science*, *211*, 103-111.

Chelluri, G. I., S. R. Ross, and K. E. Wagner. (2013). Behavioral correlates and welfare implications of informal interactions between caretakers and zoo-housed chimpanzees and gorillas. Applied Animal Behaviour Science, 147: 306-315.

Clark, F. E. (2011). Great ape cognition and captive care: Can cognitive challenges enhance well-being?. Applied Animal Behaviour Science, 135(1-2), 1-12.

Clark, F. E. (2017). Cognitive enrichment and welfare: Current approaches and future directions. Animal Behavior and Cognition, 4(1), 52-71.

Clark, F. E., Davies, S. L., Madigan, A. W., Warner, A. J., & Kuczaj, S. A. (2013). Cognitive enrichment for bottlenose dolphins (Tursiops truncatus): Evaluation of a novel underwater maze device. Zoo Biology, 32(6), 608-619.

Clark, F. E., & Smith, L. J. (2013). Effect of a cognitive challenge device containing food and non-food rewards on chimpanzee well-being. American Journal of Primatology, 75(8), 807-816.

Clegg, I. L., Domingues, M., Ström, E., & Berggren, L. (2023). Cognitive Foraging Enrichment (but Not Non-Cognitive Enrichment) Improved Several Longer-Term Welfare Indicators in Bottlenose Dolphins. Animals, 13(2), 238.

Clegg, I. L., Rödel, H. G., Boivin, X., & Delfour, F. (2018). Looking forward to interacting with their caretakers: dolphins' anticipatory behaviour indicates motivation to participate in specific events. Applied Animal Behaviour Science, 202, 85-93.

Cloutier, S., Panksepp, J., & Newberry, R. C. (2012). Playful handling by caretakers reduces fear of humans in the laboratory rat. Applied Animal Behaviour Science, 140(3-4), 161-171.

Coleman, K., & Novak, M. A. (2017). Environmental Enrichment in the 21st Century. *ILAR Journal*, *58*(2), 295-307.

Crockett, CM. (1996). Data collection in the zoo setting, emphasizing behavior. In: DG Kleiman, ME Allen, KV Thompson, S Lumpkin, H Harris, editors. Wild mammals in captivity: principles and techniques. Chicago: University of Chicago Press. p 545– 565.

Dantas-Ferreira, R. F., Dumont, S., Gourmelen, S., Cipolla-Neto, J., Simonneaux, V., Pevet, P., & Challet, E. (2015). Food-anticipatory activity in Syrian hamsters: Behavioral and molecular responses in the hypothalamus according to photoperiodic conditions. Plos One, 10, 19. doi:10.1371/journal.pone.0126519

de Azevedo, C. S., Cipreste, C. F., & Young, R. J. (2007). Environmental enrichment: A GAP analysis. Applied Animal Behaviour Science, 102(3-4), 329-343.

De Rouck, M., Kitchener, A. C., Law, G., & Nelissen, M. (2005). A comparative study of the influence of social housing conditions on the behaviour of captive tigers (Panthera tigris). Animal Welfare, 14(3), 229-238.

Fay, C., & Miller, L. J. (2015). Utilizing scents as environmental enrichment: Preference assessment and application with Rothschild giraffe. *Animal Behavior and Cognition*, *2*(3), 285-291.

Folkedal, O., Stien, L. H., Torgersen, T., Oppedal, E., Olsen, R. E., Fosseidengen, J. E., ...Kristiansen, T. S. (2012). Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon post-smolt after exposure to acute temperature fluctuation. Physiology & Behavior, 105, 350–356. doi:10.1016/j.physbeh.2011.08.008

Ghavamian, Y., Minier, D. E., & Jaffe, K. E. (2022). Effects of Complex Feeding Enrichment on the Behavior of Captive Malayan Sun Bears (Helarctos malayanus). Journal of Applied Animal Welfare Science, 1-15.

Gilloux, I., Gurnell, J., & Shepherdson, D. (1992). An enrichment device for great apes. Animal Welfare, 1(4), 279-289.

Hemsworth, P. H., and G. J. Coleman. (2010). Human-livestock interactions: The stockperson and the productivity of intensively farmed animals. CABI.

Hinde, R. A. (1976). On describing relationships. Child Psychology & Psychiatry & Allied Disciplines, 17: 1-19.

Hosey, G. (2008). A preliminary model of human–animal relationships in the zoo. Applied Animal Behaviour Science, 109: 105-127.

Hosey, G. (2013). Hediger revisited: how do zoo animals see us? Applied Animal Welfare Science, 16: 338-359.

Hosey, G. R., and P. L. Druck. (1987). The influence of zoo visitors on the behaviour of captive primates. Applied Animal Behaviour Science, 18: 19-29.

Hosey, G., & Melfi, V. (2014). Human-animal interactions, relationships and bonds: A review and analysis of the literature. International Journal of Comparative Psychology.

Hoy, J. M., Murray, P. J., and Tribe, A. (2010). Thirty years later: enrichment practices for captive mammals. Zoo Biology, 29: 303-316.

Jensen, A. L. M., Delfour, F., & Carter, T. (2013). Anticipatory behavior in captive bottlenose dolphins (Tursiops truncatus): A preliminary study. Zoo Biology, 32, 436–444. doi:10.1002/zoo.21077

Kells, A., Dawkins, M. S., & Borja, M. C. (2001). The effect of a ‘freedom food’enrichment on the behaviour of broilers on commercial farms. Animal Welfare, 10(4), 347-356.

Krebs, B. L., Torres, E., Chesney, C., Kantoniemi Moon, V., & Watters, J. V. (2017). Applying behavioral conditioning to identify anticipatory behaviors. Journal of Applied Animal Welfare Science, 20(2), 155-175.

Krebs, B. L., & Watters, J. V. (2017). Simple but temporally unpredictable puzzles are cognitive enrichment. Animal Behavior and Cognition, 4: 119-134.

Kuczaj, S. A., Lacinak, T., Fad, O., Trone, M., Solangi, M., & Ramos, J. (2002).Keeping environmental enrichment enriching. Comparative Psychology,15, 127–137

Lauderdale, L. K., & Miller, L. J. (2020). Efficacy of an interactive apparatus as environmental enrichment for common bottlenose dolphins (Tursiops truncatus). *Animal Welfare*, *29*(4), 379-386.

Lutz, C. K., & Novak, M. A. (1995). Use of foraging racks and shavings as enrichment tools for groups of rhesus monkeys (Macaca mulatta). Zoo Biology, 14(5), 463-474.

Manteuffel, G. J., Langbein, and Puppe,B. (2009). From operant learning to cognitive enrichment in farm animal housing: bases and applicability. Animal Welfare, 18: 87-95.

Martin, R. A., & Melfi, V. (2016). A comparison of zoo animal behavior in the presence of familiar and unfamiliar people. Applied Animal Welfare Science, 19(3), 234-244.

Meehan, C. L., & Mench, J. A. (2007). The challenge of challenge: can problem solving opportunities enhance animal welfare?. Applied Animal Behaviour Science, 102(3-4), 246-261.

Mellor, D. J., & Beausoleil, N. J. (2015). Extending the 'Five Domains' model for animal welfare assessment to incorporate positive welfare states. Animal Welfare, 24(3), 241-253.

Mellor, D. J., Hunt, S. & Gusset, M. (eds). (2015). Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy. Gland: WAZA Executive Office, 69 pp.

Nagabaskaran, G., Skinner, M., & Miller, N. (2022). Western Hognose Snakes (Heterodon nasicus) Prefer Environmental Enrichment. *Animals*, *12*(23), 3347.

Patel, F., Wemelsfelder, F., & Ward, S. J. (2019). Using Qualitative Behaviour Assessment to Investigate Human-Animal Relationships in Zoo-Housed Giraffes (Giraffa camelopardalis). *Animals*, *9*(6), 381.

Phillips, C., & Peck, D. (2007). The effects of personality of keepers and tigers (Panthera tigris tigris) on their behaviour in an interactive zoo exhibit. Applied Animal Behaviour Science, 106(4), 244-258.

Podturkin, A. A., Krebs, B. L., & Watters, J. V. (2022). A quantitative approach for using anticipatory behavior as a graded welfare assessment. Journal of Applied Animal Welfare Science, 1-15.

Puppe, B., Ernst, K., Schön, P. C., & Manteuffel, G. (2007). Cognitive enrichment affects behavioural reactivity in domestic pigs. Applied Animal Behaviour Science, 105(1-3), 75-86.

Quadros, S., Goulart, V. D. L., Passos,L., Vecci, M. A. M., and Young, R. J. (2014). Zoo visitor effect on mammal behaviour: Does noise matter? Applied Animal Behaviour Science, 156: 78-84.

Sanders, K., & Fernandez, E. J. (2020). Behavioral implications of enrichment for golden lion tamarins: A tool for ex situ conservation. Applied Animal Welfare Science, 1-10.

Shepherdson, D. (1994). The role of environmental enrichment in the captive breeding and reintroduction of endangered species. Creative conservation: Interactive management of wild and captive animals, 167-177.

Sherwen, S. L., Hemsworth, P. H., Butler, K. L., Fanson, K. V., & Magrath, M. J. (2015). Impacts of visitor number on Kangaroos housed in free-range exhibits. Zoo Biology, 34(4), 287-295.

Sherwen, S. L., Magrath, M. J., Butler, K. L., & Hemsworth, P. H. (2015). Little penguins, Eudyptula minor, show increased avoidance, aggression and vigilance in response to zoo visitors. Applied Animal Behaviour Science, 168, 71-76.

Shettleworth, S. J. (2001). Animal cognition and animal behaviour. Animal Behaviour, 61(2), 277-286.

Špinka, M., & Wemelsfelder, F. (2011). Environmental challenge and animal agency. Animal Welfare, 2, 27-44.

Spruijt, B. M., van den Bos, R., & Pijlman, F. T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behavior Science, 72: 145-171.

Swaisgood, R. R., & Shepherdson, D. J. (2005). Scientific approaches to enrichment and stereotypies in zoo animals: what's been done and where should we go next?. Zoo Biology, 24: 499-518.

van der Harst, J. E., Baars, A. M., & Spruijt, B. M. (2003). Standard housed rats are more sensitive to rewards than enriched housed rats as reflected by their anticipatory behaviour. Behavioural Brain Research, 142(1-2), 151-156.

Van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. Nature Reviews Neuroscience, 1(3), 191-198.

Vick, S. J., Anderson, J. R., & Young, R. (2000). Maracas for Macaca? Evaluation of three potential enrichment objects in two species of zoo-housed macaques. Zoo Biology, 19(3), 181-191.

Waiblinger, S., Boivin, X., Pedersen, V., Tosi, M. V., Janczak, A. M., Visser, E. K., & Jones, R. B. (2006). Assessing the human–animal relationship in farmed species: a critical review. Applied animal behaviour science, 101(3-4), 185-242.

Ward, S. J., Sherwen, S., & Clark, F. E. (2018). Advances in applied zoo animal welfare science. Applied Animal Welfare Science, 21(sup1), 23-33.

Watters, J. V. (2014). Searching for behavioral indicators of welfare in zoos: Uncovering anticipatory behavior. Zoo Biology, 33, 251–256. doi:10.1002/zoo.21144

Watters, J. V., Krebs, B. L., & Pacheco, E. (2019). Measuring Welfare through Behavioral Observation and Adjusting It with Dynamic Environments. In Kaufman, A. B., Bashaw, M. J., & Maple, T. L. (Eds.), Scientific Foundations of Zoos and Aquariums: Their Role in Conservation and Research (pp. 212-240). Cambridge University Press, Cambridge.

Watters, J. V., Margulis, S. W., & Atsalis, S. (2009). Behavioral monitoring in zoos and aquariums: a tool for guiding husbandry and directing research. Zoo Biology, 28(1), 35-48.

Wells, D. L. (2009). Sensory stimulation as environmental enrichment for captive animals: A review. Applied Animal Behaviour Science, 118(1-2), 1-11.

Wielebnowski, N. C., Fletchall, N., Carlstead, K., Busso, J. M., & Brown, J. L. (2002). Noninvasive assessment of adrenal activity associated with husbandry and behavioral factors in the North American clouded leopard population. Zoo Biology, 21: 77-98.

Young, R.J. (2003). *Environmental Enrichment for Captive Animals*. Blackwell Science, Oxford.

Chapter 2:
An Introduction to Species-Independent Combined-Individual Mixed-Model Analysis

Introduction

Like any scientific endeavor, animal behavior studies are designed around hypotheses and the research question determines the methodology best to answer it. Most research studies are conducted on a single species to gain knowledge of basic biology of organisms or for applied purposes. Research questions about single species require random samples from one or more populations. Examples of species-oriented studies of animals under human care have investigated reducing aggression in group-housed pigs (Verdon et al., 2018) and behavioral assessments at pet shelters and their prediction of length of stay (McGuire, 2021). Other behavior studies have focused on sampling units below the biological population level by investigating family groups, dyads or individuals as the sampling unit over time (Lehner, 1987; Martin & Bateson, 2007). For example, a study in agriculture focused on feeding laying hens from perches to reduce aggression (Sirovnik et al., 2018), and a study in zoos on mother-calf dyads assessing proximity and suckling behaviors between giraffes (Nakamichi et al., 2015). Studies can also focus on individual differences in behavior due to factors such as sex, age, or personality. Species-oriented research questions are insightful at each sampling level. When the research question is not about a single species, however, the animals studied can be diverse.

Research questions about general behavioral theories examine larger concepts outside a single species or individual. They aim to explore and understand fundamental principles of animal behavior. Because these theories are conceptually general, they can be demonstrated across multiple species and applied to many animals. The first step is to generate predictions based on the theoretical underpinnings, and then researchers can identify study animals best suited to test the predictions. Investigating general theories has allowed for much progress in behavioral ecology (Dugatkin, 2002) and other behavior fields (Shettleworth, 2001). This research can help us understand how animals adapt, respond, and interact with their environment.

The goals of zoo research vary based on the specific area of focus but aim to improve the understanding of behavior, biology, ecology, welfare, and conservation of animals. However, populations of animals in zoos differ from animal populations in the wild. Zoo populations are all the animals of the same species collectively managed across zoos, and within individual zoos there are animal groups. Despite these differences in how sampling populations are organized, zoo researchers are also interested in investigating general theories. For example, we may be interested in the impacts of giving animals choices or control over their environment or the different human-animal relationships in zoos (Hosey, 2013; Egelkamp & Ross, 2018). Zoos have relied heavily on experimental designs that carefully evaluate behavioral change in a single or small group of subjects (Plowman, 2008; Alligood et al., 2017). Given that any single zoo has many individuals of varied species in one location, they are poised to address questions aimed at elucidating general behavioral responses to the conditions prevalent in these facilities.

This chapter aims to introduce Species-Independent Combined-Individual Mixed-Model Analysis (SICIMMA) and encourage researchers to test general behavioral theories by studying the same theory across multiple species within a single institution. First, I will provide a brief description of how general behavioral theories are used to drive research and how they are expanded to more specific areas of research. Then, I will discuss the limitations of study designs in zoos and the value of single-subject experimental designs. Next, I will introduce SICIMMA. This approach will support researchers observing individuals of many species to answer general behavior hypotheses. Following its introduction, I will provide a guide for using SICIMMA with generalized linear mixed models. I review examples of studies that have used generalized linear mixed models in species-oriented research and recent zoo studies that examine a theory outside a single species. Ultimately I discuss the benefits of using SICIMMA to help expand our knowledge of animals in captivity.

General Theory Studies

Behavioral scientists are interested in understanding patterns of behavior and often ask if these patterns occur in a similar fashion independent of species. In such cases, we can use general theories to predict how animals should behave in a specific context. These predictions help develop research questions aimed at measuring behavioral patterns in a given study system. Once the theorized relationship has been observed in one species, the theory is expanded and applied to additional study systems or species. In this sense, studies of general theories are not species-specific.

For example, optimality theory predicts animals will modify their behavioral responses under different conditions to maximize their net benefits when trade-offs are present. Optimality modeling is the tool to analyze an individual's decisions regarding costs and benefits and predict how animals should perform in the face of trade-offs to achieve the maximum net benefit (Reviewed in Giraldeau & Hogan, 2022). This model is interested in two sampling levels, inter-individual variation and variation in responses across populations. The optimality theory has been extended to various decisions animals make, such as foraging (Pyke, 2019), copulation behavior (Parker, 2006), parental care (Davis et al., 1999; O'Rourke & Renn, 2015), mate choice (Ryan et al., 2019), and courtship (Aranha & Vasconcelos, 2018). Using the optimality theory, researchers can design studies to examine whether the theory applies to a given situation for different species.

There are also general predictions around welfare concepts such as indicators of affective state. For instance, anticipatory behavior is goal-directed activity before acquiring rewards such as food, sex, and drugs (Craig, 1918; van der Harst & Spruijt, 2007; Webb et al., 2009). It takes place before rewarding events occurring on fixed schedules or after reliable cues that indicate the opportunity to acquire reward is coming. Anticipatory behavior occurs when an animal perceives that a reward is likely to be available and appears to reflect the animal's reward sensitivity (Spruijt et al., 2001; Watters, 2014). It has been recorded in multiple species, such as rats and domestic cats (*Rattus*, *Felis catus*; van den Bos et al., 2003), cichlid fish (*Cichlidae*, Galhardo et al., 2011), a Western lowland gorilla and red panda (*Gorilla gorilla gorilla*, *Ailurus fulgens*; Krebs et al., 2017). Anticipatory behavior is typically measured at the individual level, and researchers are interested in the variation within individuals. Another example related to affective state and tested at the individual level is cognitive bias. Cognitive bias testing involves training animals to one cue, where the animal receives a reward, and another cue, where the animal either receives a lower-valued reward or receives a punishment. Once animals are trained to respond to a positive cue and to not respond to a negative cue, researchers observe how they react to an ambiguous cue (Horback, 2019). An ambiguous or intermediate cue is a stimulus between the positive and negative cue, which the animal has no trained response to. If the animals respond to the ambiguous cue with behaviors that suggest an expectation of a reward they are considered "optimistic," or if animals display behaviors of expecting a punishment they are "pessimistic" (Harding et al., 2004; Mendl et al., 2009; Horback, 2019). Cognitive bias testing has been broadly applied across taxa to similar results (Matheson et al., 2008; Doyle et al., 2010; Douglas et al., 2012). Thus, by establishing a foundation in one species, researchers have extended this principle to other species, evaluating whether they exhibit the predicted patterns. These behavioral theories are tested at the individual level and can demonstrate broad generalizations that apply to numerous species of animals.

Meta-analysis is another method used for discerning general patterns from multiple tests of hypotheses including those derived from general theories. It is frequently used in ecology, evolutionary biology, conservation, and behavior. For example, Risely et al. (2017) assessed how infection status and intensity affect migration using studies of birds, fish, and butterflies. Another meta-analysis looked at individual differences in behavior on survival using studies of mammals, fish, birds, reptiles, and insects (Moiron et al., 2020). Testing general theories via meta-analyses has also been conducted at zoos. Some examples are the effect of enrichment on stereotypic behavior across mammals (Shyne, 2006) or the cranial morphology of captive mammals (Siciliano-Martina et al., 2021). Meta-analyses help answer larger theoretical questions beyond one study and species. They identify trends, weigh the evidence for theories and hypotheses, and generalize common findings or observations (Gurevitch et al., 2018). To conduct a meta-analysis, theories must have already been proposed and tested in many study systems so that results can be combined and analyzed. Meta-analyses can help weigh the preponderance of evidence for or against a specific theory across a large number of studies of the same theory but do not help researchers examine whether a behavioral theory applies to a specific individual animal or population. Meta-analysis is a powerful tool for assessing questions of general theory but is best applied once many studies of a theory have been conducted already.

Study Designs in Zoos

Zoo and aquarium scientists are interested in theory-driven research questions about animal behavior, but due to differences in the structure of zoo populations and wild populations zoo researchers face several challenges in applying commonly used research methods from other fields. Small sample sizes and lack of independence violate statistical assumptions making it difficult to use inferential statistical methods in zoo research (Cohen & Cohen, 1983; Kuhar, 2006; Plowman, 2008; Alligood et al., 2017). In most singular zoos, large numbers of individuals of any species are unavailable, and there is typically, at best, only one multi-sex and age-diverse group of any species at an institution. Consequently, multi-institutional studies are often recommended to increase sample size and to test hypotheses about factors important for a given species. Examples of multi-institutional studies include investigations of the effects of environmental factors on the welfare of Asian and African elephants (Carlstead et al., 2013), low starch diet on gorilla behavior (Less et al., 2014), factors affecting locomotion and pacing in okapis (Bennet et al., 2015), and the effect of sex and season differences in wounding rates in Japanese macaques (Cronin et al., 2020). They are also often used to direct the husbandry or care of specific species. For example, Cronin et al. (2020) mentioned above was the first wounding study on Japanese macaques and suggested caretakers prioritize training females to present body parts as they are statistically more likely to be wounded than males. Multi-institutional studies are insightful but expensive, requiring intensive labor and logistical coordination. In addition, they provide little information generalizable to distantly- or unrelated species and have typically taken a back seat to determine factors important for managing individuals.

Single-Subject Designs

Zoos have relied on single-subject experimental designs (SSDs) to evaluate behavioral change in a single subject or small group of subjects who operate as their own control (Backman & Harris, 1999). SSDs are valuable when the research question is about changes in individuals' responses over time, or investigating the needs or concerns of individuals. This type of study design requires collection of repeated behavioral or physiological measures over time, usually consisting of baseline and intervention phases. These designs favor high internal validity and can establish relationships between environmental variables and individual measures. SSDs are fitting to experimentally test research questions in zoos due to the number of individual animals housed on location, all study phases can be conducted within the typical husbandry routines of the subjects, and (reviewed by Saudargas & Drummer, 1996; Alligood et al., 2017). Examples of SSDs in zoos are assessing and treating aggressive behavior in a lemur (Farmer-Dougan, 2014), training animals to interact with, or assessing animals' use of enrichment (Neto et al., 2016; Krebs & Watters, 2017). SSDs are beneficial and viable in zoos as they allow researchers to investigate potential factors contributing to the individual's behavior; however, external validity or generality with SSDs is a concern. Yet, external validity is less of a concern in SSDs since they focus on in-depth changes in individual subjects rather than trying to generalize to larger populations.

Concerns about the generality of SSDs arise due to the lack of inferential statistics typically used. Inferential statistics are not appropriate as they are based on comparing variation between subjects and looking for population level differences. Therefore, SSDs cannot make population-level inferences with one individual. Alligood et al. (2017) discuss that the critical concerns about generality in SSDs are the question of repeatable results and how applicable the situations are outside of the specific experiment conducted, but note that these concerns should not hinge on statistics. The advent of advanced statistical models, such as generalized linear mixed models, allows for more creative ways of accounting for sources of variation in data sets in ways that have not been possible before. The increased accessibility of advanced statistical methods provides a unique opportunity for testing general theories in zoos.

For a theory to be accepted, it must be proposed and tested in multiple species, individuals, and conditions and the predicted outcomes based on theory should be observed consistently across. It has been proposed that the answer to finding support for general theories should be through replications of the proposed effect (Branch & Pennypacker, 2013; Kazdin, 2011). I propose for generally applicable theories these replicates can be accomplished by studying multiple individuals of different species. We can apply a general behavior theory to individuals of different species for whom we have asked the same question and analyze their behavioral response together, using statistical methods that can account for individual-level and species-level differences among subjects. Considering multiple individuals of different species as replicates in a single study allows us to use a valid and feasible method yet still uses inferential statistics to gain confidence in the patterns observed. Therefore, I propose and encourage researchers to test the same questions on multiple individuals of different species to assess the generality of hypotheses associated with animal behavior. I refer to this statistical analysis as Species-Independent Combined-Individual Mixed-Model Analysis (SICIMMA).

Species-Independent Combined-Individual Mixed-Model Analysis

Research questions guided by general theory are typically tested within a species or by using a synthesis of multiple species to determine similar patterns as previously discussed (Figure 1a-b). I suggest that using analyses to answer a general behavioral question from data collected at the individual level from multiple individuals of different species is also valid (Figure 1c, SICIMMA). This analysis investigates if similar conditions evoke similar behavioral patterns or changes across individual subjects, regardless of their species. Using advanced statistical analyses that can factor out variation between species, SICIMMA can be accomplished by pooling data of individuals of multiple species since they are subjects who also serve as their own control. Combining multiple individuals into one study effectively increases the sample size and study systems examined, which is important for generalizing results (Branch & Pennypacker, 2013; Kazdin, 2011). This would provide sufficient data to allow the use of inferential statistics. This analysis is suited for assessing broader research hypotheses associated with animal behavior.

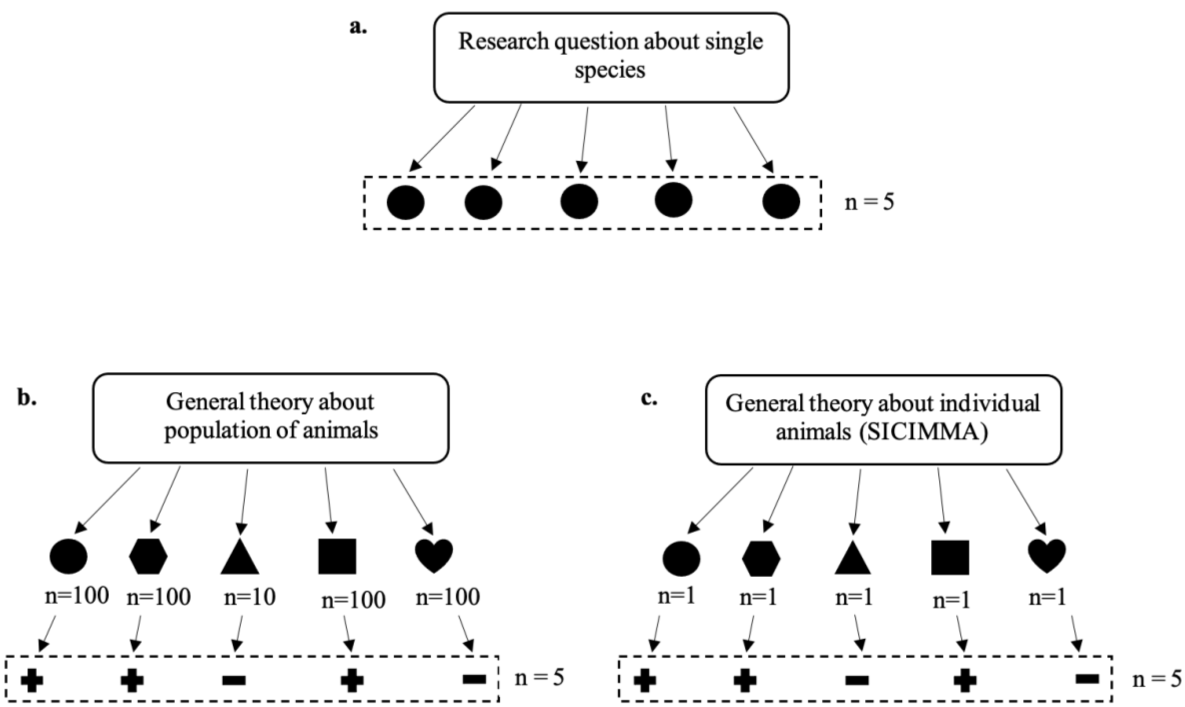

Figure 1. Diagram of research designs based on research questions (shapes represent different species). a. Research question about single species and the variation within, n = 5 individuals of the same species b. General theory at the population level, n = 5 populations of different species c. Proposed design of SICIMMA for general theory at the individual level, n = 5 individuals of different species. Positive and negative signs represent observed effects for each species (i.e. theory holds or theory does not hold).

A zoo is an ideal location for this analysis as it houses hundreds to thousands of individuals of various species on one property. These next sections provide details on conducting a SICIMMA study for a general animal behavior research question, a description of generalized linear mixed models (GLMMs) along with examples of how they are currently used, and recent zoo examples of SICIMMA being applied.

Any general behavior hypothesis about individuals can be tested in any species. For behavioral studies, specific behaviors or full time budgets can be collected, but these data may require extensive time and labor. Studies should use consistent data collection methods across species, and the subjects should be maintained under typical care regimes during the study (Mellor et al., 2018). Species-specific ethograms should be used for individuals of different species. To focus the analyses on a general theory, species-specific ethograms may need to be aggregated into broad classes of behaviors performed by most species (e.g., locomotion, stereotypies, activity vs. inactivity). Differences in facilities or animal management can be accounted for, since data will be collected in one location and individuals serve as their own control. An alternative to direct behavioral observations can be keeper records or zoo-datasets (e.g., Species360) if the data is recorded at the individual level. The following step is statistical analysis.

A possible statistical approach for SICIMMA is using generalized linear mixed models (GLMMs). They are commonly used in fields such as behavior, biology, psychology, and social sciences, where data often have multiple sources of variability. GLMMs are beneficial for multiple reasons, (1) they can handle different types of data such as binary, count, and continuous variables. Thus, they can accommodate a wide range of distributions, which allows researchers to model and analyze non-normal data appropriately. GLMMs can (2) accommodate hierarchical structures, where data has a nested organization. This organization is necessary when observations are clustered within larger units (e.g., individuals may be nested within social groups like herds or colonies). Measurements within clusters are likely not independent since they can be more homogeneous than measurements from different clusters, thus violating most parametric statistical assumptions (Tuerlinckx et al., 2006). GLMMs accommodate hierarchical structures by incorporating random effects in the model. Random effects are parameters that vary across different levels or groups in the data. They capture the variability specific to each group, unit, or unit within larger groups, and control for non-random error sources in the observed data. In the case of SICIMMA, using random effects allows researchers to account for both differences among individuals of the same species, and differences between species, in the same statistical model. The most common random effects encompass variation among blocks or studies that are replicated across sites and encompass variation among individuals when repeated measures are taken (Bolker et al., 2009). Thus, another benefit of GLMMs is to (3) handle repeated measures that often occur in longitudinal studies and within-subject designs. Because it is a mixed model it can incorporate fixed effects as well. Fixed effects are parameters that represent the effects of specific variables or factors whose levels are experimentally determined and constant across all groups or units (e.g., treatments/interventions, categorical variables: presence or absence of humans). They capture the differences associated with these variables and represent the overall influence on the response variables in the analysis (McElreath, 2016). For SSDs, a fixed effect of interest would be whatever intervention is being tested for an individual.

GLMMs are commonly used in animal personality studies that investigate consistent differences in an individual's behavior across time and contexts (Réale et al., 2007; Dingemanse & Dochtermann, 2012; Dingemanse & Wright, 2020). These studies require a set of individuals to be observed repeatedly to test whether various factors influence behavior in general, whether individuals differ consistently in their behavior, and whether individuals differ consistently in their response to the factors (Réale et al., 2007). Studies in animal personality and zoo research use the tools and flexibility GLMMs can provide. GLMMs can handle structured random effects, which allow researchers to specify explicitly how study subjects are grouped and control for variation within subjects and within groups. Thus random effects allow the analysis to control for variation by individual, group, and individual in the group. An example of GLMMs in animal personality is researchers investigating how the personalities of individuals and their sexual partners impact sexual selection and mating success and how environmental factors influence these effects (Montiglio et al., 2016; Roth et al., 2021). GLMMs can handle repeated measures or longitudinal data where multiple observations are made from the same individuals at multiple time points by allowing researchers to specify grouping variables in random effects. The model can account for individual variation which is a focus of animal personality studies, understanding consistent behavioral differences among individuals within a population. Random effects in GLMMs can capture individual variation and allow researchers to model and account for these differences.

GLMMs are already commonly used in zoo research, investigating various species-oriented research questions. For example, some use them to study the effects of visitor presence (Scott et al., 2017; Huskisson et al., 2021; Kidd et al., 2022) and abnormal behaviors in single species (Wagman, 2018; Laméris et al., 2021). For a more detailed example of a zoo study using GLMMs, Bastian et al. (2020) investigated the impact of a month-long night event (ZooLights) on the behavior and fecal glucocorticoid of six gorillas. We will only focus on the behavioral data from this example. Researchers collected behavioral observations 1-month prior to the event, during the 1-month event, and 1-month post-event. Observations were balanced throughout the day and evening during these study periods and across all the individuals. In addition, observers recorded crowd size at the start of each observation session. Researchers used GLMMs for the statistical analysis with the proportion of scans of each behavior (aggression, resting, and abnormal behaviors) as the response variable, each modeled separately. Periods (pre-event, event, and post-event) and crowd size (0, < 15, >15) were the fixed effects. Individual and observation day were used as random effects to control for repeated measures. The results were that the late-night event reduced evening resting behavior and increased daytime abnormal behavior during and post-event. This study suggests that caretakers must consider the consequences of late-night events as they can disrupt routine gorilla behavior.

GLMMs can be used for SICIMMA for the benefits discussed previously as this model combines the properties of generalized linear models, which can handle non-normal data, and linear mixed models, which can include both random and fixed effects (McElreath, 2016). This allows researchers to extrapolate statistical results to individuals or populations outside the study sample (Bolker et al., 2009). In addition, we can incorporate nested individuals within group levels as random effects in SICIMMA to account for combining multiple individuals of different species. GLMMs best fit SICIMMA to account for the changes over time and provide a more accurate representation of the underlying behavioral patterns. With an increased sample size from pooling individuals and the use of inferential statistics, we can assess commonalities in behavioral changes in response to the same management factors regardless of species. In turn, this improves our understanding of general theoretical questions about managing animals under human care. Recent zoo studies have used a species independent approach to answer research questions about more than one individual or species.

Species-Independent Zoo Studies

A recent zoo study investigated multiple individuals of different species to answer a general behavior question. Cairo-Evans et al. (2022) collected behavioral data on 16 individuals of 5 primate species to test how visitors' presence influences how primates use exhibit space. Researchers collected individual-level behavioral observations, the subject's location and distance from the enclosure glass, and the number of visitors present. These data were collected by direct observation from routine behavioral monitoring by volunteers over three years. For the behavioral data, they investigated if visitor presence impacted two behaviors (pacing and self-scratching). Although they only collected data on primate species, their question did not necessitate measuring differences in behavioral responses among species. Thus, their statistical analysis used linear and logistic regression models and included individuals nested in species as random effects. Their mixed model uses all observations of all the individuals and tests (1) whether visitor presence and the number of visitors affect the behavior of primates in general, (2) examines whether individuals differ in their behavior generally, and (3) whether individuals differ in their response to visitor presence. Their results showed that the primates' distance from the viewing glass decreased as the number of visitors increased, the animals in their study did not retreat, and they observed no differences in behavioral indicators when comparing visitor presence and complete absence. Therefore, Cairo et al. (2022) concluded that zoo visitors did not negatively influence primates' affective state. This study answered a general behavioral hypothesis applicable to all zoo primates, as visitor presence is a daily occurrence.

A second example, Krebs et al. (2022) presented a new model that suggests that the visitor effect is more likely a "dither effect" for some zoo animals than the standard framework of visitors influencing animals in a positive, negative, or neutral way. A dither stimulus is defined as a group of animals, unlike the focal subjects, that move continuously without pattern in the environment. This leads to decreased hypervigilance and increased normal behaviors in focal subjects (Barlow, 1968), though this effect might drop off with increased stimuli. Due to the prominent and consistent presence of visitors in zoos, the researchers wanted to test this nonlinear model on multiple species as an alternative to the linear ones previously suggested. They proposed that if visitors were truly providing a dither stimulus for zoo animals, the animals would show greater rates of comfortable behaviors and fewer anxiety-like behaviors as visitor numbers increased, though this might drop off with very large visitor numbers. This was tested by observing nine species (reticulated giraffes, greater kudus, Chilean flamingos, Magellanic penguins, meerkats, prairie dogs, and three species of pelicans) during two COVID-19 closures. The data was not compared between species but focused on whether a dither effect was evident in any of the study species. Their results showed a nonlinear relationship between human numbers and animal behavior. At intermediate levels of visitors, some animals decreased vigilance or other indicators of anxiety. The study discovered evidence for a dither effect across several species in a zoo and that human presence might sometimes support animals acting at ease.

Another recent study investigated multiple individuals to investigate if the presence of keepers influenced animal behavior. Pacheco et al. (Chapter 3) collected behavioral data on 15 individuals of six species when the primary keeper was present and when the keeper was absent. Observers collected time budgets for each group and sorted behaviors into active or inactive categories. Researchers completed observations over six months, and the keepers participated in the study within their schedule. We collected data at the individual level on six species (western lowland gorilla, mandrill, Coquerel’s sifaka, greater one-horned rhino, black bears, and wolverine). Researchers used generalized linear mixed models on active and inactive behaviors for statistical analysis, including individuals nested in species as random effects. The study concluded that animals are more active in the presence of keepers than when absent—suggesting that zoo animals have a cognitive bias towards their keepers and perceive them as a positive stimulus. This may be because keepers are the primary source of positive reinforcement for animals as they provide daily food, enrichment, and training sessions. By studying various species, researchers answered a general behavioral hypothesis that applies to all captive animals. This study suggests that animals' perceptions of their surroundings can be better understood by observing their response to their daily caretakers.

Conclusion

I hope this paper provides the premise for asking general behavioral theories at the individual level and encourages scientists to use SICIMMA by observing many individuals of multiple species. This framework can help us identify underlying principles shared across individuals or contexts. SICIMMA analysis can benefit animals in captivity as individuals of the same species or groups do not perceive nor interact with their environments similarly (Boyle et al., 2020). However, by using advanced statistical models, scientists can focus on the differences that matter. Combining multiple individuals of different species can identify the key factors responsible for general patterns, even if potential individual differences occur. While individual differences can be important for specific research questions, investigating general theories can provide us with knowledge for understanding the behavior as a whole, which can inform management strategies for individuals and species. Managing captive animals involves many of the same factors across settings, whether in farms, laboratories, or zoos, such as providing basic needs (e.g., food, water, health), proper environments (e.g., space, protection from weather, enrichment), and human-animal relationships. Animals in the same location or type of housing can respond to management changes differently, but it is valuable to understand general patterns to help us manage more efficiently. SICIMMA can be used in any setting but is well-suited for use in zoos. A medium-sized zoo has 168-394 species within a range on one property (Marcy, 2021), and SICIMMA can aid scientists in controlling for environmental factors. Testing general theories about captive animals can be important for management, welfare, and conservation. Changes in the husbandry in captivity and the animal's response to these changes can impact animal welfare and the institution's conservation goals. Even though conservation goals are at a species level, those goals cannot be met without the management and welfare of individuals being a priority. By embracing SICIMMA, scientists can discover a new understanding of animal behavior, inform evidence-based management strategies, and enhance animal welfare.

References

Alligood, C. A., Dorey, N. R., Mehrkam, L. R., & Leighty, K. A. (2017). Applying behavior-analytic methodology to the science and practice of environmental enrichment in zoos and aquariums. Zoo biology, 36(3), 175-185.

Aranha, M. M., & Vasconcelos, M. L. (2018). Deciphering Drosophila female innate behaviors. *Current Opinion in Neurobiology*, *52*, 139-148.

Backman, C. L., & Harris, S. R. (1999). Case studies, single-subject research, and N of 1 randomized trials: comparisons and contrasts. American Journal of Physical Medicine & Rehabilitation, 78: 170-176.

Barlow, G. W. (1968). Dither—A way to reduce undesirable fright behavior in ethological studies. *Zeitschrift für Tierpsychologie*, 25(3), 315–318.

Bastian, M. L., Glendinning, D. R., Brown, J. L., Boisseau, N. P., & Edwards, K. L. (2020). Effects of a recurring late-night event on the behavior and welfare of a population of zoo-housed gorillas. *Zoo Biology*, *39*(4), 217-229.

Bennett, C., Torgerson-White, L., Fripp, D., Watters, J., & Petric, A. (2015). A multi-institutional assessment of factors influencing locomotion and pacing in captive okapis (Okapia johnstoni). Applied Animal Welfare Science, 18(sup1), S43-S61.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127-135.

Boyle, S. A., Berry, N., Cayton, J., Ferguson, S., Gilgan, A., Khan, A., ... & Reichling, S. (2020). Widespread behavioral responses by mammals and fish to zoo visitors highlight differences between individual animals. Animals, 10(11), 2108.

Branch, M. N., & Pennypacker, H. S. (2013). Generality and generalization of research findings. In G. J. Madden, W. V. Dube, T. D. Hackenberg, G. P. Hanley, & K. A. Lattal (Eds.), APA handbooks in psychology®. APA handbook of behavior analysis, Vol. 1. Methods and Principles (p. 151–175). American Psychological Association.

Cairo-Evans, A., Wierzal, N. K., Wark, J. D., & Cronin, K. A. (2022). Do zoo-housed primates retreat from crowds? A simple study of five primate species. American Journal of Primatology, 84(10), e23386.

Carlstead, K., Mench, J. A., Meehan, C., & Brown, J. L. (2013). An epidemiological approach to welfare research in zoos: The elephant welfare project. Applied Animal Welfare Science, 16(4), 319-337.

Cohen J & Cohen P. (1983). Applied multiple regression/correlation analysis for the behavioural sciences, 2nd edition. Hillsdale: Lawrence Erlbaum Associates. 512p.

Craig, W. (1918). Appetites and aversions as constituents of instincts. The Biological Bulletin, 34(2), 91-107.

Cronin, K. A., Tank, A., Ness, T., Leahy, M., & Ross, S. R. (2020). Sex and season predict wounds in zoo-housed Japanese macaques (Macaca fuscata): A multi-institutional study. Zoo Biology, 39(3), 147-155.

Davis, J. N., Todd, P. M., & Bullock, S. (1999). Environment quality predicts parental provisioning decisions. *Procee. R. Soc. B Biol. Sci.* 266, 1791–1797. doi: 10.1098/rspb.1999.0848

Dingemanse, N. J., & Dochtermann, N. A. (2012). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *Journal of Animal Ecology*, *82*(1), 39-54. https://doi.org/10.1111/1365-2656.12013

Dingemanse, N. J., & Wright, J. (2020). Criteria for acceptable studies of animal personality and behavioural syndromes. *Ethology*, *126*(9), 865-869.

Douglas, C., Bateson, M., Walsh, C., Bédué, A., & Edwards, S. A. (2012). Environmental enrichment induces optimistic cognitive biases in pigs. Applied Animal Behaviour Science, 139(1-2), 65-73.

Doyle, R. E., Fisher, A. D., Hinch, G. N., Boissy, A., & Lee, C. (2010). Release from restraint generates a positive judgement bias in sheep. Applied Animal Behaviour Science, 122(1), 28-34.

Dugatkin, L. (2002). *Model Systems in Behavioral Ecology: Integrating Conceptual, Theoretical, and Empirical Approaches*. Princeton: Princeton University Press.

Egelkamp, C. L., & Ross, S. R. (2018). A review of zoo-based cognitive research using touchscreen interfaces. *Zoo Biology*, *38*(2), 220-235.

Farmer-Dougan, V. (2014). Functional analysis of aggression in a black- and-white Ruffed Lemur (Varecia variegate variegata). Applied Animal Welfare Science, 17, 282–293.

Galhardo, L., Vital, J., & Oliveira, R. F. (2011). The role of predictability in the stress response of a cichlid fish. Physiology & Behavior, 102(3-4), 367-372.

Giraldeau, L., & Hogan, J.A. (2022). The Function of Behavior. In J.J. Bolhuis, L. Giraldeau, J.A. Hogan (Eds.). *The Behavior of Animals: Mechanisms, Function and Evolution* (pp. 281-314). Wiley Blackwell.

Gurevitch, J., Koricheva, J., Nakagawa, S., & Stewart, G. (2018). Meta-analysis and the science of research synthesis. Nature, 555(7695), 175-182.

Harding, E. J., Paul, E. S., & Mendl, M. (2004). Cognitive bias and affective state. Nature, 427(6972), 312-312.

Horback, K. M. (2019). The emotional lives of animals. In *The Routledge Handbook of Animal Ethics* (pp. 55-70). Routledge.

Hosey, G. (2013). Hediger revisited: how do zoo animals see us?. *Applied Animal Welfare Science*, 16: 338-359.

Huskisson, S. M., Doelling, C. R., Ross, S. R., & Hopper, L. M. (2021). Assessing the potential impact of zoo visitors on the welfare and cognitive performance of Japanese macaques. *Applied Animal Behaviour Science*, *243*, 105453.

Kazdin, A. E. (2011). Single-case research designs: Methods for clinical and applied settings. Oxford University Press.

Kidd, P., Ford, S., & Rose, P. E. (2022). Exploring the Effect of the COVID-19 Zoo Closure Period on Flamingo Behaviour and Enclosure Use at Two Institutions. *Birds*, *3*(1), 117-137.

Krebs, B. L., Eschmann, C. L., & Watters, J. V. (2022). Dither: A unifying model of the effects of visitor numbers on zoo animal behavior. Zoo Biology.

Krebs, B. L., Torres, E., Chesney, C., Kantoniemi Moon, V., & Watters, J. V. (2017). Applying behavioral conditioning to identify anticipatory behaviors. Journal of Applied Animal Welfare Science, 20(2), 155-175.

Krebs, B. L. & Watters, J. V. (2017). Simple but temporally unpredictable puzzles are cognitive enrichment. Animal Behavior and Cognition, 4: 119-134.

Kuhar, CW. (2006). In the deep end: pooling data and other statistical challenges of zoo and aquarium research. Zoo Biology, 25:339–352.

Laméris, D. W., Staes, N., Salas, M., Matthyssen, S., Verspeek, J., & Stevens, J. M. (2021). The influence of sex, rearing history, and personality on abnormal behaviour in zoo-housed bonobos (Pan paniscus). *Applied Animal Behaviour Science*, *234*, 105178.

Less, E. H., Bergl, R., Ball, R., Dennis, P. M., Kuhar, C. W., Lavin, S. R., ... & Lukas, K. E. (2014). Implementing a low-starch biscuit-free diet in zoo gorillas: The impact on behavior. Zoo Biology, 33(1), 63-73.

Lehner, P. N. (1987). Design and execution of animal behavior research: an overview. *Journal of animal science*, *65*(5), 1213-1219.

Marcy, K. (2021, May 26). Interesting Zoo and Aquarium Statistics. AZA.org.

Martin, P., & Bateson, P. (2007). Measuring behavior: an introductory guide (3rd ed.). Cambridge, UK: Cambridge University Press.

Matheson, S. M., Asher, L., & Bateson, M. (2008). Larger, enriched cages are associated with 'optimistic' response biases in captive European starlings (Sturnus vulgaris). Applied Animal Behaviour Science, 109(2-4), 374-383.

McElreath, R. (2016). Statistical Rethinking: A Bayesian Course with Examples in R and Stan CRC Press.

McGuire, B., Chan, J., Jean-Baptiste, K., Kok, P., & Rosenbaum, E. (2021). Results of behavioral evaluations predict length of stay for shelter dogs. Animals, 11(11), 3272.

Mellor, E., McDonald Kinkaid, H., & Mason, G. (2018). Phylogenetic comparative methods: Harnessing the power of species diversity to investigate welfare issues in captive wild animals. Zoo Biology, 37(5), 369-388.

Mendl, M., Burman, O. H., Parker, R. M., & Paul, E. S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. Applied Animal Behaviour Science, 118(3-4), 161-181.

Moiron, M., Laskowski, K. L., & Niemelä, P. T. (2020). Individual differences in behaviour explain variation in survival: A meta-analysis. *Ecology Letters*, *23*(2), 399-408.

Montiglio, O., Wey, T. W., Chang, A. T., Fogarty, S., & Sih, A. (2016). Correlational selection on personality and social plasticity: Morphology and social context determine behavioural effects on mating success. *Journal of Animal Ecology*, *86*(2), 213-226.

Nakamichi, M., Murata, C., Eto, R., Takagi, N., & Yamada, K. (2015). Daytime mother–calf relationships in reticulated giraffes (Giraffa cameloparadalis reticulate) at the Kyoto City Zoo. Zoo Biology, 34(2), 110-117.

Neto, M. P., Silveira, M., & dos Santos, M. E. (2016). Training bottlenose dolphins to overcome avoidance of environmental enrichment objects in order to stimulate play activities. Zoo Biology, Early view https://doi. org/10.1002/zoo.21282

O'Rourke, C. F., & Renn, S. C. (2015). Integrating adaptive trade-offs between parental care and feeding regulation. *Current Opinion in Behavioral Sciences*, *6*, 160-167.

Parker, G. A. (2006). Sexual conflict over mating and fertilization: an overview. Philosophical Transactions of the Royal Society B: Biological Sciences, 361(1466), 235-259.

Plowman, A. B. (2008). BIAZA statistics guidelines: toward a common application of statistical tests for zoo research (Vol. 27, No. 3, pp. 226-233). Hoboken: Wiley Subscription Services, Inc., A Wiley Company.

Pyke, G. H. (2019). Optimal foraging theory: An introduction. In J. C. Choe (Ed.), *Encyclopedia of Animal Behavior* (2nd ed., pp. 111–117). Academic Press: Elsevier.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291-318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Risely, A., Klaassen,M., & Hoye, B. J. (2017). Migratory animals feel the cost of getting sick: A meta-analysis across species. Journal of Animal Ecology, 87: 301-314.

Roth, A. M., Dingemanse, N. J., Nakagawa, S., McDonald, G. C., Løvlie, H., Robledo-Ruiz, D. A., & Pizzari, T. (2021). Sexual selection and personality: Individual and group-level effects on mating behaviour in red junglefowl. *Journal of Animal Ecology*, *90*(5), 1288-1306.

Ryan, M. J., Page, R. A., Hunter, K. L., & Taylor, R. C. (2019). 'Crazy love': Nonlinearity and irrationality in mate choice. *Animal Behaviour*, *147*, 189-198.

Saudargas, R. A., & Drummer, L. C. (1996). Single subject (small N) research designs and zoo research. Zoo Biology, 15: 173-181.

Scott, K., Heistermann, M., Cant, M. A., & Vitikainen, E. I. (2017). Group size and visitor numbers predict faecal glucocorticoid concentrations in zoo meerkats. *Royal Society open science*, *4*(4), 161017.

Siciliano-Martina, L., Light, J. E., & Lawing, A. M. (2021). Cranial morphology of captive mammals: a meta-analysis. Frontiers in Zoology, 18(1), 1-13.

Sirovnik, J., Stratmann, A., Gebhardt-Henrich, S. G., Würbel, H., & Toscano, M. J. (2018). Feeding from perches in an aviary system reduces aggression and mortality in laying hens. Applied Animal Behaviour Science, 202, 53-62.

Shettleworth, S. J. (2001). Animal cognition and animal behaviour. *Animal behaviour*, *61*(2), 277-286.

Shyne, A. (2006). Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biology, 25(4), 317-337.

Spruijt, B. M., van den Bos, R., & Pijlman, F. T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behavior Science, 72: 145-171.

Tuerlinckx, F., Rijmen, F., Verbeke, G. & De Boeck, P. (2006). Statistical inference in generalized linear mixed models: A review. *British Journal of Mathematical and Statistical Psychology*, 59: 225-255.

van den Bos, R., Meijer, M. K., van Renselaar, J. P., van der Harst, J. E., & Spruijt, B. M. (2003). Anticipation is differently expressed in rats (Rattus norvegicus) and domestic cats (Felis silvestris catus) in the same Pavlovian conditioning paradigm. *Behavioural Brain Research*, 141(1), 83-89.

Van der Harst, J., & Spruijt, B. (2007). Tools to measure and improve animal welfare: Reward-related behaviour. Animal Welfare, 16(S1), 67-73. doi:10.1017/S0962728600031742

Verdon, M., & Rault, J. L. (2018). Aggression in group housed sows and fattening pigs. In Advances in pig welfare (pp. 235-260). Woodhead Publishing.

Wagman, J. D., Lukas, K. E., Dennis, P. M., Willis, M. A., Carroscia, J., Gindlesperger, C., & Schook, M. W. (2018). A work-for-food enrichment program increases exploration and decreases stereotypies in four species of bears. *Zoo Biology*, *37*(1), 3-15.

Watters, J. V. (2014). Searching for behavioral indicators of welfare in zoos: Uncovering anticipatory behavior. Zoo Biology, 33, 251–256. doi:10.1002/zoo.21144

Webb, I.C., Baltazar, R.M., Wang, X., Pitchers, K.K., Coolen, L.M. & Lehman, M.N. (2009). Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. *Biological Rhythm*s, 24, 465–476.

Chapter 3:

Keeper Effect: Animals are more active in the presence of keepers

Introduction

Animals in captivity rely on humans for daily care. Human-animal interactions impact animal behavior and wellbeing (reviewed in Hosey & Melfi, 2014; Hosey, 2008; Hemsworth, 2003; Bayne, 2002). Zoo animals have daily interactions with their caretakers as well as visitors. The presence of zoo visitors and its impact on animal behavior and welfare has been studied thoroughly (reviewed in Sherwen & Hemsworth, 2019; Davey, 2007). Studies have shown that visitors can have positive, negative, or no effect on animals (e.g., Rose et al., 2020; Jones et al., 2016; Sherwen et al., 2015); however, a more recent study showed the same individuals could exhibit all three responses to visitors under certain conditions (Krebs et al., 2022). Animal behavior may vary with the nature of the interactions between animals and humans. While guest presence at zoos is frequent, the human that the zoo animal has the most opportunity to become familiar with and develop a human-animal relationship is its keeper.

As the primary caretakers of animals in zoos and aquariums, keepers have frequent and close contact interactions with animals in their care. These daily interactions entail feedings, training sessions, enrichment, and social interactions, which are likely to have some form of primary reinforcement value for the animal (Chelluri et al., 2013; Ward & Melfi, 2013; Young, 2003). These positively reinforced interactions are more likely to result in a positive human-animal relationship with keepers than with other humans, such as veterinarians or visitors (Melfi & Thomas, 2005). Daily interactions with keepers are predictable for animals as they occur around the same time of day and result in a change of behavior around these times (Podturkin et al., 2022; Clegg et al., 2018; Ward & Melfi, 2015). Animals also show behavior changes in response to unscheduled and unstructured interactions with their caretakers (Chelluri et al., 2013; Baker, 2004). Differences in keeper personalities and management styles elicit different responses from animals (Phillips & Peck, 2007; Carlstead, 2009). Animals learn to associate with their specific caretakers over time and connect these humans with positive/rewarding outcomes. Evidence suggests animals can distinguish between familiar and unfamiliar people in zoos and other settings (as reviewed in Hosey & Melfi, 2014). Martin & Melfi (2016) observed a decrease in avoidance behavior by animals towards familiar keepers compared to unfamiliar keepers, suggesting that the behavioral changes an animal exhibits can be specific to the human involved. These studies show that animals can recognize keepers, and their interactions lead to changes in behavior, demonstrating a keeper effect. As behavioral observations by keepers are often used in assessments of animal welfare, assessing whether or how a keeper effect influences animal behavior in zoos has important implications for understanding zoo animal welfare.

Since animals can recognize their caretakers and may associate them with positive events, they may present appetitive behaviors when keepers are nearby. Anticipatory behavior is a behavioral indicator of animal welfare gaining interest in zoos due to animals living in highly predictable environments (Ward et al., 2018). It is a goal-directed behavior that occurs when an animal perceives that a reward is likely to be available and appears to reflect the animal's reward sensitivity (Spruijt et al., 2001; Watters, 2014). Anticipatory behavior can be expressed differently across species, individuals and contexts, and may depend on the manner in which they acquire rewards (Watters et al., 2019). Anticipation prior to scheduled feedings has been seen in different species with increased activity, such as lambs (Anderson et al., 2015), hamsters (Dantas-Ferreria et al., 2015), and salmon (Thomassen & Fjaera, 1991). Along with increased activity, some animals may also express ‘searching’ behaviors (e.g., sniffing and looking) or focus activity near the location of the expected reward (Krebs et al., 2017).

In this study, we investigated if the presence of the primary keeper standing in the public viewing area of the exhibit impacts the behavior of individuals from six species. Zoo animals are likely to associate keeper presence with positive outcomes, as keepers are the source of primary reinforcers, such as food, access to social companions, enrichment, etc. Therefore, we hypothesize that animals behave differently in the keepers’ presence than in the keeper’s absence. Specifically, because their caretakers are associated with primary reinforcers, we suggest that animals will act in an appetitive fashion as if they are seeking a reward. If so, we predict that animal behavior in the keeper’s presence will show more active behaviors than when the keeper is not present.

Materials & Methods

Subjects

We conducted our study between August 2019 – December 2019 at the San Francisco Zoo and Gardens (San Francisco, California). The subjects for data collection were 15 individuals of five species: Western Lowland gorillas (*Gorilla gorilla gorilla*), Coquerel’s sifakas (*Propithecus coquereli*), mandrills (*Mandrillus sphinx*), American black bears (*Ursus americanus*), wolverine (*Gulo gulo*) and Greater one-horned rhinoceros *(Rhinoceros unicornis*) (age and sex of each in Table 1). Each species occupied its own exhibit and, apart from the rhinoceros and wolverine, were housed socially. Each exhibit varied in size, and we conducted all observations from the public viewing areas. The primary keeper of each study species participated in this study (a total of six keepers). Primary keepers work five days a week with the same individuals and we expect them to develop a strong human-animal relationship.

Table 1. Study population of individuals at San Francisco Zoo and Gardens

ID	Species	Sex	Age at time of study (years)
Gorilla 1	Western Lowland Gorilla	M	39
Gorilla 2	Western Lowland Gorilla	F	40
Gorilla 3	Western Lowland Gorilla	F	22
Gorilla 4	Western Lowland Gorilla	F	20
Mandrill 1	Mandrill	M	9
Mandrill 2	Mandrill	F	27
Mandrill 3	Mandrill	F	29
Mandrill 4	Mandrill	F	17
Mandrill 5	Mandrill	M	1
Lemur 1	Coqueral Sifaka	M	6
Lemur 2	Coqueral Sifaka	F	8
Rhino	Greater One-horn Rhino	M	24
Bear 1	American Black Bear	M	2
Bear 2	American Black Bear	F	2
Wolverine	Wolverine	M	10

Behavioral Observations

We collected observations during zoo operating hours, approximately 1000 – 1700. To compare how the animals behaved in the absence of keepers, we took observations each day and split them into one-hour intervals (e.g., 1000 – 1059, 1100 – 1159, etc.). We balanced observations across all groups for each hour interval. This split resulted in seven observation sessions per day/per species for 24 days of data collection. Data was collected using an ethogram for each species on the ZooMonitor application (Lincoln Park Zoo, 2020). The gorillas, sifakas, bears, and wolverine occasionally had access to indoor, off-exhibit space in the afternoons and were thus recorded as out of view in this circumstance. If features of the exhibit obstructed the view of animals, they were out of view. Each observation session was five minutes, with instantaneous scan sampling behavioral data collected at one-minute intervals (Martin & Bateson, 2007). The same observer (E.P.) collected data and rotated between each exhibit every hour; we randomized the order of exhibits throughout the day.

To test the effect of the keeper's presence on behavior, keepers stood in the public viewing area of the exhibit for a 5,10, or 20-minute period. The durations varied by keeper and across sampling periods for a cumulative 20-30 minutes a day to accommodate the caretaker's schedule. The keepers' presence was visible to the animals, but they did not interact with them or give any reinforcement during observations. Keepers participated in these sessions three times a week for four weeks (a total of 24-36 formal observation periods per keeper). Each keeper determined the timing of sessions based on their schedule. We did not set constraints on whether keepers should do observations before or after servicing the exhibit or feeding the animal. To account for variations in when keepers chose to do observations in relation to animal husbandry activities, we took notes on whether the timing of sessions was associated with keepers offering an opportunity to the animals. An opportunity could include feeding, training session, or enclosure shift. If keepers provided an opportunity within 30 minutes of a session, we noted whether the session was "Before" or "After" the animals were given an opportunity. These opportunities corresponded to a normal time period of when the keeper conducts husbandry activities throughout the day. If sessions were not associated with opportunities provided by keepers (not within 30 minutes), we recorded them as "None." We expected keepers to choose times which fit into their typical daily routines for each species as they would be close to the exhibit, but keepers were allowed to set their session schedules.

Statistical Analysis

We categorized behaviors on the ethogram and lumped them into either “Active” or “Inactive.” Inactive behaviors included lying down and sitting (Table 2). Self-directed and potentially abnormal behaviors were excluded from analysis as these behaviors are not positive, active behaviors, and occurred infrequently or less likely on the interval (see supplemental table for complete ethogram for each species). Active behaviors included all other behaviors not included as inactive or self-directed/abnormal (Table 2). We expected the presence of a keeper would be a cue to animals for positive events, and thus animal behavior in the keeper’s presence would reflect appetitive behaviors. Studies in a variety of species suggest appetitive behaviors are expressed as an increase in activity levels or locomotion (Anderson et al., 2015; Dantas-Ferreria et al., 2015). Therefore, we expected anticipatory behaviors would be reflected in the “Active” behavior category. We included the standing behavior, as some animals expressed it as waiting and focused in a particular area (Table 2). Each behavior was also analyzed separately. As some behaviors were removed for analysis, Active and Inactive do not sum to 1.

We analyzed the data with a generalized linear mixed model to test the effect of the keeper’s presence on observed behavior using the package “lme4” (with a logit link) in R v. 4.2.1. GLMMs can handle structured random effects, which allows researchers to specify explicitly how study subjects are grouped and control for variation within subjects and within groups. GLMMs are commonly used in other fields (e.g., animal personality where animals are already grouped by age or sex) as random effects allow the analysis to control for variation by individual, group, and individual in a group (Bolker et al., 2009). For each behavior, we calculated the total counts of observations per day. Then the proportion of each behavior was calculated using counts observed and counts of total observations per day. Individuals were identifiable for all species and thus data were collected at the level of individual animals. We expect individuals to respond differently to keeper presence since there is individual variation in behavioral responses, and animals can distinguish specific caretakers (Boyle et al., 2020; Martin & Melfi, 2016). Thus, individual nested in species was included as a random effect to account for repeated measures of individuals and expected differences among species. Keeper's presence was recorded as binary data and set as the fixed effect. When investigating the effect of the timing of opportunities and keeper presence on observed behavior, we added a fixed effect of Timing to our models above. Separate models were created for each activity level, "Active" and "Inactive," and individual behaviors.

Table 2. Ethogram of behaviors used in the analysis.

Active Behaviors	
Locomote	Moves from one location to another on the ground via walking, jumping, or running.
Eat/Forage	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with the mouth or hands. Includes eating grass or leaves in the exhibit and walking slowly with head down looking for food.
Interact with enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or limbs to count.
Wade	In pool, locomoting, standing, or splashing but not playing with enrichment items in the pool.
Stand	Standing with all limbs on the ground or standing at full height on two rear feet. No other behavior is occurring
Drink	Consumes water.

Climb	Climbs or jumps either onto structures, trees, or logs in exhibit.
Sniff	Puts nose onto or near an object, includes lifting head or upper lip in air.
Hang	Hanging from mesh enclosure or branches.
Dig	Digging in dirt or ground, does not include keeper provided substrates.
Other	An active behavior not included in the ethogram.
Play	Includes patting or pulling of body parts (arms, legs, fingers, clapping, chest-beating). This includes active playing such as twirling, somersaults, rolling.
Affiliative	Rubs against, lies against, or engages in friendly behaviors besides grooming towards another individual.
Aggress	Chases, growls at, snaps/bites at or otherwise aggressively interacts with another individual.
Inactive Behaviors	
Lie Down	Lying on side, belly or back. No other behavior is occurring.
Sit	Rear is on the ground or rocks, upper body upright. No other behavior is occurring.

Results

Overall Keeper Presence

The generalized linear mixed model of the effect of keeper presence revealed that animals were significantly more active when the keeper was near the animal’s exhibit than when the keeper was not present, and inactivity decreased in the presence of the keeper (Figure 1, $p<0.0001$). A time budget of all active behaviors (Figure 2) shows that animals display greater proportions of each in the keeper's presence than when the keeper is absent. This keeper effect occurs for all active behaviors. When analyzing each behavior independently, eating/foraging, interacting with enrichment, and wading were significantly higher in the keeper's presence than in the keeper's absence (Figure 2, p <0.0001).

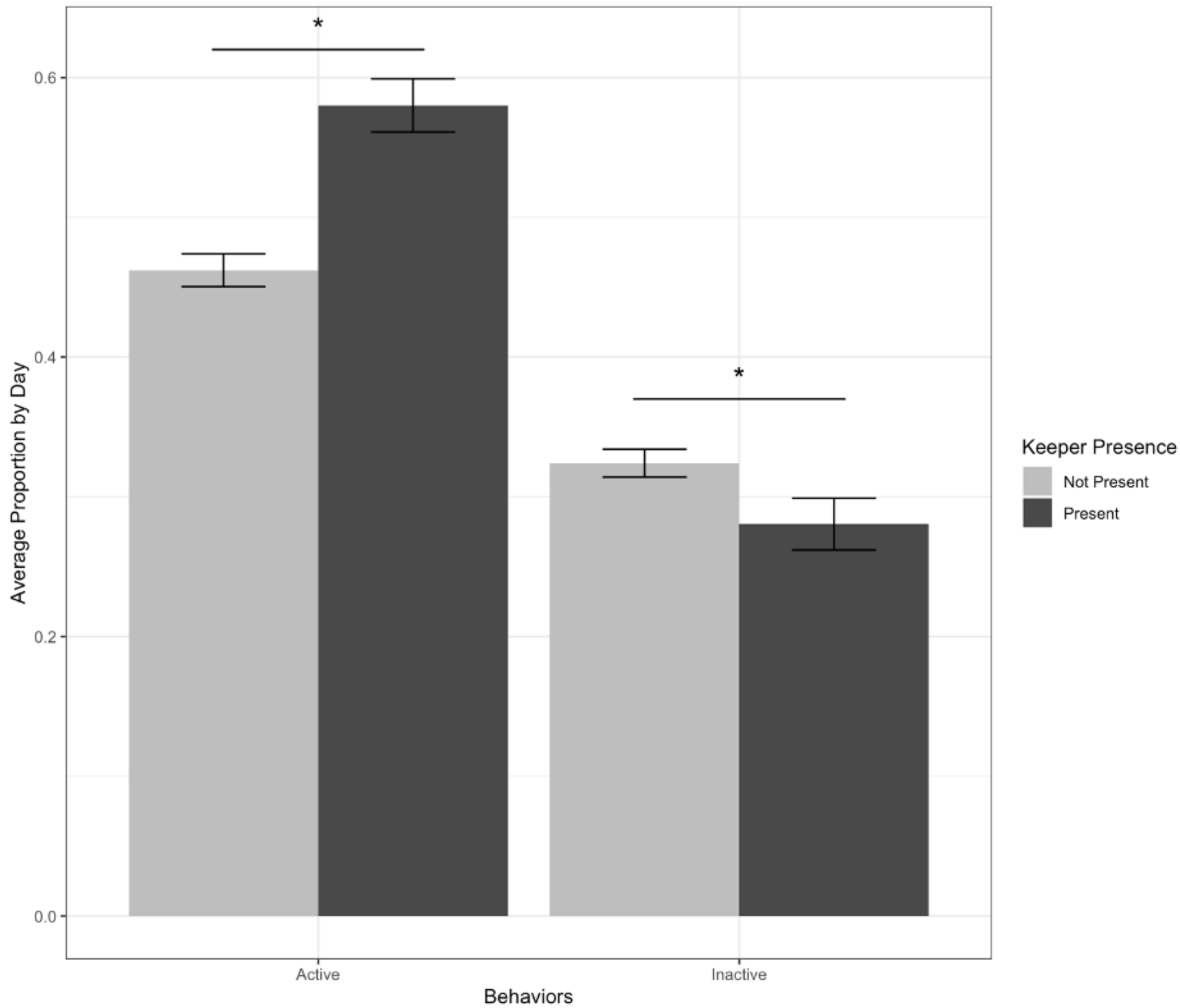

Figure 1. Average daily proportion of active and inactive behaviors when keeper is present and not present. Asterisk denotes significant difference and error bars represent ± one standard error.

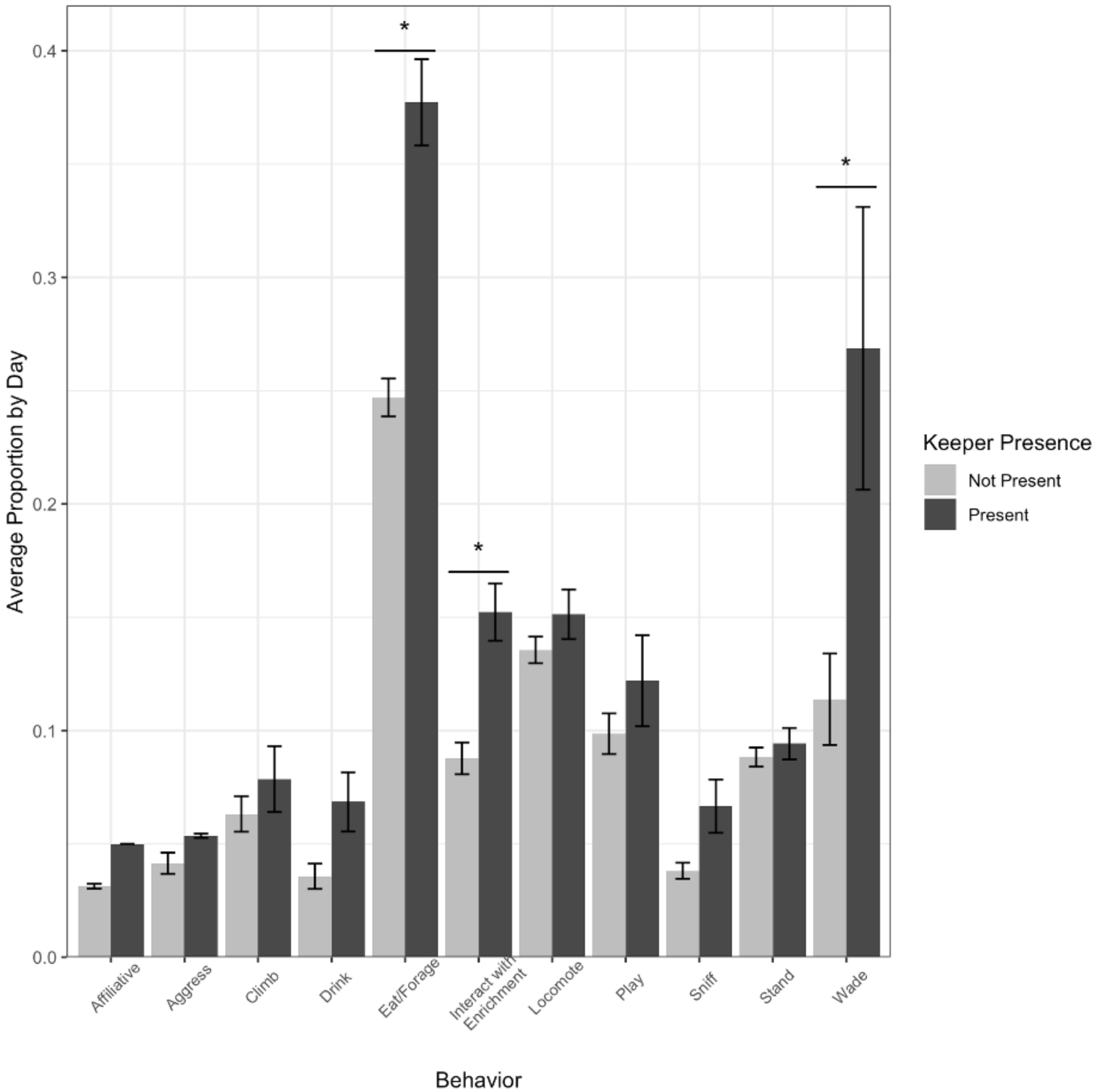

Figure 2. Average daily proportion of all active behaviors when keeper was present and not present. Only active behaviors that were observed across more than one species were graphed for simplicity. Asterisk denotes significant difference ($p < 0.0001$) for specific behavior analyzed separately across all species with that behavior in its ethogram. Error bars represent ± one standard error.

Keeper Presence by Timing of Opportunities

We examined keeper presence data by whether opportunities were offered to the animals. Based on caretaker schedule, sessions testing keeper presence occurred 15% of the time before an opportunity, 44% after an opportunity, and 41% when no opportunity was offered to the animals. This resulted in activity in each time period being significantly different compared to when the keeper was not present regardless of the timing the keeper was present (after: $p < 0.01$, before: $p<0.0001$, none: $p<0.0001$).

We observed greater activity when the keeper was present after giving an opportunity to animals compared to activity levels before or when no opportunity was offered (Figure 3, $p<0.0001$). Animals were equally as active when the keeper was present before providing an opportunity and when the keeper was present but offered no opportunity (Figure 3, $p<0.0001$).

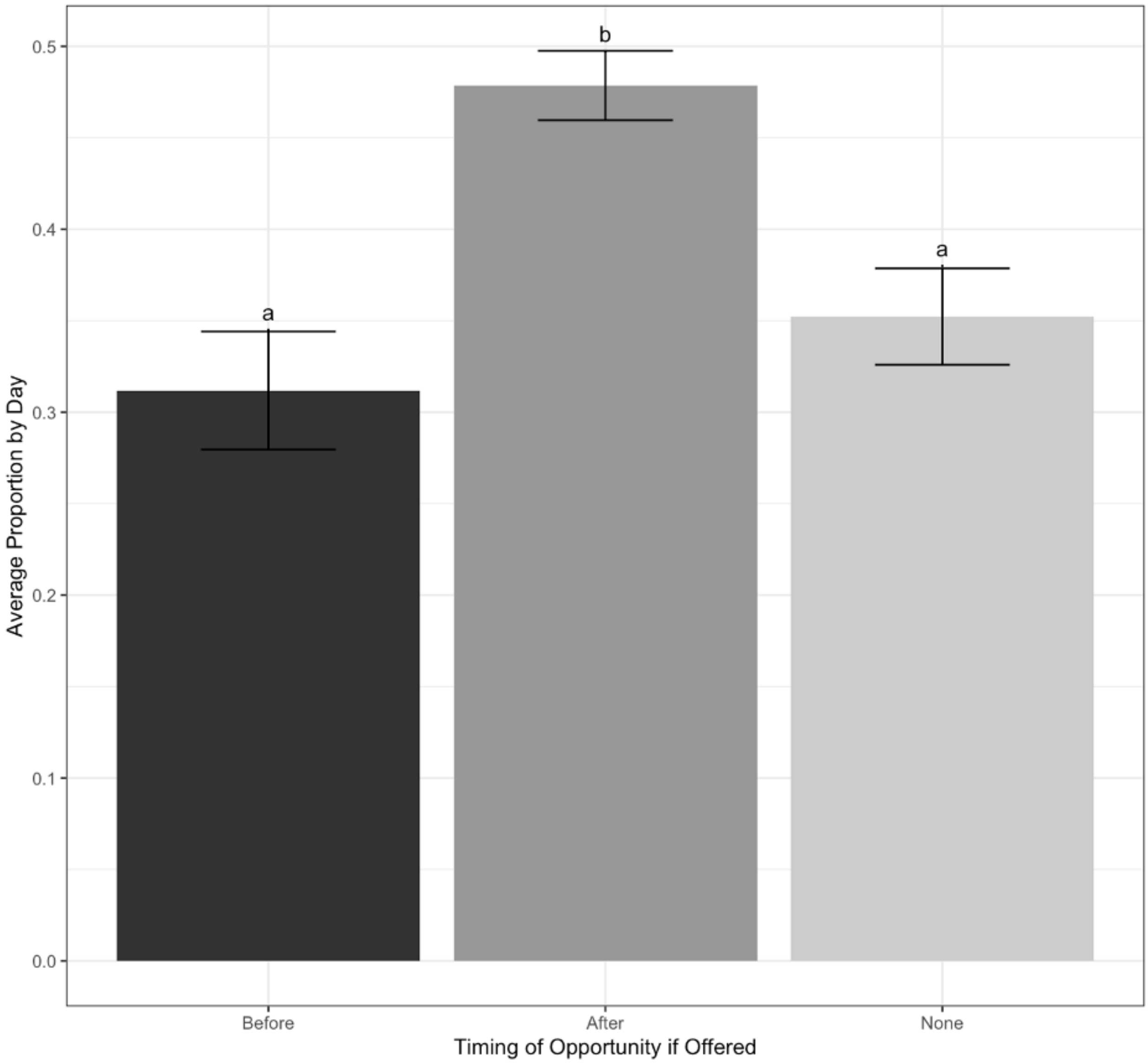

Figure 3. Average daily proportion of active behaviors for keeper present data by timing of session in relation to opportunities offered to animals. Keepers arrived for a session prior to giving an opportunity to animals “Before”, post giving an opportunity “After”, or at a time that no opportunity was offered at all “None.” Letters denote significant differences between the three times and error bars represent ± one standard error.

Discussion

In this study, we investigated if the presence of caretakers influences animal behavior. We observed multiple individuals of different species in the presence and absence of their primary keeper. Our results imply that zoo animals, in some cases behave differently around their caretakers compared to when their caretakers are absent. Thus, keepers may see a different behavioral repertoire than observers who monitor animals outside of times when their caretakers are not present. Active behaviors were significantly higher in the keeper's presence than when the keeper was not present. Caretakers and animals engage in a high frequency of positive interactions, and human-animal relationships can form in captive settings. Due to this positive relationship keepers have formed with the animals under their care, they observe more active behaviors. In this study, we chose the primary keeper of each species to participate as they work the closest with these animals five days out of the week and have frequent interactions. Most daily interactions between keepers and animals are positive and negative interactions are infrequent (e.g., medical procedures, isolation). Consistent, reliable interactions develop a human-animal relationship where familiarity with each other's behavior is acquired (Hinde, 1976; 1987). This relationship is mutually beneficial; animals expect a reward from the keeper, and keepers expect efficient management of animals and experience affective benefits (Hosey & Melfi, 2012). Thus because of this positive relationship, the presence of keepers is a context that results in a behavior change for the animals they care for (Krebs et al., 2022).

Our results showed that the timing keepers were present in association with opportunities offered was significant. Active behavior levels were equal before an opportunity and when no opportunities were offered to animals. Activity levels before an opportunity and within an animal's regular schedule can reflect anticipatory behavior, as the keeper's presence is a reliable cue indicating an event is coming. When the keeper arrives outside the animal's regular schedule and does not intend to provide an opportunity, the same activity level is seen as prior to when the keeper will provide an opportunity, indicating the keeper's presence was inadvertently stimulating the same level of anticipatory behavior. This suggests the keeper's presence in and of itself is a mostly reliable cue for a future opportunity (Watters & Krebs, 2019) and supports our hypothesis. An increase in the active behaviors observed in this study reflects anticipatory behavior and suggests that animals behave "optimistically", or as if they are about to receive a reward (Matheson et al., 2008; Brydges et al., 2011) in the presence of their caretaker. This is due to the keeper frequently indicating a potentially positive opportunity and can redirect animals to the opportunities they have in their current environment. Our results showed a significant increase in specific behaviors: eating/foraging, interacting with enrichment and wading in the presence of their caretaker. Animals engaged with existing opportunities in their environments, such as uneaten food, enrichment items, or environmental features, when keepers were present. These behaviors represent consummatory behaviors, but the keeper's presence appears to have stimulated appetitive behaviors that were then able to be consummated because of the good environmental conditions. The context a zookeeper's presence adds to the animal's day results in a change in anticipatory and optimistic behaviors.

Our results provide additional evidence of a keeper effect in zoo settings. However, more research is needed to determine whether this effect positively or negatively impacts animal behavior and welfare. A potential consequence of this keeper effect is behavioral assessments conducted by keepers may differ from those conducted by observers unfamiliar to the animals, yet still be accurate for a specific context. Institutions often rely on caretakers for behavioral or welfare assessments of their animals due to their knowledge of individual animals and being the first to observe changes in an animal’s behavior. There are contradicting studies investigating the reliability of keeper surveys or ratings against researcher-collected behavioral data. Keeper welfare surveys and behavioral profiles completed for animals have matched with equivalent surveys and behavioral tests (novel object tests) completed by researchers (Carlstead et al., 1999; Less, 2012; Brouwers & Duchateau, 2021). When comparing keeper surveys to behavioral observations by researchers, however, there was a significant correlation between frequencies of aggression but no significant correlation between frequencies of abnormal behaviors, inactivity, and enrichment use (Brouwers & Duchateau, 2021). This suggests that keepers observe some behaviors more readily/frequently than others. Keepers have busy schedules, and their most significant constraint is time (Less et al., 2012; Kuhar, 2006). In this study, keepers spent an average of 22 minutes per day from their schedule to participate in sessions (ranging from 10-30 minutes/per day). Most sessions keepers participated in were within a short period to offering animals an opportunity as it is probably easier to observe animals during their schedule when they are in proximity to their exhibit. However, animals are on exhibit for at least 6-7 hours daily. Even though keepers observe infrequently, we believe they are accurate in their behavioral observations. A study shows good percent agreement when testing inter-rater reliability between most keepers and researchers on behavioral observations (unpublished data). Thus, keepers are not observing animals incorrectly; they are a context that needs to be considered.

These results help expand our understanding of the prominent relationship between animals and keepers. More research is needed on these positive relationships developed in zoos to aid in daily management practices, precisely the unintended effect on behavior and welfare assessments. Management needs to consider keepers as a context in the animal’s environment that causes changes in behavior. If they are concerned by this effect, zoos could try to minimize it by adding more visits to the exhibit when no positive reinforcement is given or indicating a location for observations only or via cameras. On the other side of that coin, however, the positive human-animal relationship and trust built between the two can be essential to lower stress management and thus support animal welfare.

References

Anderson, C., Yngvesson, J., Boissy, A., Uvnäs-Moberg, K., & Lidfors, L. (2015). Behavioural expression of positive anticipation for food or opportunity to play in lambs. Behavioural Processes, 113, 152-158.

Baker, K. C. (2004). Benefits of positive human interaction for socially housed chimpanzees. Animal Welfare, 13(2), 239-245.

Bayne, K. (2002). Development of the human-research animal bond and its impact on animal well-being. ILAR journal, 43(1), 4-9.

Boyle, S. A., Berry, N., Cayton, J., Ferguson, S., Gilgan, A., Khan, A., ... & Reichling, S. (2020). Widespread behavioral responses by mammals and fish to zoo visitors highlight differences between individual animals. Animals, 10(11), 2108.

Brouwers, S., & Duchateau, M. J. (2021). Feasibility and validity of the Animal Welfare Assessment Grid to monitor the welfare of zoo-housed gorillas Gorilla gorilla gorilla. Journal of Zoo and Aquarium Research, 9(4), 208-217.

Brydges, N. M., Leach, M., Nicol, K., Wright, R., & Bateson, M. (2011). Environmental enrichment induces optimistic cognitive bias in rats. Animal Behaviour, 81(1), 169-175.

Carlstead, K. (2009). A comparative approach to the study of keeper–animal relationships in the zoo. Zoo Biology, 28(6), 589-608.

Carlstead, K., Mellen, J., & Kleiman, D. G. (1999). Black rhinoceros (Diceros bicornis) in US zoos: I. Individual behavior profiles and their relationship to breeding success. Zoo Biology, 18(1), 17-34.

Chelluri, G. I., Ross, S. R., & Wagner, K. E. (2013). Behavioral correlates and welfare implications of informal interactions between caretakers and zoo-housed chimpanzees and gorillas. Applied Animal Behaviour Science, 147: 306-315.

Clegg, I. L., Rödel, H. G., Boivin, X., & Delfour, F. (2018). Looking forward to interacting with their caretakers: Dolphins' anticipatory behaviour indicates motivation to participate in specific events. Applied Animal Behaviour Science, 202, 85-93.

Dantas-Ferreira, R. F., Dumont, S., Gourmelen, S., Cipolla-Neto, J., Simonneaux, V., Pévet, P., & Challet, E. (2015). Food-anticipatory activity in Syrian hamsters: behavioral and molecular responses in the hypothalamus according to photoperiodic conditions. PLoS One, 10(5), e0126519.

Davey, G. (2007). Visitors' effects on the welfare of animals in the zoo: A review. Journal of Applied Animal Welfare Science, 10(2), 169-183.

Hemsworth, P. H. (2003). Human–animal interactions in livestock production. Applied Animal Behaviour Science, 81(3), 185-198.

Hinde, R. A. (1976). On describing relationships. Journal of Child Psychology and Psychiatry, 17: 1-19.

Hinde, R. A. (1987). Individuals, relationships & culture: Links between ethology and the social sciences. Cambridge University Press, New York, NY, US.

Hosey, G. (2008). A preliminary model of human–animal relationships in the zoo. Applied Animal Behaviour Science, 109: 105-127.

Hosey, G., & Melfi, V. (2012). Human–animal bonds between zoo professionals and the animals in their care. Zoo Biology, 31(1), 13-26.

Hosey, G., & Melfi, V. (2014). Human-animal interactions, relationships and bonds: A review and analysis of the literature. International Journal of Comparative Psychology.

Jones, H., McGregor, P. K., Farmer, H. L. A., & Baker, K. R. (2016). The influence of visitor interaction on the behavior of captive crowned lemurs (Eulemur coronatus) and implications for welfare. Zoo Biology, 35(3), 222-227.

Krebs, B. L., Chudeau, K. R., Eschmann, C. L., Tu, C. W., Pacheco, E., & Watters, J. V. (2022). Space, time, and context drive anticipatory behavior: Considerations for understanding the behavior of animals in human care. Frontiers in Animal Science, 9.

Krebs, B. L., Eschmann, C. L., & Watters, J. V. (2022). Dither: A unifying model of the effects of visitor numbers on zoo animal behavior. Zoo Biology.

Krebs, B. L., Torres, E., Chesney, C., Kantoniemi Moon, V., & Watters, J. V. (2017). Applying behavioral conditioning to identify anticipatory behaviors. Journal of Applied Animal Welfare Science, 20(2), 155-175.

Kuhar, CW. (2006). In the deep end: pooling data and other statistical challenges of zoo and aquarium research. Zoo Biology, 25:339–352.

Less, E. H., Kuhar, C. W., Dennis, P. M., & Lukas, K. E. (2012). Assessing inactivity in zoo gorillas using keeper ratings and behavioral data. Applied Animal Behaviour Science, 137(1-2), 74-79.

Lincoln Park Zoo. (2020). ZooMonitor [Mobile app]. https:// zoomonitor.org

Martin, P., & Bateson, P. (2007). Measuring behavior: an introductory guide (3rd ed.). Cambridge, UK: Cambridge University Press.

Martin, R. A., & Melfi, V. (2016). A comparison of zoo animal behavior in the presence of familiar and unfamiliar people. Applied Animal Welfare Science, 19(3), 234-244.

Matheson, S. M., Asher, L., & Bateson, M. (2008). Larger, enriched cages are associated with 'optimistic' response biases in captive European starlings (Sturnus vulgaris). Applied Animal Behaviour Science, 109(2-4), 374-383.

Melfi, V. A., & Thomas, S. (2005). Can training zoo-housed primates compromise their conservation? A case study using Abyssinian colobus monkeys (Colobus guereza). Anthrozoös, 18(3), 304-317.

Phillips, C., & Peck, D. (2007). The effects of personality of keepers and tigers (Panthera tigris tigris) on their behaviour in an interactive zoo exhibit. Applied Animal Behaviour Science, 106(4), 244-258.

Podturkin, A. A., Krebs, B. L., & Watters, J. V. (2022). A quantitative approach for using anticipatory behavior as a graded welfare assessment. Journal of Applied Animal Welfare Science, 1-15.

Rose, P. E., Scales, J. S., & Brereton, J. E. (2020). Why the "visitor effect" is complicated. Unraveling individual animal, visitor number, and climatic influences on behavior, space use and interactions with keepers—A case study on captive hornbills. Frontiers in veterinary science, 7, 236.

Sherwen, S. L., & Hemsworth, P. H. (2019). The visitor effect on zoo animals: Implications and opportunities for zoo animal welfare. Animals, 9(6), 366.

Sherwen, S. L., Hemsworth, P. H., Butler, K. L., Fanson, K. V., & Magrath, M. J. L. (2015). Impacts of visitor number on Kangaroos housed in free-range exhibits. Zoo Biology, 34: 287-295.

Spruijt, B. M., R. van den Bos, & Pijlman, F. T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behavior Science, 72: 145-171.

Thomassen, J. M., & Fjæra, S. O. (1991). Use of light signalling before feeding of salmon (Salmo salar). Aquacultural Engineering, 10(1), 65-71.

Ward, S. J., & Melfi, V. (2013). The implications of husbandry training on zoo animal response rates. Applied Animal Behaviour Science, 147(1-2), 179-185.

Ward, S. J., & Melfi, V. (2015). Keeper-animal interactions: Differences between the behaviour of zoo animals affect stockmanship. PloS one, 10(10), e0140237.

Ward, S. J., Sherwen, S., & Clark, F. E. (2018). Advances in applied zoo animal welfare science. Journal of Applied Animal Welfare Science, 21(sup1), 23-33.

Watters, J. V. (2014). Searching for behavioral indicators of welfare in zoos: Uncovering anticipatory behavior. Zoo Biology, 33(4), 251-256.

Watters, J. V., & Krebs, B. L. (2019). Assessing and enhancing the welfare of animals with equivocal and reliable cues. Animals, 9(9), 680.

Watters, J. V., B. L. Krebs, and E. Pacheco. (2019). Measuring Welfare through Behavioral Observation and Adjusting It with Dynamic Environments. In: A. B. Kaufman, M. J. Bashaw and T. L. Maple (eds.) Scientific Foundations of Zoos and Aquariums: Their Role in Conservation and Research. p 212-240. Cambridge University Press, Cambridge.

Young, R.J., (2003). Environmental Enrichment for Captive Animals. Blackwell Science, Oxford.

Supplemental Table. Detailed ethogram for each species and categorization of active or inactive behaviors.

Behavior	**Definition**	**Categorization for analysis**
Western lowland gorillas, Mandrills, and Sifaka		
Locomote	Moves from one location to another on the ground via walking, jumping, or running. Head is upright	Active
Eat/Forage	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with the mouth or hands. Includes eating grass or browse in the exhibit and walking slowly with head down looking for food.	Active
Interact with enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.	Active
Stand	Standing with all limbs on the ground or standing at full height on two rear feet. No other behavior is occurring	Active
Drink	Consumes water.	Active
Climb	Climbs or jumps either onto structures or trees in exhibit.	Active
Sniff	Puts nose onto or near an object, includes lifting head in air and smelling air	Active
Play	Includes patting or pulling of body parts (arms, legs, fingers, clapping, chest-beating). This includes active playing such as twirling, somersaults, rolling.	Active
Affiliative	Rubs against, lies against, or engages in friendly behaviors besides grooming towards another individual.	Active
Aggress	Chases, growls at, snaps/bites at or otherwise aggressively interacts with another individual.	Active
Other	An active behavior not included in the ethogram.	Active

Hang	Hanging from mesh or branches.	Active
Lie Down	Lying on side, belly or back. No other behavior is occurring.	Inactive
Sit	Rear is on the ground or rocks, upper body upright. No other behavior is occurring.	Inactive
Groom	Licks, chews, scratches or otherwise grooms self or other.	Excluded from analysis
Pull Hair	Self-manipulation of pulling hair, different from grooming.	Excluded from analysis
Lick	Licking object or structure that is not food or enrichment item.	Excluded from analysis
Out of View	Unobservable	Excluded from analysis
Greater one- horn rhino		
Locomote	Moves from one location to another on the ground via walking or running.	Active
Eat	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with mouth.	Active
Interact with enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.	Active
Stand	Standing with all limbs on the ground. No other behavior is occurring	Active
Drink	Consumes water.	Active
Sniff	Puts nose onto or near an object, includes waving upper lip in air.	Active
Wade	In pool, locomoting, standing, or splashing but not playing with enrichment in the pool.	Active

Scratch	Rubs body against an object or wall of enclosure	Excluded from analysis
Lie Down	Lying on side, belly or back. No other behavior is occurring.	Inactive
Out of View	Unobservable	Excluded from analysis
American black bears		
Locomote	Moves from one location to another on the ground via walking, jumping, or running. Head is upright	Active
Eat/Forage	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with the mouth. Includes eating grass or browse in the exhibit and walking slowly with head down looking for food.	Active
Interact with enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.	Active
Stand	Standing with all limbs on the ground or standing at full height on two rear feet. No other behavior is occurring	Active
Drink	Consumes water.	Active
Climb	Climbs onto structures or logs in exhibit.	Active
Sniff	Puts nose onto or near an object, includes lifting head in air and wiggling nose.	Active
Play	Includes somersaults, rolling, etc. It can be towards another individual (wrestling, swatting, biting).	Active
Wade	In pool, locomoting, standing, or splashing but not playing with enrichment in the pool.	Active
Affiliative	Rubs against, lies against, or engages in friendly behaviors besides grooming towards another individual.	Active

Aggress	Chases, growls at, snaps/bites at or otherwise aggressively interacts with another individual.	Active
Lie Down	Lying on side, belly or back. No other behavior is occurring.	Inactive
Sit	Rear is on the ground or rocks, upper body upright. No other behavior is occurring.	Inactive
Groom	Includes scratches, licks, picks at, shakes fur, or otherwise grooms itself. Includes stretching behaviors.	Excluded from analysis
Pace	Walks back and forth for at least one full loop (A to B to A) or walks the same complete circle at least once	Excluded from analysis
Out of View	Unobservable	Excluded from analysis
Wolverine		
Locomote	Moves from one location to another on the ground via walking, jumping, or running. Head is upright	Active
Eat	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with the mouth or paws.	Active
Interact with enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.	Active
Stand	Standing with all limbs on the ground or standing at full height on two rear feet. No other behavior is occurring	Active
Drink	Consumes water.	Active
Climb	Climbs onto structures or logs in exhibit.	Active
Sniff	Puts nose onto or near an object, includes lifting head in air and wiggling nose.	Active
Play	Includes somersaults, rolling, etc. It can be towards another individual (wrestling, swatting, biting).	Active

Wade	In pool, locomoting, standing, or splashing but not playing with enrichment in the pool.	Active
Dig	Digging in dirt or ground, does not include keeper provided substrates	Active
Lie Down	Lying on side, belly or back. No other behavior is occurring.	Inactive
Sit	Rear is on the ground or rocks, upper body upright. No other behavior is occurring.	Inactive
Groom	Includes scratches, licks, picks at, shakes fur, or otherwise grooms itself. Includes stretching behaviors.	Excluded from analysis
Pace	Walks back and forth for at least one full loop (A to B to A) or walks the same complete circle at least once	Excluded from analysis
Out of View	Unobservable	Excluded from analysis

Chapter 4:
Puzzle feeders decrease anticipatory and anxiety-like behavior and increase social behavior more than object enrichment

Introduction

Environmental enrichment is a component of animal husbandry that aims to provide a dynamic environment through varied behavioral opportunities (Shepherdson, 1994; Swaisgood & Shepherdson, 2005; Watters et al., 2019). It promotes an increase in behavioral diversity and species-specific behaviors, a reduction in abnormal behaviors, an expansion of space use, more natural time-budgets and animals' ability to cope with challenges (Lutz & Novak, 1995; Vick et al., 2000; Kells et al., 2001; Young, 2003; Fay & Miller, 2015: Lauderdale & Miller, 2020; Nagabaskaran et al., 2022). As a result, providing environmental enrichment is now an industry standard best practice for accredited zoos and aquariums (Mellor et al., 2015; Association of Zoos and Aquariums, 2022). There are many enrichment categories, including feeding, sensory, social, tactile, and cognitive opportunities (Young, 2003; de Azevedo et al., 2007). Enrichment programs in zoos typically utilize items from multiple categories daily. However, some are applied more than others due to ease of use, availability, time constraints on care staff, or a perception that they are more effective. One study found that the highest frequency of enrichments provided by caretakers were feeding, human-animal interactions, and manipulatable objects (Hoy et al., 2010). Providing enrichment from different categories at variable schedules keeps animals interested and avoids the effects of habituation (Kuczaj et al., 2002; Soriano et al., 2019). Although using multiple enrichment categories to provide positive behavioral opportunities for animals is the best practice for animal management, doing so may lead to difficulty in understanding which ones lead to more robust changes in animal behavior (de

Azevedo et al., 2007). Assessing the effects of different enrichment categories will help better understand how to support the animal's behavioral needs.

Cognitive enrichment provides problem-solving and learning opportunities. It includes mechanical apparatuses, computer tasks, mazes and other means to generate problem solving (Swaisgood & Shepherdson, 2005; Meehan & Mench, 2007; Clark, 2017). One basic type of cognitive enrichment, the puzzle feeder, is widely used. Puzzle feeders offer a problem-solving opportunity to extract food using complex manipulations instead of more traditional methods, such as being fed from a dish or scatter fed throughout the enclosure. Studies have demonstrated that filled puzzle feeders can increase activity, foraging, and interaction with enrichment compared to empty puzzle feeders (Gilloux et al., 1992; Sanders & Fernandez, 2020). Providing a problem-solving opportunity with a reward allows animals to use cognitive skills for food acquisition (Shettleworth, 2001; Meehan & Mench, 2007). Using cognitive skills via tasks or enrichment decreases stress and abnormal behaviors and increases exploration and positive affect (Puppe et al., 2007; Manteuffel et al., 2009). Successfully solving cognitive challenges results in greater mRNA expression in brain receptors used for processing positive affect in pigs (Kalbe & Puppe, 2010), and the engagement of challenges generates positive affective states (Clegg et al., 2023). Providing animals a cognitive challenge they can solve successfully is important as it can stimulate environmental engagement outside of enrichment use; animals may seek opportunities to explore and problem-solve in their environment (Spinka & Wemelsfelder, 2011). An increase in positive behavioral indicators and affective states by problem-solving has been recognized as a critical behavioral opportunity for supporting animal wellbeing (Meehan & Mench, 2007; Clark, 2011; Clark & Smith, 2013; Clark et al., 2013). Therefore, cognitive challenges result in behavioral changes, and these sustained behavioral changes can be indicative of improved welfare. As enrichment is a primary and resource-consumptive component of daily husbandry, comparisons of different enrichment categories must be further studied. Thus, studying them separately should lead to a deeper understanding of the best techniques for applying them.

Studies of manipulable objects have mainly focused on their impact on behavioral responses observed immediately after their presentation. Some studies measure the number and duration of interactions (Kuczaj et al., 2002; Delfour & Beyer, 2011), behavioral tests (Meehan & Mench, 2002; Podturkin, 2021), or exploratory behaviors (Averos et al., 2010; Franks et al., 2013). As increasing activity and exploration is a common behavioral goal of environmental enrichment (Young, 2003), assessing these behaviors over the course of an animal's day and whether they are directly engaged with enrichment or not may help clarify the long-term effectiveness of a specific enrichment category. However, assessments of the behavioral impacts of cognitive enrichment have received less attention than other categories. A statistical analysis for identifying gaps in the enrichment literature by de Azevedo et al. (2007) discovered that 3.5% of zoo studies utilized cognitive enrichment between 1985 and 2004. Additionally, while cognitive enrichment is crucial for animal welfare, it is still not widely implemented across different species (Hall et al., 2021).

One potential method for assessing an animal's welfare state is the observation of anticipatory behavior. Anticipatory behavior is a goal-directed behavior that occurs when an animal perceives that a reward is to come. This behavior is of interest as it reflects animals' reward sensitivity and their perception of their environment (Spruijt van den Bos & Pijlman, 2001; Watters, 2014). In lab animals, enriched environments compared to standard housing reduce the expression of anticipatory behavior (van der Harst et al., 2003; Makowska & Weary, 2016). Similarly, increasing the frequency of enrichment opportunities reduces anticipatory behavior in zoo animals (Swaisgood et al., 2001; Krebs & Watters, 2017). This evidence suggests that enrichment can modulate anticipatory behavior. For example, puzzle feeders can provide additional rewarding opportunities to animals and the welfare benefits of problem-solving opportunities. Therefore, using puzzle feeders could decrease anticipation by decreasing overall reward sensitivity (Watters, 2014; Krebs & Watters, 2017). Assessing an animal's anticipatory behaviors under different management conditions, such as enrichment categories, can help determine how their wellbeing changes with changes in husbandry.

The study presented here investigated how two different categories of enrichment influence animal behavior and welfare indicators. We hypothesized that compared to manipulable objects, problem-solving opportunities would result in more activity and exploration throughout the day and would have a greater impact on anticipatory behavior that occurs at the end of the day prior to a reliable reward. Specifically, we predict puzzle feeder enrichment will result in more exploratory behaviors and interaction with enrichment throughout the day compared to object enrichment. We also expect puzzle feeder enrichment to decrease anticipatory behavior more than object enrichment at the end of the day before animals receive their evening meal.

Materials & Methods

Subjects

We conducted our study between June and September 2021 at the San Francisco Zoo and Gardens (San Francisco, California). The subjects were eight individuals of three species (age and sex of each in Table 1): five reticulated giraffes (*Giraffa reticulata*), one guanaco (*Lama guanicoe*), and two North American river otters (*Lontra canadensis*). Giraffes and otters were housed socially while the guanaco lived individually. The reticulated giraffes were in a mixed species exhibit shared with ostriches (*Struthio camelus*), Grant's zebras (*Equus quagga boehmi*), and East African crowned cranes (*Balearica regulorum*). Although animal husbandry varied by species, there were commonalities in the daily patterns of all animals included in this study. Every morning keepers shifted animals to the outdoor, public viewing exhibit between 1000-1100. At the end of the day, between 1630-1700, keepers again shifted or gave animals access to overnight quarters along with their last meal of the day. Daily husbandry stayed consistent within species throughout the study apart from providing the designated enrichment treatment every day.

Table 1. Species, age, and sex of study subjects

ID	Species	Sex	Age at time of study (years)
Giraffe 1	Giraffe	F	18
Giraffe 2	Giraffe	F	12
Giraffe 3	Giraffe	F	11
Giraffe 4	Giraffe	F	8
Giraffe 5	Giraffe	F	5
Guanaco	Guanaco	M	6
River Otter 1	River Otter	M	4
River Otter 2	River Otter	M	6

Treatments

At the start of each treatment day, keepers added either puzzle feeders or object enrichments to each enclosure. Most often, the following morning, keepers removed any enrichment items from the day before and placed new enrichment items of the same designated treatment. The treatment continued for seven consecutive days. Then, there was a 2-week break with no observations where animals followed a regular enrichment schedule. Husbandry staff determined this schedule, and animals could receive any of their approved enrichment items during this time. After the 2-week break, keepers added the second treatment for seven consecutive days. A second replicate of seven days with each enrichment type and the subsequent breaks between treatments followed. There were approximately 14 days of each treatment per enclosure/ per species.

Enrichment items used during treatments were from a pre-approved list and routinely given to the animals. No enrichments used in this study were novel to the recipients. Puzzle feeder treatment included items that animals needed to manipulate in some way to retrieve the food inside. Keepers filled puzzle feeders with a ration of food from their daily allowance, typically provided on exhibit daily (outside of regular meals), such as produce, formulated pelleted diet, or meat. As the food placed in the puzzle feeders was part of what would normally be used for enrichment or feeding around the enclosure, the animals received the same amount of food during each treatment. The bulk of the animals' diets was still available to them at regular feeding times. Therefore, the amount of food offered did not differ between treatments, which served to minimize variations in food motivation between treatments. Object treatment included items intended for manipulation (e.g., boomer balls, rope, cones; Table 2 for examples). Object enrichment items could be similar in shape, color, or texture to puzzle enrichment items, but the main difference was that puzzle feeders offered food via a problem-solving opportunity. The shape or size of enrichments varied between groups due to species differences and safety precautions, but all items belonged to the two categories of enrichment tested. The number of items was approximately equivalent every treatment day within groups.

Table 2. Examples of enrichment items for each treatment for each species. Food was placed inside puzzle feeders, while no food was placed in object enrichment.

	Puzzle Feeders	Objects
Giraffes	 	 

Guanaco	 	 
River Otters		

Behavioral Observations

We collected observations while animals were on exhibit during zoo operating hours, approximately 1000 - 1700. Because each animal was readily identifiable, we collected data at the level of individual animals. We balanced observations across the zoo hours, splitting the day into one-hour intervals (e.g., 1000 – 1059, 1100 – 1159…1600 - 1659). Each observation session was 10 minutes with instantaneous scan sampling at one-minute intervals (Martin & Bateson, 2007). This split resulted in seven observation sessions per day/per species for 28 days of data collection. We scheduled each day's last ten-minute observation session immediately before the animals shifted indoors or received access to their final meal of the day to capture anticipatory behavior (occurred between 1620 – 1700, group dependent). Although anticipatory behavior can occur throughout the day with events such as training or meals, the timing of events would also differ across species. As we expect animals to be hungry by 1700, they are more motivated than at 1100, assuming they get breakfast immediately after shifting out. Thus testing the evening shift controls for food motivation due to the zoo closing daily at 1700 (Podturkin et al., 2022). We used the ZooMonitor application (Lincoln Park Zoo, 2020) to collect data (Supplemental Table includes behaviors observed for each species). The same observer (E.P.) collected data and rotated between each exhibit every hour; we randomized the order of exhibits throughout the day.

Statistical Analysis

We analyzed any behaviors observed in at least two of the three species in our study. The behaviors in the analysis comprised the following categories and corresponding behaviors: maintenance (eat), investigative (explore & interact with enrichment), social (affiliative/play), inactive (lie down), anxiety-like (groom), abnormal (lick/chew), and anticipatory (locomote & stand; Table 3). We included standing behavior as anticipatory as some animals expressed it as waiting and focused in a particular area. First, we calculated the total counts of all observations per day for each behavior. Then the proportion of each behavior was calculated using counts observed and counts of total observations per day. Next, we calculated two proportions, the first from all the observation sessions per day, referred to as "Full Day" from now on, to investigate behavior differences throughout the day. The second proportion calculated observations from the last 10-minute session separately, referred to as “Last Ten,” to examine potential anticipatory behavior at the end of the day. Finally, we analyzed the data with a generalized linear mixed model (with a logit link) on observed behavior using the package “lme4” in R v. 4.2.1.

We created models for each behavior at Full Day and Last Ten. Individual nested in species was included as a random effect to account for repeated measures of individuals and expected differences among species. We set enrichment treatment (puzzle feeders or objects) as a fixed effect. We also included potential explanatory factors in our full regression models: day of treatment, replicate, number of enrichment items, new enrichment, and consecutive days in all models. Day of treatment was the number of days since the treatment started, ranging from 1-7. Replicate indicated which week number of the repeated treatment we were on, 1 or 2. The number of enrichment items was the exact count of how many items keepers provided that day. New enrichment was a binomial factor regarding whether new enrichment items were given that day, 0 or 1. This factor was due to keepers being unable to provide new enrichment items some days due to unexpected animal management needs. Consecutive days were the number of consecutive days clustered within each treatment week, ranging from 1-7. Although we aimed for seven consecutive days of treatments, keepers did not provide enrichment on some days throughout the treatment week due to management reasons. We used a backward stepwise procedure to remove fixed effects from the full model one at a time according to the BIC and AIC decreases (Heinze et al., 2018). Final models were declared when BIC and AIC values reached a minimum without considering the p-values of individual factor estimates (Supplemnental table of final model for each behavior).

Table 3. Categorization of behaviors used in analysis. Behaviors were observed in at least two of the three species.

Behavior	Description	Categorization
Affiliative/Play	Rubs against, lies against, or engages in friendly behaviors besides grooming towards another individual. Includes otters tumbling or wrestling.	Social
Eat	Consumes food. Includes processing behaviors like biting, pulling, or otherwise manipulating food with the mouth or paws. Includes eating grass or browse in the exhibit and walking slowly with head down looking for food.	Maintenance
Explore	Sniffing, licking (less than 5 seconds), pawing, or manipulating environment that is not enrichment.	Investigative
Groom	Animal scratches itself against an object such as a tree, or otherwise bites, rubs, or licks itself.	Anxiety- Like
Interact with Enrichment	Interacts with the keeper provided enrichment item. Must contact the item with face, mouth, or limbs to count.	Investigative
Lick/Chew	Giraffe or Guanaco is repeatedly licking its face or non-enrichment object for more than 5 seconds.	Abnormal
Lie Down	Lying on side, belly or back. No other behavior is occurring.	Inactive
Locomote	Moves from one location to another on the ground via walking or running.	Anticipatory
Stand	Standing with all limbs on the ground or standing at full height on two rear feet. No other behavior is occurring.	Anticipatory

Results

Full Day Behavioral Observations

Animals interacted more with puzzle feeder enrichment and for a longer duration than with object enrichment ($p < 0.0001$, 41 seconds vs. 12 seconds, respectively). In addition, we found significant differences between treatments for affiliative/play, eating, grooming, lying down, and standing behaviors (Table 4). Animals spent proportionally more time expressing affiliative/play ($p < 0.05$) and standing ($p < 0.0001$) behaviors during days with puzzle feeders than with object enrichment. We also observed lower proportions of eating ($p < 0.0001$), grooming ($p < 0.01$), and lying down ($p < 0.0001$) behaviors during days with puzzle feeders than with objects. There were no significant differences between treatments for behaviors of exploring and locomotion (Table 5).

Table 4. Results of generalized linear mixed models of behaviors for Full Day and Last Ten. This table only includes behaviors observed in at least two out of the three species.

	Full Day	Last Ten	Categorization of behaviors
Affiliative/Play	-↑ Puzzle Feeders ↓ Objects	Not Significant	Social
Eat	↓ Puzzle Feeders ↑ Objects	↑ Puzzle Feeders ↓ Objects	Maintenance
Explore	Not Significant	Not Significant	Investigative
Groom	↓ Puzzle Feeders ↑ Objects	↓ Puzzle Feeders ↑ Objects	Anxiety- Like
Interact with Enrichment	↑ Puzzle Feeders ↓ Objects	Not Significant	Investigative
Lick/Chew	Not Significant	Not Significant	Abnormal
Lie Down	↓ Puzzle Feeders ↑ Objects	Not Significant	Inactive
Locomote	Not Significant	↓ Puzzle Feeders ↑ Objects	Anticipatory
Stand	↑ Puzzle Feeders ↓ Objects	↑ Puzzle Feeders ↓ Objects	Anticipatory

- ↑ = significant increase ($p < 0.05$), ↓ = significant decrease ($p < 0.05$)

Last Ten Behavioral Observations

We observed significant differences in eating, grooming, locomotion, and standing (Table 5). Proportions of eating ($p < 0.01$) and standing ($p < 0.05$) were higher at the end of the day with puzzle feeder enrichment than object enrichment. In addition, animals performed less grooming ($p < 0.0001$) and locomotion ($p < 0.0001$) at the end of the day with puzzle feeders than with object enrichment.

Table 5. Detailed results of generalized linear mixed models of behaviors for Full Day and Last Ten. Asterisks indicate significant differences ($p < 0.05$) between puzzle feeders and objects.

	Full Day			Last Ten		
Behavior	Estimate	Standard Error	p-Value	Estimate	Standard Error	p-Value
Affiliative/Play	-0.258	0.128	0.0431*	-0.208	0.296	0.4808
Eat	0.431	0.042	<0.0001*	-0.488	0.175	0.0053*
Explore	-0.162	0.127	0.2027	0.098	0.344	0.7767
Groom	0.412	0.139	0.0031*	1.859	0.481	<0.0001*
Interact with Enrichment	-1.517	0.155	<0.0001*	-1.294	0.923	0.1607
Lick/Chew	-0.141	0.084	0.0925	0.207	0.287	0.471
Lie Down	0.250	0.067	<0.0001*	-0.125	0.219	0.5689
Locomote	0.042	0.042	0.318	0.475	0.142	<0.0001*
Stand	-0.293	0.040	<0.0001*	-0.275	0.129	0.0333*

Discussion

In this study we investigated how the type of enrichment, puzzle feeders or objects, influenced animal behavior throughout the day. We observed eight individuals of three species on days when they were only given puzzle feeders or objects as enrichment. Our results suggest that the provision of puzzle feeders positively affected daily behavior more than object only enrichments. Thus, providing a problem-solving opportunity to retrieve food had a behavioral effect seen throughout the day, not only when the feeders had food in them. Strikingly, these effects appear strong even given the relatively small proportion of the day that animals interacted with the enrichment items.

As we predicted, animals were engaged more and for longer durations with the puzzle feeders than with objects, impacting daily behavior. Our results are consistent with previous studies showing behavioral changes with cognitive enrichment in various species (e.g., Lumeji & Hommers, 2008; Clark et al., 2013; Krebs & Watters, 2017; Sanders & Fernandez, 2020; Clegg et al., 2023). These positive behavioral changes observed when animals engaged in a problem-solving opportunity support the provision of cognitive enrichment to promote positive welfare more than only object enrichment. We asked care staff to keep their protocols the same as they would any day when providing enrichment, only differing in the category of items provided to the animals. This was to keep our study in line with routine daily management; thus, we gave the animals the same amount and type of food in both treatments. Keepers placed only a portion of their meals provided in the mornings inside the puzzle feeders; the remainder was, per usual, scatter fed or placed in dishes for the animals. Thus, the differences we observed were likely not only due to the food in the puzzle feeders since the animals had other options to eat simultaneously but the extraction of the food through a challenge. Anecdotally, most animals approached the puzzle feeders first (personal observation by E.P.), perhaps indicating a preference to problem solve over free feed (Sasson-Yenor & Powell, 2019).

An unexpected result was that the giraffes and otters engaged in more prosocial behaviors when offered puzzle feeders than when given objects. This is consistent with previous studies with cognitive challenges, which saw greater engagement in social play in chimpanzees (*Pan troglodytes*: Clark & Smith, 2013), dolphins (Delphinidae: Clark et al., 2013), and pro-social behaviors in guinea baboons (*Papio papio*: Fagot & Bonté, 2010; Fagot et al., 2014). These results support the idea that cognitive enrichment may benefit social groups and could be used to facilitate group bonding. Furthermore, prosocial behaviors observed with cognitive enrichments are contrary to a common management worry when giving feeding enrichments to animals where there is a concern of potentially causing aggression in groups. In this study, we did not observe increased aggression over puzzle feeders. However, to decrease the risk of intra-group aggression or one individual dominating all the feeders, caretakers might provide more than one item per animal and/or secure them within the environment.

We also observed that animals groomed themselves less on days of puzzle feeder enrichment than on days with objects. Grooming behaviors have been considered a proxy for anxiety in some species (Aureli & de Waal, 1997; Baker & Aureli, 1997). Animals lacking opportunities for environmental interaction can lead to negative subjective states, resulting in self-directed behaviors such as grooming (Rowan, 1988; Wood-Gush & Vestergaard, 1989). On the contrary, anxiety-like behaviors have also been observed when cognitive tasks are present (Clark & Smith, 2013) or when an animal does not have the cognitive skills to cope with the challenge (Duncan & Wood-Gush, 1972). Therefore, it is vital to make the problem-solving opportunity manageable for the animal (Hintze & Yee, 2022; Clark, 2023). Finding the appropriate difficulty level for different animals takes time and resources; results can vary by enrichment items, and increasing difficulty does not always result in positive behavioral changes (Ghavamian et al., 2022). In this study, all animals had previous experience with the puzzle feeders provided, and therefore we knew the challenge was manageable for them. This minimized the risk of frustration for the study subjects in this case. Our study supports simple problem-solving opportunities, and the puzzle feeders provided were a good level of difficulty for the animals based on the observed behavioral changes.

At the end of the day, when the zoo closes, most animals are shifted to their indoor enclosure or given access to another to receive their final meal before the husbandry team leaves the zoo grounds. This feeding is concurrent as the zoo closes at the same time every day, thus predictable for the animals. Closing times at zoos are usually signaled to guests by speaker announcements and, by default, a reliable signal to the animals. In certain species, anticipatory behavior of scheduled feedings is observed as an increased activity prior to the feeding time (Folkedal et al., 2012; Anderson et al., 2015; Dantas-Ferreira et al., 2015; Podturkin et al., 2022). Our results showed that at the end of the day (last ten minutes before feeding), animals expressed lower locomotion and higher standing behaviors with puzzle feeders than on days with object enrichment. This suggests that puzzle feeders successfully stimulated animals to decrease anticipatory behavior at the end of the day. Although we did not measure the intensity of anticipatory behavior, our results may represent a decrease in intensity by observing lower locomotion and greater standing behaviors at the end of the day. Our results are consistent with a previous zoo study that showed decreased anticipatory behavior with cognitive enrichment in dolphins via spy hopping and looking behaviors (Clegg et al., 2023). In addition, Krebs & Watters (2017) observed less locomotion and overall activity in a black rhino (*Diceros bicornis*) prior to its evening feeding. Our results from this study also show that at the end of the day, animals spent more time eating food provided earlier when they received puzzle feeders than on days with object enrichment. Allowing animals to achieve a goal via puzzle feeders early in the day might satisfy psychological motivations other than feeding motivation and promote stopping feeding before consuming all the food. While with object enrichment, the animals may have eaten the food instantaneously and had no more food left to consume at the end of the day, which leads to animals spending more time anticipating their last reward. Therefore, our cognitive enrichment has met the behavioral goal of extending animal foraging time throughout the day while decreasing anticipatory behavior ahead of evening feedings.

Enrichment standards for the Association of Zoos & Aquariums require programs to promote species-appropriate behavioral opportunities (Association of Zoos and Aquariums, 2022). The "how" this is completed with enrichment is left to each institution to decide the category of items, frequency, and duration. These decisions vary within a zoo, across animals, and can vary by keepers where personal experiences or beliefs can influence their enrichment practices (Tuite et al., 2022). Our study supports prioritizing cognitive enrichment within enrichment programs, even for animals not typically considered "problem-solving" species. Although we did not vary the difficulty of the puzzle feeders as suggested to reach an "appropriate level of challenge," as discussed in Meehan & Mench (2007), we believe the simple puzzle feeders allowed animals to use cognitive skills for food acquisition. Previous studies have also implemented simple feeders that provide a foraging challenge to animals and have been considered cognitive devices (Gronqvist et al., 2013; Krebs & Watters, 2017; Clegg et al., 2023). The cognitive enrichment we used were not novel items nor complex devices which provided an opportunity that was familiar and solvable by individuals. Husbandry teams should not defer due to funding, designing a complex device, or buying new items, as they can create simple challenges with everyday items. Therefore, we hope this evidence will encourage husbandry teams to provide even simple problem-solving or goal-based opportunities to animals, potentially outweighing the common reasons for steering away from cognitive enrichment (e.g., time and financial constraints; Hall et al., 2021).

We completed this study with eight individuals of three species, two camelid and one carnivore species. We observed our expected predictions regardless of the differences in the life histories of these animals. We observed the benefits of cognitive opportunities for animals under human care outside of a single species. Although the definition and approach to cognitive enrichment are still debated and not widely agreed upon (Clark, 2017), its importance is evident for animal welfare. One definition states that cognitive enrichment should engage cognitive skills by providing opportunities to problem-solve and control an aspect of the environment, and its response should correlate to one or more validated measures of wellbeing (Clark, 2011). Here, puzzle feeders met this definition as they provided an opportunity to extract food using cognitive skills and were more stimulating to the individuals than object enrichment, as seen through behavioral changes. The frequency and duration of interactions the animals took to achieve the goal varied depending on the type of puzzle feeder offered. However, in our study, we found that the benefits of achieving the goal of extracting food were more significant than the action itself. These benefits are longer lasting and seen throughout the day. We will learn more about the positive effects of cognitive enrichment and their generality as more studies test multiple individuals of different species and observe behaviors outside of utilizing the enrichment.

We found that the eight individuals of three species interacted significantly more with puzzle feeders than objects. During the days with puzzle feeders, animals exhibited less anticipatory and anxiety-like behaviors and more social behaviors. The behavioral changes observed in this study suggest that providing problem-solving opportunities via puzzle feeders can potentially impact animal welfare. While we studied eight individuals of three species, the results of this study could be used to inform enrichment programs for other species housed in zoos. We encourage using simple problem-solving opportunities as they can promote positive behaviors and impact wellbeing more than the common enrichment objects offered.

References

Anderson, C., Yngvesson, J., Boissy, A., Uvnas-Moberg, K., & Lidfors, L. (2015). Behavioural expression of positive anticipation for food or opportunity to play in lambs. Behavioural Processes, 113, 152–158. doi:10.1016/j.beproc.2015.02.003

Association of Zoos and Aquariums. (2022). The Accreditation Standards & Related Policies. https://assets.speakcdn.com/assets/2332/aza-accreditation-standards.pdf

Aureli, F., & de Waal, F. (1997). Inhibition of social behavior in chimpanzees under high-density conditions. American Journal of Primatology, 41, 213-228.

Baker, K. C., & Aureli, F. (1997). Behavioural indicators of anxiety: An empirical test in chimpanzees. Behaviour, 134, 1031-1050.

Averós, X., Brossard, L., Dourmad, J. Y., de Greef, K. H., Edge, H. L., Edwards, S. A., & Meunier-Salaün, M. C. (2010). A meta-analysis of the combined effect of housing and environmental enrichment characteristics on the behaviour and performance of pigs. Applied Animal Behaviour Science, 127(3-4), 73-85.

Clark, F. E. (2011). Great ape cognition and captive care: Can cognitive challenges enhance well-being?. Applied Animal Behaviour Science, 135(1-2), 1-12.

Clark, F. E. (2017). Cognitive enrichment and welfare: Current approaches and future directions. Animal Behavior and Cognition, 4(1), 52-71.

Clark, F. (2023). In the Zone: Towards a Comparative Study of Flow State in Primates. Animal Behavior and Cognition, 10(1), 62. https://doi.org/10.26451/abc.10.01.04.2023

Clark, F. E., & Smith, L. J. (2013). Effect of a cognitive challenge device containing food and non-food rewards on chimpanzee well-being. American Journal of Primatology, 75(8), 807-816.

Clark, F. E., Davies, S. L., Madigan, A. W., Warner, A. J., & Kuczaj, S. A. (2013). Cognitive enrichment for bottlenose dolphins (Tursiops truncatus): Evaluation of a novel underwater maze device. Zoo Biology, 32(6), 608-619.

Clegg, I. L., Domingues, M., Ström, E., & Berggren, L. (2023). Cognitive Foraging Enrichment (but Not Non-Cognitive Enrichment) Improved Several Longer-Term Welfare Indicators in Bottlenose Dolphins. Animals, 13(2), 238.

Dantas-Ferreira, R. F., Dumont, S., Gourmelen, S., Cipolla-Neto, J., Simonneaux, V., Pevet, P., & Challet, E. (2015). Food-anticipatory activity in Syrian hamsters: Behavioral and molecular responses in the hypothalamus according to photoperiodic conditions. Plos One, 10, 19. doi:10.1371/journal.pone.0126519

de Azevedo, C. S., Cipreste, C. F., & Young, R. J. (2007). Environmental enrichment: A GAP analysis. Applied Animal Behaviour Science, 102(3-4), 329-343.

Delfour, F., & Beyer, H. (2011). Assessing the effectiveness of environmental enrichment in Bottlenose dolphins (Tursiops truncatus). Zoo Biology, 29,1–14.

Duncan, I.J., Wood-Gush, D.G.M. (1972). Thwarting of feeding behaviour in the domestic fowl. Animal Behavior, 20, 444–451.

Fagot, J., & Bonté, E. (2010). Automated testing of cognitive performance in monkeys: Use of a battery of computerized test systems by a troop of semi-free-ranging baboons (Papio papio). Behavior Research Methods, 42, 507-516.

Fagot, J., Gullstrand, J., Kemp, C., Defilles, C., & Mekaouche, M. (2014). Effects of freely accessible computerized test systems on the spontaneous behaviors and stress level of Guinea baboons (Papio papio). American Journal of Primatology, 76, 56-64.

Fay, C., & Miller, L. J. (2015). Utilizing scents as environmental enrichment: Preference assessment and application with Rothschild giraffe. *Animal Behavior and Cognition*, *2*(3), 285-291.

Folkedal, O., Stien, L. H., Torgersen, T., Oppedal, E., Olsen, R. E., Fosseidengen, J. E., ...Kristiansen, T. S. (2012). Food anticipatory behaviour as an indicator of stress response and recovery in Atlantic salmon post-smolt after exposure to acute temperature fluctuation. Physiology & Behavior, 105, 350–356. doi:10.1016/j.physbeh.2011.08.008

Franks, B., Champagne, F. A., & Higgins, E. T. (2013). How enrichment affects exploration trade-offs in rats: implications for welfare and well-being. PLoS One, 8(12), e83578.

Ghavamian, Y., Minier, D. E., & Jaffe, K. E. (2022). Effects of Complex Feeding Enrichment on the Behavior of Captive Malayan Sun Bears (Helarctos malayanus). Journal of Applied Animal Welfare Science, 1-15.

Gilloux, I., Gurnell, J., & Shepherdson, D. (1992). An enrichment device for great apes. Animal Welfare, 1(4), 279-289.

Gronqvist, G., Kingston-Jones, M., May, A., & Lehmann, J. (2013). The effects of three types of environmental enrichment on the behaviour of captive Javan gibbons (Hylobates moloch). Applied Animal Behaviour Science, 147(1-2), 214-223.

Hall, B. A., McGill, D. M., Sherwen, S. L., & Doyle, R. E. (2021). Cognitive enrichment in practice: a survey of factors affecting its implementation in zoos globally. Animals, 11(6), 1721.

Heinze, G., Wallisch, C., & Dunkler, D. (2018). Variable selection - A review and recommendations for the practicing statistician. Biometrical journal. Biometrische Zeitschrift, 60(3), 431–449.

Hintze, S., & Yee, J. R. (2022). Animals in flow–Towards the scientific study of intrinsic reward in animals. Biological Reviews.

Hoy, J. M., Murray, P. J., & Tribe, A. (2010). Thirty years later: enrichment practices for captive mammals. Zoo Biology, 29: 303-316.

Kalbe, C., & Puppe, B. (2010). Long-term cognitive enrichment affects opioid receptor expression in the amygdala of domestic pigs. Genes, Brain and Behavior, 9, 75-83.

Kells, A., Dawkins, M. S., & Borja, M. C. (2001). The effect of a 'freedom food'enrichment on the behaviour of broilers on commercial farms. Animal Welfare, 10(4), 347-356.

Krebs, B. L., & Watters, J. V. (2017). Simple but temporally unpredictable puzzles are cognitive enrichment. Animal Behavior and Cognition, 4: 119-134.

Kuczaj, S. A., Lacinak, T., Fad, O., Trone, M., Solangi, M., & Ramos, J. (2002). Keeping environmental enrichment enriching. International Journal of Comparative Psychology,15, 127–137

Lauderdale, L. K., & Miller, L. J. (2020). Efficacy of an interactive apparatus as environmental enrichment for common bottlenose dolphins (Tursiops truncatus). *Animal Welfare*, *29*(4), 379-386.

Lincoln Park Zoo. (2020). ZooMonitor [Mobile app]. https:// zoomonitor.org

Lumeij, J. T., & Hommers, C. J. (2008). Foraging 'enrichment' as treatment for pterotillomania. Applied Animal Behaviour Science, 111(1-2), 85-94.

Lutz, C. K., & Novak, M. A. (1995). Use of foraging racks and shavings as enrichment tools for groups of rhesus monkeys (Macaca mulatta) (Vol. 14, No. 5, pp. 463-474). New York: Wiley Subscription Services, Inc., A Wiley Company.

Makowska, I. J., & Weary, D. M. (2016). Differences in anticipatory behaviour between rats (Rattus norvegicus) housed in standard versus semi-naturalistic laboratory environments. PLoS ONE, 11, e0147595.

Manteuffel, G., Langbein, J., & Puppe, B. (2009). From operant learning to cognitive enrichment in farm animal housing: bases and applicability. Animal Welfare, 18: 87-95.

Martin, P., & Bateson, P. (2007). Measuring behavior: an introductory guide (3rd ed.). Cambridge, UK: Cambridge University Press.

Meehan, C. L., & Mench, J. A. (2002). Environmental enrichment affects the fear and exploratory responses to novelty of young Amazon parrots. Applied Animal Behaviour Science, 79(1), 75-88.

Meehan, C. L., & Mench, J. A. (2007). The challenge of challenge: can problem solving opportunities enhance animal welfare?. Applied Animal Behaviour Science, 102(3-4), 246-261.

Mellor, D.J., Hunt, S. & Gusset, M. (eds). (2015). Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy. World Association of Zoos and Aquariums (WAZA) Executive Office. Gland, Switzerland.

Nagabaskaran, G., Skinner, M., & Miller, N. (2022). Western Hognose Snakes (Heterodon nasicus) Prefer Environmental Enrichment. *Animals*, *12*(23), 3347.

Podturkin, A. A. (2021). In search of the optimal enrichment program for zoo-housed animals. Zoo Biology, 40(6), 527-540.

Podturkin, A. A., Krebs, B. L., & Watters, J. V. (2022). A Quantitative Approach for Using Anticipatory Behavior as a Graded Welfare Assessment. Journal of Applied Animal Welfare Science, 1-15.

Puppe, B., Ernst, K., Schön, P. C., & Manteuffel, G. (2007). Cognitive enrichment affects behavioural reactivity in domestic pigs. Applied Animal Behaviour Science, 105(1-3), 75-86.

Rowan, A.N. (1988). Animal anxiety and animal suffering. Applied Animal Behaviour Science, 20, 135–142.

Sanders, K., & Fernandez, E. J. (2020). Behavioral implications of enrichment for golden lion tamarins: A tool for ex situ conservation. Journal of Applied Animal Welfare Science, 1-10.

Sasson-Yenor, J., & Powell, D. M. (2019). Assessment of contrafreeloading preferences in giraffe (Giraffa camelopardalis). Zoo Biology, 38(5), 414-423.

Shepherdson, D. (1994). The role of environmental enrichment in the captive breeding and reintroduction of endangered species. Creative Conservation: Interactive Management of Wild and Captive Animals, 167-177.

Shettleworth, S. J. (2001). Animal cognition and animal behaviour. Animal Behaviour, 61(2), 277-286.

Soriano, A. I., Vinyoles, D., & Maté, C. (2019). Patterns of animal–enrichment interaction in captive brown bears. Zoo Biology, 38(3), 239-247.

Špinka, M., & Wemelsfelder, F. (2011). Environmental challenge and animal agency. Animal Welfare, 2, 27-44.

Spruijt, B. M., van den Bos, R., & Pijlman, F. T. (2001). A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behavior Science, 72: 145-171.

Swaisgood, R. R., & Shepherdson, D. J. (2005). Scientific approaches to enrichment and stereotypies in zoo animals: what's been done and where should we go next?. Zoo Biology, 24: 499-518.

Swaisgood, R. R., White, A. M., Zhou, X., Zhang, H., Zhang, G., Wei, R., ... & Lindburg, D. G. (2001). A quantitative assessment of the efficacy of an environmental enrichment programme for giant pandas. Animal Behaviour, 61(2), 447-457.

Tuite, E. K., Moss, S. A., Phillips, C. J., & Ward, S. J. (2022). Why Are Enrichment Practices in Zoos Difficult to Implement Effectively?. Animals, 12(5), 554.

Van der Harst, J. E., Fermont, P. C. J., Bilstra, A. E., & Spruijt, B. M. (2003). Access to enriched housing is rewarding to rats as reflected by their anticipatory behaviour. Animal Behaviour, 66(3), 493-504.

Vick, S. J., Anderson, J. R., & Young, R. (2000). Maracas for Macaca? Evaluation of three potential enrichment objects in two species of zoo-housed macaques. Zoo Biology, 19(3), 181-191.

Watters, J. V. (2014). Searching for behavioral indicators of welfare in zoos: Uncovering anticipatory behavior. Zoo Biology, 33, 251–256. doi:10.1002/zoo.21144

Watters, J. V., Krebs, B. L., & Pacheco, E. (2019). Measuring Welfare through Behavioral Observation and Adjusting It with Dynamic Environments. In: A. B. Kaufman, M. J. Bashaw and T. L. Maple (eds.) Scientific Foundations of Zoos and Aquariums: Their Role in Conservation and Research. p 212-240. Cambridge University Press, Cambridge.

Wood-Gush, D.G.M., Vestergaard, K. (1989). Exploratory behavior and the welfare of intensively kept animals. Journal of Agricultural Ethics, 2, 161–169.

Young, R.J., 2003. Environmental Enrichment for Captive Animals. Blackwell Science, Oxford.

Supplemental Table. Detailed ethogram for each species.

Behavior	Description
Giraffes	
Stand	Giraffe is standing on all four legs, on the ground. Includes the giraffe ruminating while standing.
Lie Down	Giraffe is laying down on the ground, all four legs tucked underneath body
Locomote	Giraffe moves from one place to another via walking. Includes the giraffe ruminating while walking.
Eat	Giraffe consumes food. Includes processing behaviors like, biting, pulling, or otherwise manipulating food with mouth.
Explore	Sniffing, licking (less than 5 seconds), manipulating environment that is not enrichment.
Affiliative	Giraffe rubs against or engages in other friendly behaviors towards another giraffe.
Interact with enrichment	Giraffe interacts with keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.
Lick/Chew	Giraffe is repeatedly licking its face or object for more than 5 seconds. Record modifier: barn, tree, or other.
Groom	Giraffe scratches itself against an object such as a tree, or otherwise bites, rubs, or licks itself.
Drink	Giraffe consumes water.
Out of View	Giraffe is not in view.
Guanaco	
Stand	Guanaco is standing on all four legs, on the ground. Includes ruminating while s

Lie Down	Guanaco is laying down on the ground, all four legs tucked underneath body
Locomote	Guanaco moves from one place to another via walking. Includes ruminating while walking.
Eat	Guanaco consumes food. Includes processing behaviors like, biting, pulling, or otherwise manipulating food with mouth. Includes walking slowly with head close to ground searching for food.
Explore	Sniffing, licking (less than 5 seconds), manipulating environment that is not enrichment.
Interact with enrichment	Guanaco interacts with keeper provided enrichment item. Must contact the item with face, mouth, or feet to count.
Lick/Chew	Guanaco repeatedly licks or chews on non-food objects for more than 5 seconds.
Groom	Guanaco scratches itself against an object such as a tree, or otherwise bites, rubs, or licks itself.
Drink	Guanaco consumes water.
Out of View	Guanaco is not in view.
River Otters	
Swimming	Otter is in water, moving from one place to another. Head can be above or under water.
Stand	Otter is standing out of the water on all four feet or bipedal. No other behavior is occurring.
Lie Down	Otter is laying down, out of the water.
Locomote	Otter is moving from one place to another out of the water. Includes walking, trotting, or running.
Eat	Otter is consuming food. Includes processing behaviors, such as gathering food or chewing.
Explore	Sniffing, pawing, licking, or manipulating environment that is not enrichment.

Interact with enrichment	Otter interacts with any enrichment item, including pushing, pulling, rolling, sitting on chewing on, or any other physical contact with a keeper provided item.
Affiliative	Otter is swimming, wrestling, or otherwise exhibiting affiliative behaviors toward conspecific.
Look/Climb	Otter climbs partially out of water near glass at front of exhibit, looks out.
Door	Otter is digging at, pawing at, or chewing on keeper door at back of exhibit.
Groom	Otter scratches itself against an object or otherwise licking, bites, pawing at self.
Drink	Otter consumes water.
Out of View	Otter is not in view.

Supplemental Table. Final model for each behavior used in the analysis for FULL DAY and LAST TEN

FULL DAY	
Behavior	**Final Model**
Affiliative/Play	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Number of Enrichment Items + New Enrichment + Consecutive Days + (1|Species/ID), data=TB_Aff, weights = Total Counts, family = binomial(link="logit")
Eat	Proportion ~ Enrichment Type + Replicate + Consecutive Days + (1|Species/ID), data=TB_EF, weights = Total Counts, family = binomial(link="logit"))
Explore	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Number of Enrichment Items + New Enrichment + Consecutive Days + (1|Species/ID), data=TB_Explore, weights = Total Counts, family = binomial(link="logit")
Groom	Proportion ~ Enrichment Type + Replicate + Consecutive Days + (1|Species/ID), data=TB_Groom, weights = Total Counts , family = binomial(link="logit")
Interact with Enrichment	Proportion ~ Enrichment Type + New Enrichment + (1|Species/ID), data=TB_IE, weights = Total Counts, family = binomial(link="logit")
Lick/Chew	Proportion ~ Enrichment Type + Day of Treatment + Number of Enrichment Items + New Enrichment + (1|Species/ID), data=TB_LickChew, weights = Total Counts, family = binomial(link="logit")
Lie Down	Proportion ~ Enrichment Type + Day of Treatment + Replicate+ Number of Enrichment Items + New Enrichment + (1|Species/ID), data=TB_LieDown, weights = Total Counts, family = binomial(link="logit")
Locomote	Proportion ~ Enrichment Type + Number of Enrichment Items + (1|Species/ID), data=TB_Locomote, weights = Total Counts, family = binomial(link="logit")

Stand	Proportion ~ Enrichment Type + Day of Treatment + Replicate + (1|Species/ID), data=TB_Stand, weights = Total Counts, family = binomial(link="logit")
LAST TEN	
Affiliative/Play	Proportion ~ Enrichment Type + Day of Treatment + Number of Enrichment Items+ New Enrichment + (1|Species/ID), data=AB_Aff, weights = Total Counts, family = binomial(link="logit")
Eat	Proportion ~ Enrichment Type + Consecutive Days + (1|Species/ID), data=AB_EF, weights = Total Counts, family = binomial(link="logit")
Explore	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Number of Enrichment Items + New Enrichment + Consecutive Days + (1|Species/ID), data=AB_Explore, weights = Total Counts, family = binomial(link="logit")
Groom	Proportion ~ Enrichment Type + Replicate + (1|Species/ID), data=AB_Groom, weights = Total Counts, family = binomial(link="logit")
Interact with Enrichment	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Number of Enrichment Items + New Enrichment + Consecutive Days + (1|Species/ID), data=AB_IE,weights = Total Counts, family = binomial(link="logit")
Lick/Chew	Proportion ~ Enrichment Type + Day of Treatment + Consecutive Days + (1|Species/ID), data=AB_LickChew, weights = Total Counts, family = binomial(link="logit")
Lie Down	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Number of Enrichment Items + New Enrichment + Consecutive Days + (1|Species/ID), data=AB_LieDown, weights = Total Counts, family = binomial(link="logit")
Locomote	Proportion ~ Enrichment Type + Day of Treatment + Replicate + Consecutive Days + (1|Species/ID), data=AB_Locomote, weights = Total Counts, family = binomial(link="logit")

Stand	Proportion ~ Enrichment Type + Day of Treatment + (1|Species/ID), data=AB_Stand, weights = Total Counts, family = binomial(link="logit")