INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

2025, 38
Charles I. Abramson
Editor
Peer-reviewed

Assessing Judgment Bias in Ambassador Animals: Two Case Studies

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Judgment bias tasks assess optimism and pessimism through responses to ambiguous stimuli. When interacting with ambiguous stimuli, optimistic individuals anticipate receiving a reward whereas pessimistic individuals anticipate a lack of reward, with these differing expectations reflected in approach time. Researchers have used these tests to assess animals' reactions to assumed positive and negative contexts, but rarely to assess the effects of participation in ambassador programs. We tested two ambassador animals—a domestic chicken (*Gallus domesticus*) and a red tegu (*Salvator rufescens*)—after exposure to zoo visitors. Once they learned that a container on the left contained food whereas a container on the right contained no food, we introduced an ambiguous container equidistant from the left and right locations. We assessed the chicken's judgment biases when she was perched or held. We assessed the tegu's judgment bias when visitors were allowed to touch him or not. The chicken displayed pessimism whether she was held or perched, but the tegu displayed pessimism only when no visitor touch occurred, suggesting that touch may not be aversive to the tegu, but that interacting with visitors may have deleterious effects on the chicken. We encourage the use of these tests to inform the use of animals in ambassador programs.

Keywords: domestic chicken, optimism, pessimism, red tegu, visitor interactions

アンバサダーアニマルにおける判断バイアスの評価：2つの事例研究

バイアス課題は、あいまいな刺激に対する反応を通じて動物の楽観性と悲観性を評価する。曖昧な刺激に接するとき、楽観的な個体は報酬があることを期待し、悲観的な個体は報酬がないことを予期し、これらの違いは反応時間に反映される。研究者たちはこのようなテストを、想定した肯定的および否定的な状況に対する動物たちの反応を評価することに使用してきたが、アンバサダープログラムへの参加が与える影響を評価する目的で行われたことはほとんどなかった。私たちは、2種類のアンバサダーアニマル（ニワトリ（*Gallus domesticus*）とレッドテグー（*Salvator rufescens*））を用いて、彼らを動物園の来園者と接触させた後に以下のテストを行った。左の容器には食べ物があり、右の容器には食べ物がないことを学習させた後、左右の位置から等距離にある曖昧な容器を設置した。ニワトリの判断バイアスは、ニワトリが止まり木にいるときと抱かれているときで評価した。レッドテグーの判断バイアスは、来園者に触ることを許可した場合と許可しなかった場合で評価した。ニワトリは抱かれても止まっても悲観的な反応を示したが、レッドテグーは来園者による接触がなかったときのみ悲観的な反応を示した。このことから、レッドテグーにとって触られることは嫌悪ではないものの、来客と触れ合うことはニワトリに悪影響を及ぼす可能性が示唆された。我々は、アンバサダープログラムにおける動物の使用についての情報を提供するうえで、これらのテストを活用することを奨励する。

キーワード：ニワトリ、楽観性、悲観性、レッドテグー、来園者とのふれあい

Evaluación del sesgo de juicio en animales embajadores: dos estudios de caso

Las tareas de sesgo evalúan el optimismo y el pesimismo a través de respuestas a estímulos ambiguos. Cuando interactúan con este tipo de estímulos, los individuos optimistas anticipan recibir una recompensa, mientras que los pesimistas anticipan una ausencia de recompensa; estas expectativas diferenciales se reflejan en los tiempos de aproximación. Estas pruebas se han utilizado en la investigación para evaluar las reacciones de los animales ante contextos asumidos como positivos o negativos, aunque no es común su uso para evaluar los efectos de la participación en programas de animales embajadores. Tras una exposición a visitantes del zoológico, se evaluaron dos animales embajadores: un gallina doméstica (Gallus domesticus) y un tegu rojo (Salvator rufescens). Una vez que aprendieron que un recipiente a la izquierda contenía comida y uno a la derecha no, se introdujo un recipiente ambiguo en una posición equidistante entre ambas ubicaciones. Evaluamos los sesgos de juicio del pollo cuando estaba posado o era sostenido. Por su parte, el sesgo de juicio del teju se evaluó según los visitantes pudieran tocarlo o no. Se encontró que la gallina mostró pesimismo tanto al ser sostenida como al estar posada, mientras que el teju solo mostró pesimismo cuando no había contacto con los visitantes. Esto sugiere que la interacción con los visitantes podría no ser aversiva para el teju, pero sí tener efectos perjudiciales para la gallina. Se recomienda el uso de estas pruebas para informar las decisiones sobre el uso de animales en programas de embajadores.

Keywords: Gallina doméstica, optimismo, pesimismo, tegu rojo, interacciones con visitantes

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A focus on animal cognition has grown in recent years (Whissell et al., 2013), with an ever-increasing diversity of species and topics (Vonk, 2016), yet a consensus on the operationalization of cognition and related traits remains elusive (Abramson, 2013). Similarly, research in particular areas of focus, such as animal emotion, is also proliferating despite a lack of consensus as to how emotion should be operationalized in nonhumans. There is general agreement among researchers that nonhuman animals experience at least some emotional states (Scarantino & de Sousa, 2021). Yet, the research regarding animal emotional states is also rife with inconsistencies, specifically regarding definitions of emotions and relevant terminology (Nematipour et al., 2022; see Vonk et al., 2024 for a review). What is certain is that presuming emotional states allows researchers to predict animal behavior (Mendl et al., 2009); thus, researchers in various fields, such as neuroscience, psychopharmacology, and animal welfare, have worked to develop valid assessments of these states, in addition to confirmation that animals experience these states (Kremer et al., 2020). Much of the past research on animal emotional states has focused on negative states (Proctor et al., 2013), which makes detection of positive emotional states—a necessary focus for the field of animal welfare (Mellor, 2016)—difficult.

A recent welfare assessment method allows for the assessment of emotional biases that affect how information is processed, explaining the somewhat misleading name of the tests as cognitive bias tests (Harding et al., 2004). The label is misleading in the sense that these tests do not assess an animal's cognitive ability; rather, they indicate when the animal might be experiencing a likely implicit tendency to view the world optimistically or pessimistically. The judgment bias task, one of several variations of the cognitive bias test, assesses optimism and pessimism through an organism's response to ambiguous stimuli (Bethell, 2015), thereby providing indicators of positive as well as negative emotional states. An individual's affective state can influence the perception of ambiguity through multiple means. That is, feeling optimistic or pessimistic can influence what an individual pays attention to, how they judge stimuli, what they remember, and more (e.g., Coles & Heimberg, 2002; Dunn et al., 2009; Eysenck et al., 1991; Novak et al., 2015). In addition, an individual's affect may bias their tendency towards risk aversion and prediction of expected utility (Mendl et al., 2009). An individual who is optimistic would anticipate receiving a reward when interacting with ambiguous stimuli, whereas an individual who is pessimistic would not anticipate a reward or might expect something aversive from interacting with ambiguous stimuli. Optimism reflects a positive emotional state, whereas pessimism reflects a negative emotional state. Therefore, assessing an animal's judgment bias might shed light on their affective state in various contexts.

A multitude of animals from insects to primates have demonstrated judgment biases in response to manipulations presumed to impact affective states (Lagisz et al., 2020). However, of the non-pharmacological studies utilizing judgment bias tests, the majority assessed mammals (Lagisz et al., 2020). Fewer studies have examined the judgment biases of birds (canaries, *Serinus canaria*: Lalot et al., 2017; chickens, *Gallus domesticus*: Anderson et al., 2021; Deakin et al., 2016; Lourenço-Silva et al., 2023; Seehuus et al., 2013; Wichman et al., 2012; Zidar et al., 2018; crows, *Corvus moneduloides*: McCoy et al., 2019; European starlings, *Sturnus vulgaris*: Bateson & Matheson, 2007; Bateson et al., 2015; Brilot et al., 2010; Gott et al., 2019; Matheson et al., 2008; Japanese quails, *Coturnix japonica*: Horváth et al., 2016; ravens, *Corvus corax*: Adriaense et al., 2019; red junglefowl, *G. gallus*: Munari, 2021), although the judgment bias test is becoming a popular welfare assessment method in livestock poultry (Košťál et al., 2020). Even fewer studies have examined judgment biases of insects and fish (bumblebees, *Bombus terrestris*: Solvi et al., 2016; convict cichlid, *Amatitlania siquia*: Laubu et al., 2019; fruit flies, *Drosophila melanogaster*: Deakin et al., 2018; honeybees, *Apis mellifera*: Bateson et al., 2011; Schlüns et al., 2017), and no published studies have examined judgment biases of reptiles (Lagisz et al., 2020). The studies on poultry have primarily manipulated the environment and its complexity to understand environmental impacts on affective states but no studies have assessed the impact of human interactions directly.

Despite the increasing use of judgment bias tests in agricultural settings, it has seldom been applied to assess zoo animal welfare (Clegg, 2018). Perhaps this underutilization is due to its potential pitfalls, such as its lack of suitability for sustained testing due to the loss of ambiguity over time (Bethell, 2015) or the sometimes extensive training required, as well as challenges in the interpretation of the animal's perception of the task (Perdue, 2017). However, testing with a small number of zoo animals has validated the approach at least for mammals; Bottlenose dolphins (*Tursiops truncatus*; Clegg & Delfour, 2018; Clegg et al., 2017) Western lowland gorillas (*Gorilla gorilla gorilla,* McGuire & Vonk, 2018; McGuire et al., 2017a), an American black bear (*Ursus Americanus*, McGuire et al., 2017b; Vonk et al., 2021) and grizzly bears (Bernstein-Kurtycz et al., 2024). Other methods involving less training, such as evaluating response slowing to threatening stimuli, have been used to assess the effects of zoo environments on captive animals. For example, Cronin et al. (2018) found that Japanese macaques (*Macaca fuscata*) exhibited a response slowing towards conspecific images compared to control images during more extreme anthropogenic noise conditions, but no change was found for chimpanzees (*Pan troglodytes*) and gorillas. McGuire and Vonk (2020) found that gorillas responded slower to directly-facing versus averted gorilla faces, but this pattern did not depend upon housing conditions. None of these studies assessed the impacts of interactions with humans.

Ambassador animals are representatives of the species that can be used to demonstrate conservation goals and successes. Individual members of the species are selected to be ambassadors if they have suitable temperaments for interacting with the trainers and the public. For example, they typically have more training experience, can be separated from conspecifics, and are calm and adaptable to environmental changes. Ambassador animals participate in education presentations meant to teach visitors about animals and conservation, yet not much is known about the impact of participation on the ambassador animals themselves (Spooner et al., 2021). They are often exposed to handling by visitors, which is not a typical experience for most zoo animals and could have significant impacts on their welfare, including their emotional states.

The AZA has created an Ambassador Animal Evaluation Tool to assess an animal's suitability for education programs (AZA, n.d.), but there are minimal guidelines on judging the welfare in an ambassador animal (Spooner et al., 2021). Although other studies have assessed the welfare of ambassador animals, they have largely utilized behavioral and/or physiological measures (Spooner et al., 2021). For example, a study examining ambassador animal welfare at 17 AZA-accredited zoos for several armadillo species (*Dasypodidae* spp.), African hedgehogs (*Atelerix albiventris*), and red-tailed hawks (*Buteo jamaicensis*) showed no effect of education programs specifically, but fecal glucocorticoid metabolites and undesirable behaviors increased with greater handling durations, and rest behavior decreased (Baird et al., 2016). Another multi-zoo study demonstrated that giraffes (*Giraffa camelopardalis*) spent less time ruminating (i.e., masticating, regurgitating, and swallowing cud) and more time idle when taking part in all-day visitor feeding programs, but not in part-day programs, and there was no association with stereotypic behaviors (Orban et al., 2016). Although the monitoring of glucocorticoid levels is undoubtedly useful in assessing animal welfare, assessments of affective states have typically not been conducted in concert to aid in the interpretation of the results.

Findings from these studies can be challenging to interpret. For example, in dromedary camels (*Camelus dromedarius*), salivary cortisol levels were lower when camels took part in visitor rides compared to seasons when they did not, suggesting that either the visitor rides or the food reinforcement received during such rides were enriching (Majchrzak et al., 2015). This finding highlights an issue that may complicate the interpretation of these results and explain the apparent mixed results. It can be difficult to determine whether the effects of interactions with humans are due to the interactions themself or the fact that animals often receive reinforcement during these sessions. A recent study attempted to tease these factors apart in assessing the judgment biases of two grizzly bears (*U. arctos horribilis*). Judgment biases were assessed following four conditions that varied in two dimensions; the presence of visitors and the delivery of food rewards. One of the two bears appeared pessimistic during the absence of food reinforcement, suggesting that the presence of food was more impactful on affect than the presence of visitors (Bernstein-Kurtycz et al., 2024). Thus, manipulating specific aspects of ambassador programs could contribute to a better understanding of how education presentations might affect the welfare of ambassador animals.

To address this paucity of research and demonstrate the utility of the judgment bias test for assessing zoo animal welfare (Bethell, 2015), we assessed the effects of human interaction on judgment biases in two zoo-housed ambassador animals: a domestic chicken and a red tegu (*Salvator rufescens*). Chickens are a common subject for judgment bias research with farm animals, but this is the first study on the judgment bias of a zoo-housed chicken used in ambassador programs, and the first study to assess judgment bias in a reptile of any species. The red tegu is an omnivorous terrestrial lizard native to South America which grows to a relatively large body size (Fitzgerald et al., 1993; Jarnevich et al., 2018) and is commonly kept as a pet. Little is known about learning capacities in tegus, so it important to assess this species' ability to acquire the spatial discrimination as a test of its feasibility for further testing.

We modified a procedure developed by Harding et al. (2004), which was recently adapted for use with crows (McCoy et al., 2019). Both ambassador animals were required to learn that one location was associated with a large reward, whereas another location was associated with no reward. Then, one novel location was introduced equidistant between the previously known locations, and the latency to approach this novel ambiguous location was compared to the latencies for the known locations (i.e., reward and no reward). A latency to approach the ambiguous location similar to the no reward location indicated pessimism, and a latency to approach the ambiguous location similar to the latency to approach the large reward location indicated optimism.

We assessed the impact of experiences that were thought to be potentially aversive to each animal, as indicated by their two primary caregivers. Both the tegu and the chicken took part in visitor interactions in two different conditions. We assessed the impact of keeper handling (perch or hold) for the chicken and the impact of visitor touch (touch or no touch) for the tegu. Based on the care staff's assessment of the animals' preferences, we expected that the chicken would demonstrate greater pessimism in the hold condition (i.e., staff keeping their arms around the chicken preventing wing movement) as compared to the perch condition, and the tegu would demonstrate greater pessimism in the touch condition as compared to the no touch condition. Previous research has also indicated that visitor touch may be aversive for prolonged periods, albeit in different species (Baird et al., 2016). Thus, each animal was exposed to two experimental conditions or contexts that were anticipated to impact affect, and each session included three types of trials involving rewarded, non-rewarded, or ambiguous locations, creating a 2×3 within-subjects design. Whether the impact of these programs is positive or negative, or potentially neutral, this study provided meaningful data for zoos and demonstrated the utility of the judgment bias protocol in assessing welfare in these species.

Study 1

Both studies were reviewed and approved by Oakland University's IACUC (Protocol # 2022-1190).

Method

Subject

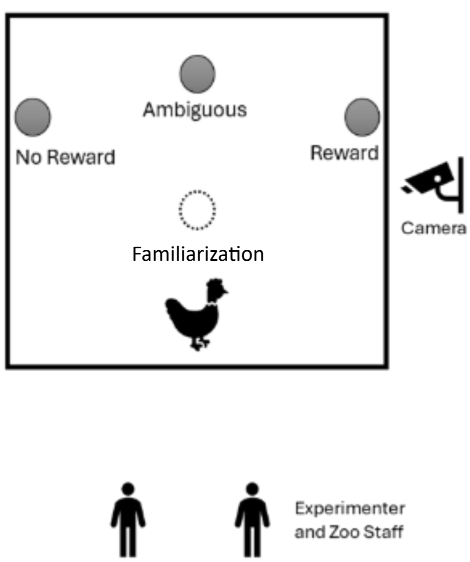
The subject was an adult female chicken (*Gallus domesticus*) that served as an education animal at the Alexandria Zoo in Alexandria, Louisiana. This chicken was housed with a crested screamer (*Chauna torquata*) in an outdoor habitat near the zoo's education buildings, which was on a path not often frequented by zoo visitors. The housing was a rectangular fenced area attached to the zoo building on one side which contained some bushes, logs, and a small pond. At the time of the study, she was a recent addition to the zoo. She was purchased as a chick from a local feed store in April 2023 specifically to be a companion for the crested screamer, thus she was approximately four to five months old at the time of the study. When she first came to the zoo, she was raised with another chick purchased at the same time. She was chosen to be an ambassador based on her temperament shortly before she was introduced to the screamer in July 2023. The chicken interacted with zoo staff at least once a day, but typically more often due to the proximity of its housing to the education buildings (i.e., keepers were often passing by, and sometimes visitors). This animal had minimal training experience from participating in previous educational programs and was naive to the judgment bias test. On days when testing occurred, daily feeding occurred after testing and education programs never occurred.

Materials

All testing occurred within an indoor education building attached to the chicken's home habitat. This area was secluded from visitors, but the chicken was exposed to random keeper entrances and noises from their two-way radios, as well as noises from the reptiles and birds housed in this building. Trials were carried out within a caged square arena made out of moveable metal fencing. This fencing was 2.4 m by 2.2 m with sides approximately 0.7 - 0.9 m tall. (see Figure 1). The height was 0.9 m in the back half of the arena, whereas the height was 0.7 m in the front half of the arena due to differences in the two portions of moveable fencing utilized. One metal bowl was utilized as a food container throughout the study. The bowl was opaque so that the chicken could not see the reward within the container until she approached the container. This bowl was placed in four different locations depending on what phase and what trial was occurring. In Familiarization trials, the container was placed in the middle center of the testing arena. During training trials, the container would be placed on either the right (no reward) or left (reward) side of the testing arena. The middle (ambiguous) location was equidistant from both of these locations in addition to being the same distance from the animal's starting point. Food was placed in the container, and then removed before trials started, so that the no reward container would smell similarly to the reward container. Rewards consisted of high value food (mealworms) as rated by the bird's keepers and approved to be used across multiple trials by the veterinarian. A GoPro HERO10 was used to film all trials.

Figure 1

Vertical View of Enclosure Setup



Note. Distance to containers (excluding Familiarization trials) was 2.1 m for the chicken and 1.6 m for the tegu.

Procedure

Sessions occurred at approximately 11:00 a.m. CST on Sundays, Mondays, and Thursdays for Familiarization and Training. These days and this time were selected due to zoo staff availability. Testing occurred twice a day on Wednesdays/Thursdays and Sundays/Mondays to allow consecutive days of testing. The researcher tested both the chicken and the tegu each day, and the order of sessions were randomly decided. The indoor trial arena, the containers, and the camera were set up prior to retrieving the chicken for sessions. The container was placed in particular locations depending on the trial (reward, no reward, and ambiguous). The reward and no reward locations were 2.3 m apart from each other, and both locations were 2.1 m from the starting point of the arena. On ambiguous trials, the same container was placed equidistant (1.2 m) from the high reward and no reward locations, and this location was also 2.1 m from the starting point of the arena. The distances between the containers were determined based on the size, gait, and speed of the chicken to encourage a difference in latency to reach the container on reward versus no reward trials. Once the trial was set up, the keeper retrieved the subject from its outdoor habitat and brought the bird to the entrance of the arena. Between trials, the bird was picked up by the keeper and positioned so that the setup of the arena was not visible to the bird. The keeper was blind as to which container location contained the reward on the first trial, although they inevitably saw the reward locations as they viewed the bird across later trials. All trials were recorded for later coding by naïve coders. The researcher recorded the bird's latency to move from the starting position to the container (i.e., touching the container or positioning their head directly over top of the container) live during testing. This latency was used as the criterion for advancing to the next phase.

Familiarization. One session (5 trials per session) of Familiarization occurred per day. The chicken was required to receive a minimum of one session of Familiarization, but Familiarization continued until she could retrieve the food in under 1 minute in 4 out of 5 trials within a session. At the start of a trial, the keeper placed the chicken at the middle front of the arena (see Figure 1) facing a baited container directly in front of her, so that this location was neutral compared to the testing locations. The experimenter (JT) and the keeper stood quietly behind the arena in the opposite direction of the baited container so that they were not visible to the chicken when she was moving toward the container. The trial was scored as successful if the chicken retrieved the food in under one minute. All Familiarization trials continued until the reward had been retrieved. If the chicken had not retrieved the reward after three minutes and was not moving toward the food, the keeper attempted to direct the animal toward the baited container. This phase ensured the chicken was familiar with the container, knew that the container held food, and retrieved the food quickly.

Training. One training session (six trials per session) occurred per day until criterion was met. Of these six trials, three trials were high reward trials and three trials were no reward trials, presented in a pseudorandomized order. The last of the six trials was always a reward trial. Before each training session, the chicken was given a single Familiarization trial to maintain motivation. At the start of a training trial, the chicken was placed at the middle front of the arena (see Figure 1). The experimenter and the keeper then stood quietly behind the arena so that they were not visible to the chicken when she was moving toward the container. All trials ended after three minutes to ensure the chicken had time to eat the reward. Trial length was kept constant in training and testing regardless of whether the reward was retrieved to ensure that the chicken did not approach more quickly on ambiguous trials in testing to hasten the next rewarded trial (not because she was optimistic). As seen in Figure 1, the locations of the high reward container and no reward container are indicated by the left and right circles. Only one container was present on each trial, so that high reward trials consisted of only the high reward container, and no reward trials consisted of only the no reward container. The experimenter set up the high and no reward containers with the same procedure so as not to cue the chicken to their difference. To move to the testing phase, the chicken was required to reach a specific time difference in their approach to the no reward and high reward conditions. Specifically, the three no reward trials were paired with the three reward trials by presentation order (i.e., first reward trial with first no reward trial and so on), and the chicken was required to demonstrate a faster time on reward trials (by any amount) in each of the three pairs of trials across two consecutive sessions to move to testing.

Testing. The chicken received eight 5-trial sessions of testing after completing training. Two sessions occurred per day. Testing sessions occurred on a separate day from the last training session to ensure consistency in having two test sessions each test day with at least one fresh Testing session each day. In this way, we could assess effects of fatigue or satiation consistently. The testing sessions consisted of 2 high reward trials, 2 no reward trials, and 1 ambiguous trial. The number of trials were limited to reduce fatigue and satiation and to maintain motivation. The locations of the high reward and no reward containers remained the same as in the previous phases, and the ambiguous container was equidistant from both of these containers and the same distance from the starting point. The ambiguous trials were randomly presented among the 5 trials within a session, with the constraint that the first trial was never an ambiguous trial, such that the first trial served to remind the chicken of previously learned contingencies. On ambiguous trials, the container was baited 50% of the time but was scented with food on every trial to maintain its unpredictability and encourage some motivation to approach. To further differentiate this ambiguous container, it was filled with newspaper shreds to cover the potential food so that the bird would not know if it contained food upon approach.

Once a trial started, the experimenter and the keeper stood quietly behind the arena so that they were not visible to the bird when it was moving toward the container. All trials ended once three minutes passed. Limiting testing to eight sessions helped to ensure that the ambiguous location remained ambiguous; however, some learning may have occurred as sessions progressed. Four sessions were conducted after the chicken experienced a football hold (i.e., keeper's arms wrapped around the wings) while interacting with zoo visitors and four sessions were conducted after the chicken experienced a perched hold (i.e., feet on top of the keeper's arm with the other arm in front of the chicken's chest) while interacting with zoo visitors. Each day of testing, the chicken received a football hold condition and a perched hold condition in randomized order (see Table 1). In both conditions, the chicken was carried by a familiar keeper around outside areas in the zoo and experienced physical contact from at least three humans. The keeper aimed for contact with three people, but if the last person was in a group, the number would sometimes exceed three. This interaction could take place in any outside area around the zoo, as the keeper looked for human interactions opportunistically. This interaction lasted around 10 min each time, and then the chicken was immediately returned to the testing arena. Once familiarization, training, and testing were complete, coders naïve to the manipulation recorded latencies to approach the food locations and foraging behavior from the video recordings.

Data Analysis

All analyses were conducted via IBM SPSS version 29.0.1.1. To assess the impact of visitors on the subjects, we conducted generalized linear mixed models (GLMM) with trial type (reward, no reward, ambiguous) and condition (perched or football hold) and their interaction as fixed factors, session as a random factor, and latencies to reach the container as the outcome variable. Session was included as a random factor to control for factors such as weather, care staff present, etc. We used planned contrasts to compare latencies of ambiguous trials to both reward and no reward trials. The gamma distribution was used as the data were not normally distributed and it led to a better fitting model than the gaussian distribution, as indicated by a lower corrected AIC. We applied a Satterthwaite approximation, as this is appropriate for small sample sizes. To probe interactions, separate GLMMs were conducted for the two conditions.

Table 1

The Chicken's Schedule of Sessions Across Phases

Date	Phase	Session	Testing Only	
			Condition	Time of Day
7.30.23	Familiarization	1		
7.31.23		2		
8.6.23		3		
8.7.23		4		
8.10.23		5		
	Training	1		
8.13.23		2		
8.14.23		3		
		4		
8.17.23		5		
		6		
8.23.23	Testing	1	Football Hold	Morning
		2	Perch	Afternoon
8.24.23		3	Perch	Morning
		4	Football Hold	Afternoon
8.27.23		5	Football Hold	Morning
		6	Perch	Afternoon
8.28.23		7	Perch	Morning
		8	Football Hold	Afternoon

Results

Reliability

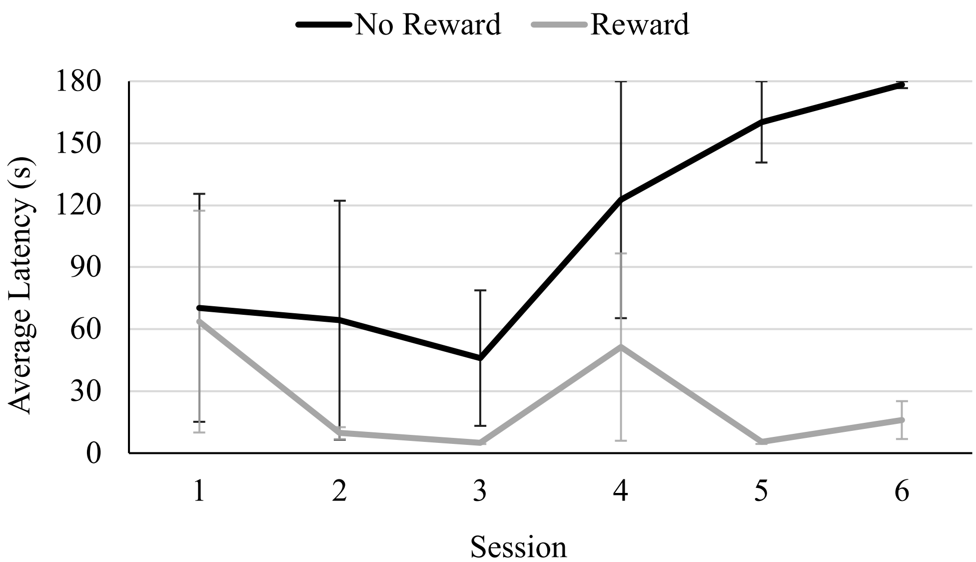
A primary coder coded all videos and a secondary coder coded 20% of the same videos. Reliability between coders for latency to approach was assessed via ICC, α = .999.

Training

The chicken passed criterion in the Familiarization phase after five sessions and then moved on to training. Figure 2 shows the chicken's average approach time on training sessions by trial type (reward and no reward). The chicken passed the training criterion in session 6, advancing to testing sessions when the discrimination was learned sufficiently.

Figure 2

Average Latency for the Chicken to Approach by Trial Type and Session



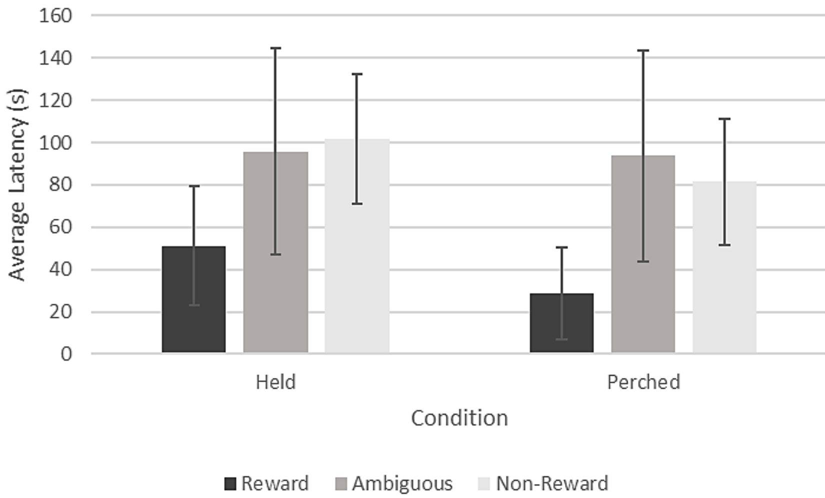
Note. Error bars depict standard error of the mean.

Testing

The model fit with a corrected AIC of 262.30. There was no main effect of condition ($F(1,34) = 0.04$, $p = .835$; held: $M = 129.40$ s, $SE = 116.14$ s, perched: $M = 45.30$ s, $SE = 8.96$ s). There was a significant main effect of trial type ($F(2,5) = 14.68$, $p < .001$) but this was qualified by a significant interaction between condition and trial type ($F(2,5) = 10.39$, $p < .001$) . Separate GLMMs for each condition revealed that the main effect of trial type was significant for both conditions (Held, $F(2,2) = 46.29$, $p = .004$, Perch, $F(2,2) = 26.50$, $p < .001$). Models fit well with corrected AICs of 110.70 and 119.31. In the Held condition, the chicken did not approach the ambiguous location significantly more slowly than she approached the reward condition (t (2) = -0.43, $p = .705$, 95% CI [-32.44, 25.66]), or more quickly than she approached the non-reward location (t (2) = 3.87, $p = .066$, 95% CI [-24.67, 310.16]). In the Perch condition, the chicken did not approach the ambiguous location significantly more slowly than she approached the reward condition (t (2) = -1.84, $p = .085$, 95% CI [-246.73, 17.51]), or significantly more quickly than she approached the non-reward location (t (2) = -1.81, $p = .090$, 95% CI [-238.54, 19.02]), although both effects approached significance. Although Figure 3 shows that the pattern of approach differed somewhat according to condition, the chicken's approach latency on ambiguous trials was more similar to her approach to the non-reward than to reward location in both conditions.

Figure 3

Average Latency to Approach Across Trial Type and Condition for the Chicken



Note. Error bars depict standard error of the mean.

Discussion

There was a significant difference between the chicken's average approach time in the ambiguous trials compared to the reward trials with the average approach latency greater in the ambiguous trials compared to the reward trials. This, coupled with the finding that the average latency to approach in ambiguous trials did not significantly differ from the average latency in no reward trials suggests pessimism (i.e., a latency to approach the ambiguous location similar to the no reward location) rather than optimism (i.e., a latency to approach the ambiguous location similar to the latency to approach the large reward location). Although the main effect of trial type interacted with condition, the overall pattern was one in which latencies to approach on ambiguous trials were numerically more similar to latencies on non-reward compared to reward trials (Figure 3), suggesting that the chicken may experience a negative affective state during those interactions that is not differentially impacted by the style of hold. However, future studies would need to include a baseline condition without human interaction to further assess whether the chicken was pessimistic due to visitor interactions, or to both hold types, or just in general. This condition was not included here because it is critical to minimize exposure to the ambiguous location to maintain the condition of ambiguity, and therefore, we limited the number of test sessions.

Study 2

Method

Subject

A single male red tegu (*Salvator rufescens*) that served as an education animal at the Alexandria Zoo in Alexandria, Louisiana was tested. Red tegus are large omnivorous lizards native to South America. This tegu arrived at the zoo in July 2010 at an estimated 7 months of age and was donated by a private owner; thus, he was approximately thirteen or fourteen years old at the time of our study. He was raised along with an Argentine black and white (*S. merianae*) female tegu for most of his time at the zoo. He began target training in September 2017. The black and white tegu left for another zoo in May 2021, and the red tegu has been housed individually since then. The tegu was housed in an indoor habitat within the same educational building that testing occurred, although testing occurred in a different room. This building was infrequently open to the public, and the tegu was housed in a back room, so the tegu's interactions with visitors were primarily limited to educational and other on-site programs. This habitat was an approximately 5' × 7' rectangular structure built into the building with branch structures for climbing. The tegu was exposed to zoo staff near its habitat at least once a day, but zoo staff would randomly go through the educational building throughout the day, albeit likely not within close proximity to the tegu. The educational programs provided the subject with minimal training experience, but he was naïve to the judgment bias test. On days when testing occurred, daily feeding occurred after testing and education programs never occurred.

Materials

All testing occurred within an indoor education building that housed the tegu's home habitat. This area was secluded from visitors, but the tegu was exposed to the same noises in this building as the chicken, although he may have been more habituated to these noises as this was his daily habitat. Trials were carried out within an approximately square cardboard arena. This arena was 1.6 m by 1.7 m with sides 0.9 m tall (set up similarly to Figure 1), smaller than the chicken's arena to account for the tegu's slower speed. One opaque blue plastic lidded soap container was utilized as a food container throughout the study. This container was placed in the same locations as the setup for the chicken for Familiarization, training, and testing trials. The reward and no reward locations were 1.5 m apart from each other, and both locations were 1.6 m from the entrance to the arena. On ambiguous trials, the container was placed equidistant (0.8 m) from the high reward and no reward locations, and this location was also 1.6 m from the starting point of the arena. The distances between the containers were determined based on the size, gait, and speed of the tegu to encourage a difference in latency to contact the containers on reward versus no reward trials. The container had food placed within it before trials started so that scent could not be used as a cue. Rewards consisted of high value food (meat cubes) as rated by the tegu's keepers and approved to be used across multiple trials by the veterinarian. A GoPro was used to film all trials.

Procedure

The details of the procedure for the tegu were similar to those of the chicken. However, the tegu received a different manipulation. During testing, the manipulation for the tegu centered around physical interaction with visitors. Four sessions were conducted after the tegu experienced physical touch from various visitors, and four sessions were conducted after the tegu experienced nonphysical interaction with various visitors (see Table 2). Each day of testing, the tegu received a visitor touch condition and a no visitor touch condition in randomized order. In both conditions, the tegu was carried by a familiar keeper around outside areas in the zoo and experienced interaction with at least three humans (aside from the keeper). The keeper aimed for contact with three people, but if the last person was in a group, the number would sometimes exceed three. This interaction could take place in any outside area around the zoo, as the keeper looked for human interactions opportunistically. Similar to the chicken, this interaction lasted around 10 minutes each time, and then the tegu was immediately returned to the testing arena to begin sessions. The analytic approach was the same as above.

Table 2

The Tegu's Schedule of Sessions Across Phases

Day	Phase	Session	Testing Only	
			Condition	**Time of Day**
7.30.23	Familiarization	1		
7.31.23		2		
8.6.23	Training	1		
8.7.23		2		
8.10.23		3		
8.13.23		4		
8.14.23		5		
		6		
8.17.23		7		
8.23.23	Testing	1	Touch	Morning
		2	No Touch	Afternoon
8.24.23		3	No Touch	Morning
		4	Touch	Afternoon
8.27.23		5	Touch	Morning
		6	No Touch	Afternoon
8.28.23		7	No Touch	Morning
		8	Touch	Afternoon

Results

Reliability

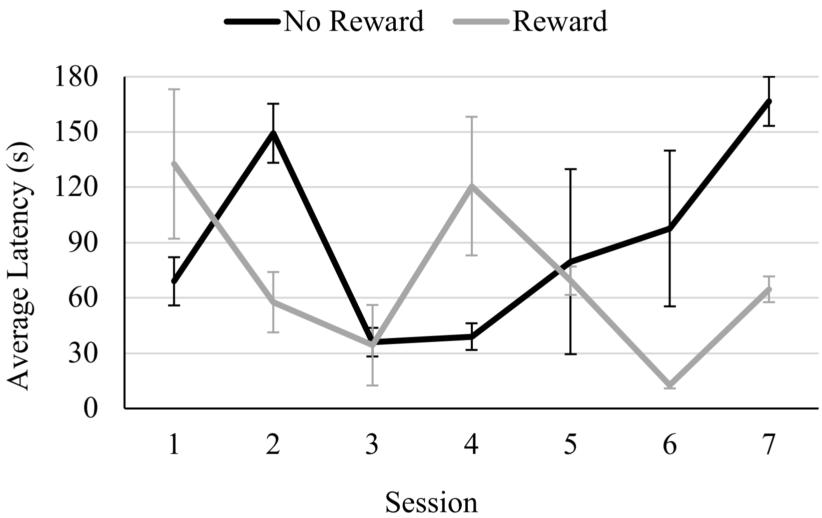
Reliability between coders for latency to approach was excellent; ICC, $\alpha = 1.00$.

Training

The tegu passed criterion in the Familiarization phase after two sessions, and then advanced to training. His average latencies to approach in the training phase on both trial types (reward, no reward) are depicted in Figure 4. The tegu passed criterion on session 7, which allowed him to advance to testing sessions after demonstrating discrimination between trial types.

Figure 4

Average Latency to Approach Across Training Trials by Trial Type and Session for the Tegu



Note. Error bars depict standard error of the mean.

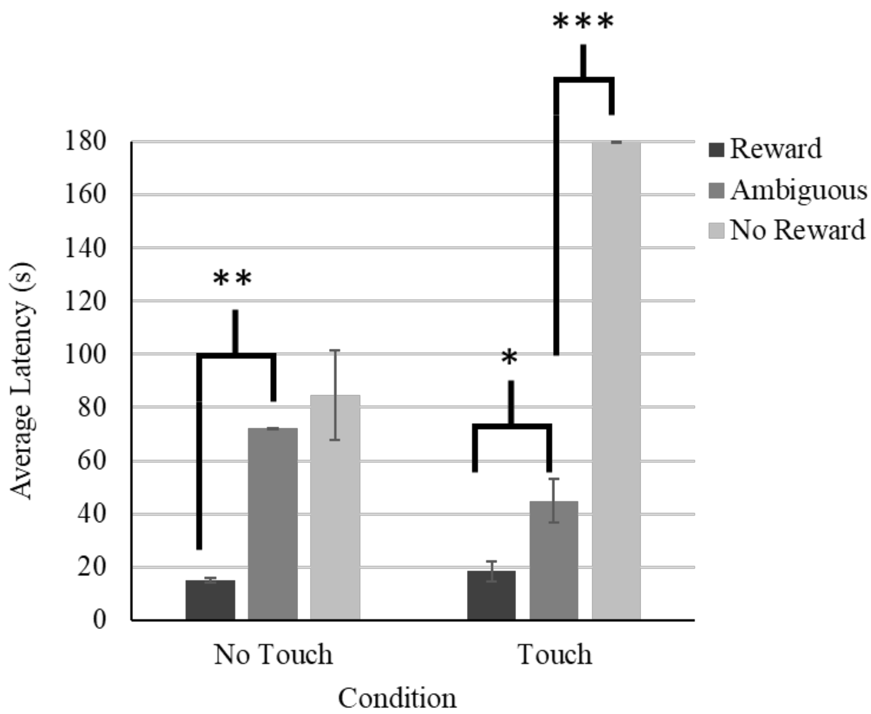
Testing

The model fit with a corrected AIC of 181.46. Results of the GLMM demonstrated no main effect of condition ($F(1,9) = 1.07$, $p = .329$; visitor touch: $M = 56.10$ s, $SE = 5.59$ s, no visitor touch: $M = 49.58$ s, $SE = 3.28$ s), but there was a main effect of trial type ($F(2,6) = 66.47$, $p < .001$). The tegu approached more slowly on ambiguous trials ($M = 56.78$ s, $SE = 5.21$ s) compared to reward trials ($M = 16.65$ s, $SE = 1.79$ s, $t = -3.19$, $p = .043$, $SE = .28$, CI [-1.72, -0.05]) and more quickly compared to no reward trials ($M = 123.43$ s, $SE = 12.21$ s, $t = 7.58$, $p = .023$, $SE = .18$, CI [0.43, 2.35]). However, these effects were qualified by a significant interaction between condition and trial type ($F(2,6) = 10.34$, $p = .012$). The tegu's approach times between the ambiguous trial type and the no reward trial ($t = -4.56$, $p = .006$, $SE = .27$, CI [-1.93, -.53]), but not the reward trial type ($t = -2.43$, $p = .079$, $SE = 0.28$, CI [-1.51, 0.13]), significantly differed across conditions (see Figure 5).

Separate GLMMs were conducted to probe the interaction further. Models fit well with corrected AICs of 194.44 and 204.03. In the no touch condition, there was a significant effect of trial type ($F(2,17) = 8.83$, $p = .002$). Latency to approach on reward trials ($t = -2.99$, $p = .008$, 95% CI [-63.12, -10.94]) but not no reward trials ($t = 1.13$, $p = .274$, 95% CI [-16.96, 56.15]) significantly differed from latency to approach on ambiguous trials. In the touch condition, there was also a significant effect of trial type ($F(2,7) = 231.32$, $p < .001$). Latency to approach on reward trials ($t = -2.67$, $p = .026$, 95% CI [-43.71, -3.54]) and no reward trials ($t = 14.57$, $p < .001$, 95% CI [-108.72, 148.89]) significantly differed from latency to approach on ambiguous trials. Thus, the tegu was somewhat pessimistic in the no touch but was neither optimistic nor pessimistic in the touch condition. However, as can be seen in Figure 5, his latency to approach on ambiguous trials was more similar to the reward, compared to the non-reward trials in the touch condition.

Figure 5

Average Latency to Approach Across Trial Type and Condition for the Tegu



Note. Error bars depict standard error.

*$p < .05$; **$p < .01$; ***$p < .001$.

Discussion

The results show for the first time how quickly a tegu can learn a spatial discrimination. Testing results also indicate that tegus are suitable for cognitive bias paradigms despite not having been assessed previously. The designation of optimism and pessimism depends upon the comparison of the subject's approach latency to the ambiguous stimulus in both reward and no reward conditions. That the tegu responded significantly more slowly to the ambiguous stimulus compared to the reward stimulus, but also significantly more quickly than to the no reward stimulus suggests neither optimism or pessimism overall. The tegu's intermediate approach latency on ambiguous trials may suggest that he perceived the intermediate spatial position of the ambiguous stimulus and was suitably uncertain as to what to expect on these trials. Interestingly, the difference in his latency to approach the location on the ambiguous and no reward trials depended upon condition. Whereas the data are suggestive of pessimism in the no touch condition, the data in the touch condition are not consistent with pessimism, with the tegu approaching the ambiguous stimulus significantly more quickly than the no reward stimulus. Somewhat surprisingly, this suggests that the tegu may have experienced less negative affect in the condition with touch compared to the condition without.

General Discussion

These studies demonstrate the suitability of assessing cognitive biases in birds and reptiles using a spatial version of the cognitive bias paradigm. These animals showed similar time to acquisition of the spatial discrimination despite being distantly related species with distinct evolutionary histories. Importantly, the studies extend previous studies to the assessment of particular elements of ambassador programs. These results indicate distinct differences between the chicken and the tegu in their affect following visitor interactions. Specifically, the tegu did not appear to be negatively affected by the touch of visitors. The lack of evidence for negative affect following the visitor touch condition indicates little cost to his psychological well-being from participating in the program. However, his response to the ambiguous stimulus in the no touch condition was indicative of pessimism, suggesting that his visitor interactions should include a touch component. On the other hand, the chicken demonstrated pessimism in general following visitor interactions regardless of the manner in which she was held by keepers. This interpretation is consistent with previous research on the negative effects of prolonged human handling in hawks, hedgehogs, and armadillos, which showed increased fecal glucocorticoid levels and undesirable behaviors in such conditions (Baird et al., 2016). Thus, the chicken's interactions with visitors may need to be reconsidered.

It is difficult to integrate these results with other findings as there is minimal research on changes in affect following human interactions in zoo animals. Of the few previous studies on cognitive biases in zoo animals, only one examined the influence of humans on three primate species, and this was an indirect effect of anthropogenic noise (i.e., air show noise). Only the macaques, compared to the gorillas and chimpanzees, showed response slowing to conspecific images compared to control images when anthropogenic noise occurred (Cronin et al., 2018). Response slowing on simple tasks in these studies is thought to indicate anxiety, similar to results in humans (Bar-Haim et al., 2007), or that subjects' attention is focused on vigilance behaviors (Cronin et al., 2018; Ebitz et al., 2013), and is expected to be more extreme with threatening content. However, the macaques did not show response slowing to forward facing faces, which were thought to be threatening, compared to averted faces, which could have been due to the researchers' use of both female and male faces or lack of statistical power.

Interestingly, the macaques were a more recent addition to the zoo (i.e., data were collected during their second year of exposure to the noise) compared to the apes that had 13 years of exposure to the air show (Cronin et al., 2018). The chicken in the current study was also a recent addition to the zoo, as she came to the zoo about four months before testing occurred. This pattern reinforces the idea that habituation to human influences is an important factor in determining whether human influences might be aversive. Both the gorillas and chimpanzees in Cronin et al. (2018), as well as the tegu here (housed at the zoo since 2010), had been housed at their respective zoos for longer periods of time and displayed neutral affects after exposure to more intense human influences (i.e., touch for the tegu and loud jet noises for the apes). These comparisons must be tempered by the fact that noises from jets in an air show substantially differ from direct human handling.

The manipulation utilized in Bernstein-Kurtycz et al.'s (2024) study of grizzly bears was more comparable to the manipulation in the current study. Grizzly bears were tested after four experimental conditions varying by the occurrence of two factors: a visitor tour behind the scenes and food delivery by a trainer. One bear responded more slowly to the ambiguous stimulus compared to the reward, but not the no reward stimulus, suggesting pessimism only in conditions where food reinforcement was withheld but neutral effects based on the presence or absence of zoo visitors during a tour. The bears displayed a lower proportion of frustration behaviors when presented with food reinforcement compared to when there was no reinforcement regardless of whether visitors were present, validating the results from the judgment bias test. It is important to note, however, that proportions of frustration behaviors were extremely low overall.

Unfortunately, we did not assess behavioral impacts in the chicken or the tegu. These subjects also did not receive food reinforcement during the visitor interactions independent of the test rewards. However, the tegu and chicken both had a history of food reinforcement when involved in training and education sessions with keepers, which could influence their perceptions of these sessions and visitor interactions. It is possible that visitor presence did not have a large impact on the bears, as compared to our chicken, because the bears were not touched by visitors. Bears may also be less likely to find humans threatening compared to the much smaller chicken and tegu. In addition, the bears were able to move around somewhat freely, potentially giving them more of an escape opportunity and preventing negative effects (Sherwen & Hemsworth, 2019), whereas the chicken and tegu were being held by care staff. Even so, our tegu showed pessimism in the no touch, compared to no pessimism in the touch condition. The bears' prior history with visitor tours also likely impacted their response in the study. The bears and tegu may have become more accustomed to visitor presence, and associated visitors with the provision of rewards, in their time at the zoos compared to the chicken.

These results must be interpreted cautiously due to the short-term interactions assessed here, as the animals were exposed to visitors for an approximately ten-minute span before being tested. These animals have been frequently exposed to visitors in their past given their ambassador animal status; thus, a ten-minute exposure period may not have been long enough to cause any of the effects found here. We did not have the capacity to manipulate the duration, timing, or frequency of the ambassador encounters at the zoo. The judgment bias task has been considered less effective for assessment of short-term effects as animals may show a positive judgment bias given the supposed stressor has come to an end (Bethell, 2015). Therefore, the results for the tegu in the touch condition could indicate relief at the removal of the stimulus (see also Doyle et al., 2010 Freymond et al., 2014). On the other hand, the pessimism across conditions demonstrated by the chicken could have been a general affective bias caused by long-term factors. Future research could benefit from assessing judgment biases following longer, more intensive, and more consistent visitor interactions in a larger sample.

It is important to note that these animals were chosen to be ambassadors based on their temperament and past history of positive human interactions. This non-random selection is a common practice with ambassador animals, meaning that interactions with humans may have minimal impact on these individuals because of sampling biases. However, we still observed potentially negative impacts on the chicken, indicating that even these sampling biases do not negate the ability of judgement bias tests to detect potentially sub-optimal human-animal interactions.

Although this study aimed to fill gaps within the literature, there were several limitations. First, assessing the impact of human interactions meant that many different variables, such as the individual keeper handling the animal, differences in visitor behavior, and testing environment could affect the judgment bias of the animals. Previous research has indicated that these variables can impact animal behavior over and above visitor effects (Rose et al., 2020). Testing took place in Louisiana in August, during an abnormal drought, and in a building without air conditioning, meaning temperature and weather could have affected the animals more than any human interaction. We attempted to control some variation by including session number in the models as a random factor. The models were also lacking power due to the minimal number of subjects and trials, which were kept to a minimum to keep the ambiguous location ambiguous over successive exposures. It could also be that further keeper interaction during the testing procedure overshadowed any effects of the change in hold during visitor interactions for the chicken. The chicken was held in a manner to prevent her from flying into the arena when she was placed inside for testing; thus, the results could indicate the chicken's general dislike of handling. It would have been ideal to include baseline conditions in which the chicken was not handled at all and/or visitors were not present, but adding an additional condition would have diminished the ambiguity of the ambiguous location. Furthermore, it is always best practice to collect other measures of welfare concurrently, such as behavioral or physiological measures when it is possible to do so, but we did not collect such data here. Lastly, this study involved an extremely small sample from one zoo, so it is important to recognize that the results may not generalize to other ambassador animals. It is also important to note that affective responses and preferences are likely to always depend upon the individual and are not necessarily expected to generalize to other members of a species, or even other individuals sharing the same experiences. Studies like this can still point to facets of human-animal interactions that warrant further exploration with regard to their impact on animals.

Despite limitations, these results demonstrate the promise of the judgment bias task for ambassador animal assessment. The chicken and the tegu both learned to discriminate between the reward and no reward location relatively quickly and comparably—the tegu in seven sessions and the chicken in six sessions. Importantly, neither subject appeared to experience neophobia in the testing situation. The chicken's rapid approach to both reward and non-reward locations during training precludes concerns about neophobia as a reason for her slower approach to ambiguous locations, compared to the tegu. Previous studies have demonstrated the usefulness of the task in chickens (Košťál et al., 2020), although its promise for reptiles had only been theorized before this project (Benn et al., 2019). The current data show that tegus may learn spatial discrimination relatively quickly, which will be useful for future explorations of their cognition. We utilized a location-based discrimination with three distinct locations; however, other discriminations could be utilized based on the needs or abilities of various animals, such as colors, sizes, sounds, etc. (see Bethell, 2015, for a review).

Conclusion

Judgment biases are just beginning to be better studied in birds (e.g., Košťál et al., 2020; McCoy et al., 2019), and the current research extends this mainly agriculturally-based literature to zoo animal welfare. In addition, this is the first lizard, to the authors' knowledge, to be assessed with a judgment bias task, although the promise of judgment bias tests as a welfare assessment tool for reptiles has been acknowledged previously (Benn et al., 2019; Bethell, 2015). Given that affect can alter cognition (Mendl et al., 2009), it is worth investigating the judgment biases of lesser studied animals and the circumstances that may impact these biases. Both tegus and chickens are clearly able to rapidly learn the spatial discrimination on which this version of the judgement bias task depends. Furthermore, ambassador animals often experience close human interaction for prolonged periods, yet not enough research has investigated their impact on the welfare of the animals (Spooner et al., 2021). The current research suggests that various aspects of human interaction could affect the judgment biases of these ambassador animals but that these impacts likely depend on the individual. We suggest that judgement bias tests can be a valuable tool for examining the psychological effects of animals participating in ambassador programs. Further research is needed to understand if this practice should be reconsidered or encouraged for ambassador animals as a whole.

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Financial conflict of interest: No stated conflicts.
Conflict of interest: No stated conflicts.

Submitted: *September 15, 2024*
Resubmitted: *January 8, 2025*
Accepted: *April 22, 2025*