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Assessing choice ability and preferences of five leopard tortoises *Stigmochelys pardalis* for three stimuli through a novel two-phase preference test

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Abstract

Preference testing has long been used in ethology and animal welfare science to assess the preferences that animals have for different resources and stimuli. The study conducted herein assessed the choice-making ability of five leopard tortoises *Stigmochelys pardalis* in a novel two-phase preference test. Phase 1 was a discrete choice test in a y-maze with two options: food in one arm, the other arm left empty, with positions fixed per tortoise, but randomised across tortoises. Multivariate ANOVA were performed on three dependent variables of time taken to make a choice in both phases. In Phase 1, four of the five tortoises clearly chose the food arm more than the empty arm. One tortoise chose the food arm and empty arm equally (50/50). Phase 2 involved opening an additional arm on the maze and offering each tortoise three choices: food (the same as Phase 1); human interaction (shell scratches and rubs using hands); or an empty arm. Positions were again fixed per tortoise but randomised across tortoises. In Phase 2, tortoise choices were more varied. Two tortoises chose human interaction more than the other two maze arms; another two chose the food maze arm most; and one did not seem to show a strong preference for any particular arm. These results suggested that some individuals of this species of tortoise may possibly prefer this form of human interaction (shell scratches and rubs) over other stimuli in certain conditions; however, further research is necessary to improve the confidence of the conclusions presented herein.

Introduction

Globally, approximately 700 million visitors attend zoos annually (Gusset and Dick 2011). Awareness of animal welfare-related issues and captive animal wellbeing have rapidly changed in the general public in the last few decades (Whitham and Wielebnowski 2013), motivating zoos worldwide to improve many practices, to transform into 'ethical zoos' (Mellor et al. 2015; Gray 2017). However, many visitors still attend zoos purely for their own entertainment or for socialising, rather than for ethical reasons (Tribe and Booth 2003; Reading and Miller 2007; Carr and Cohen 2011; Gray 2017). Many visitors also report attending zoos to 'connect' with animals (Howell et al. 2019). Animal-visitor interactions (AVIs), especially close-contact or hands-on experiences, are often reported as a major drawcard to attend zoos, and almost all zoos regularly advertise these sorts of experiences to their potential guests (D'Cruze et al. 2019). Many animal welfare issues can arise in zoo environments (especially concerning AVIs or other human-animal interactions (HAIs)), and many solutions may involve 'asking' the animal what it wants (Franks 2019), or as some authors say, endeavouring to 'listen' to an animal's attempts to express agency or make choices (Špinka and Wemelsfelder 2011; 2018; Špinka 2019). However, the way in which we ask animals what they want; whether we even ask them at all; and how we interpret the animals' answers, may all affect our decisions regarding captive animal management, for better or for worse (Franks 2019; Špinka 2019). One solution to the conundrum of how to ask animals what they want may be competitive preference testing.

Preference testing is a long-standing experimental methodology used to investigate what an animal might want or value, within a limited set of options, though the efficacy and usefulness of these tests have been both praised and criticised (Kirkden and Pajor 2006; Browne et al. 2011; Hemsworth et al. 2011; Mehrkam and Dorey 2014; Franks 2019). Animal preferences may change rapidly or over time; can be highly dependent upon other conditions, circumstances and experiences specific to each individual animal; and concurrent preferences can conflict with each other and become incommensurate (Franks 2019). Also, it is possible to conclude false preferences based on what stimuli the animals have been given to choose between and those they have not, and simple preference testing may not actually capture an animal's level of motivation for the particular resource (Fraser and Nicol 2018; Franks 2019). Despite these concerns, classical competitive-choice preference tests, such as the tests used herein, are considered a first step when initiating preference testing in a new species of animal (Fraser and Nicol 2018). Acknowledging cautions, preference tests are still some of the best methods available for assessing animal preferences for a constrained set of options, and if the options are chosen well, the observed preferences may be very meaningful for an individual's overall welfare. Understanding animal preferences may lead to more effective housing and enrichment strategies (that can be tailored for individual animals) in practice in zoo environments.

Non-avian reptiles, amphibians, fish and birds are very often overlooked in the published academic literature (Hosey and Melfi 2014; Lambert et al. 2019), with an estimated 76% of zoo animal welfare research solely focusing on mammals, especially primates (Goulart et al. 2009; Hosey and Melfi 2014, respectively). The experiments conducted herein focused on five leopard tortoises *Stigmochelys pardalis* housed at a zoo in Melbourne, Australia. A captive husbandry manual was used for general background information about the keeping of leopard tortoises (Highfield 1996). Previous preference tests and operant conditioning studies, conducted in similar circumstances with other tortoise species, were used to inform the experimental design employed here (Mattis 1994; Weiss and Wilson 2003; Gaalema and Benboe 2008; Mehrkam and Dorey 2014; Passos et al. 2014; Alba et al. 2017; Tetzlaff et al. 2018; Gutnick et al. 2019; Learmonth et al. 2020). Understanding whether preferences for human interaction, food or exploration exist within the studied leopard tortoises may be used for informing and improving husbandry, enrichment and housing of this particular group of animals, that are frequently used for visitor education and engagement activities. If some animals indeed prefer human interaction, this may be an easy and effective avenue for zoos to improve both animal welfare and visitor engagement, by offering safe, interactive experiences with animals that are known to enjoy this form of enrichment.

Aim

This study tested leopard tortoise preferences in two phases. Phase 1 investigated whether leopard tortoises were able to make a simple choice between food or nothing. Phase 2 tested the preferences of each leopard tortoise for three stimuli: food, human interaction or nothing. It was hypothesised that some of the tortoises may prefer human interaction even over food, depending on each individual's immediate level of hunger and taste preference for the offered food item, and other internal motivations at the time of testing.

Methods

Study site and animals

This study was conducted at Zoos Victoria's Werribee Open Range Zoo (WORZ; Werribee, Victoria, Australia) and was approved by the Zoos Victoria Animal Ethics Committee (ZV18003). The study subjects were five 13-year-old male leopard tortoises hatched from the same clutch, hereafter labelled LT1–LT5. Leopard tortoises are a medium-sized land tortoise, the second largest tortoise from mainland Africa (Highfield 1996). The tortoise enclosure was built in and around (split indoor/outdoor enclosure) the 'Ranger Kids' classroom and child-play building at WORZ, tailored for play-learning between young children and their parents (or early-childhood teachers). Ranger Kids was a climate-controlled building with a consistent temperature of 24°C, optimal for the few reptile species housed in separate enclosures around the room (the leopard tortoises, a snake and a few skinks). All testing was conducted indoors, adjacent to the indoor section of the tortoise enclosure, with the maze positioned so that the 'decision arms' (arms 2, 3 and 4; see Fig. 1 below) were facing towards the glass front of the home enclosure. These five tortoises were classified as 'education' animals and were regularly handled by trained personnel for educational experiences both within and outside of their enclosure, which included regular contact with visitors in the form of shell scratches and rubs.

Testing was conducted between December 2018 and August 2019. All trials were performed on reduced feeding days. Reduced feeding days consisted of small scatter feeds of lettuces and leafy greens. Fresh pawpaw (papaya) fruit segments, each approximately 3×2×2 cm in size, were selected as the food stimulus for all trials. Pawpaw was considered a high-value food for the tortoises and was a colour (orange) understood to be highly attractive to multiple tortoise species (Stoddart and Westoll 1979; Gaalema and Benboe 2008; Pellitteri-Rosa et al. 2010; Passos et al. 2014). Pawpaw was not a standard part of the tortoises' weekly diet; instead, it was sometimes used as a training or motivation food due to its perceived high value by the tortoises. The nutrient and energy content of these fruit pieces were incorporated into the regular weekly diet of the tortoises and only offered during the experimental trials. Tortoises were only tested when they were awake, alert and compliant for being handled (i.e., not resisting by flattening plastron to ground to inhibit handling) and moved to the testing arena (Y-maze), following Zoos Victoria's existing protocols for animal use for 'visitor encounters'. Resting or sleeping individuals were not tested on that day, unless they awoke and/or became active within the testing hours. All trials in all phases were conducted with one tortoise at a time.

Y-maze construction

The experiment was conducted within a four-arm maze structure placed on tarps on the floor in the Ranger Kids building. The maze was modular, made of lightweight foamed-PVC panels with plastic guiding rails (plastic skeleton) above and below for structural integrity and was erected and dismantled on each testing day (fabrication by Alternative Engineering, Melbourne, Victoria, Australia). Figure 1 depicts the maze plans and a photograph of the completed structure on the floor tarps (magnetic 'doors' for each arm not visible in photograph).

Arm 1 was always the 'starting chamber' where tortoises were first placed. Tarps (that the tortoises were already familiar with) were placed underneath the maze for hygiene and ease of cleaning. Tortoises were acclimatised to the maze by placing them in arm 1 with no doors attached (as in Fig. 1 photograph) and allowed to explore for 5 min before being returned to their enclosure. Each tortoise was given four introductions to the open maze structure over two separate days (two per day, 20 min total).

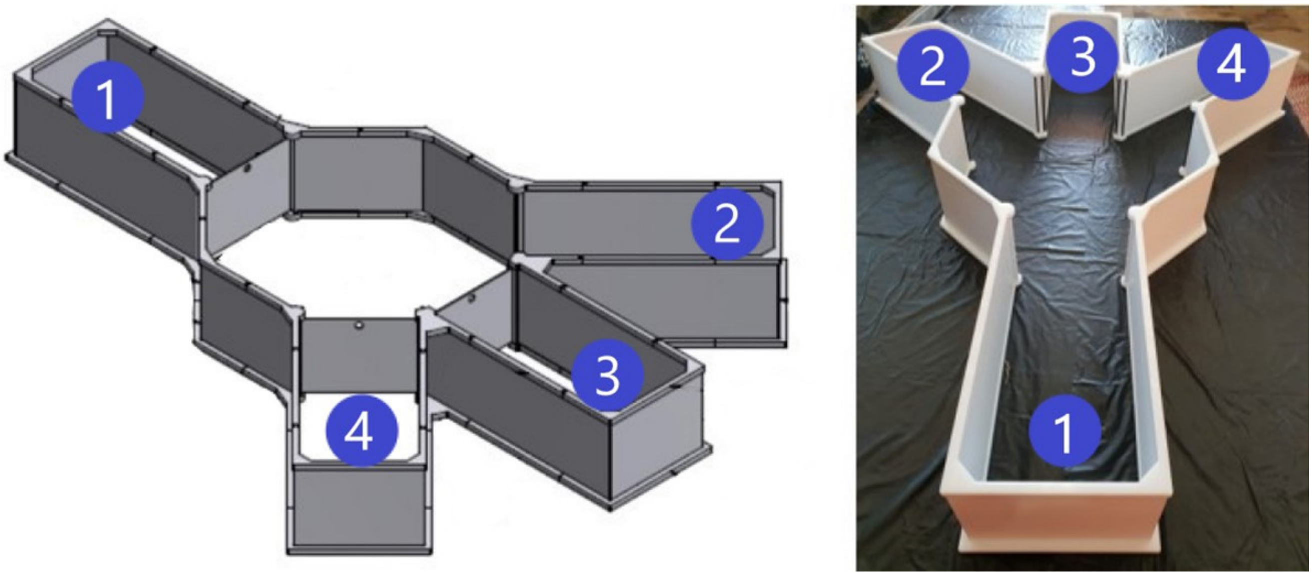

Figure 1. Design plan and photograph of foamed PVC four-arm 'maze' used for preference tests with leopard tortoises. Fabrication by Alternative Engineering, Melbourne, Victoria, Australia.

Tarps and maze walls were spot-cleaned between all trials with F10 SC Veterinary Disinfectant solution to avoid contamination or odour residue from previous trials affecting future trial outcomes.

Preference test Phase 1: Dichotomous choice

The first phase of preference testing was a simple dichotomous choice test, using only two of the three decision arms of the maze (arms 2 and 4). The choice was between an arm containing food, and one that remained empty (no stimulus). This discrete choice was designed to test the tortoises' ability to make a choice for an obvious reward over nothing, but also to test the preference of the tortoises for the chosen food stimulus, based on latency to choice and the relative number of each choice. To assist learning, the assignment of each stimulus to an arm was fixed for each tortoise but randomised across tortoises. The assignment of stimulus sides is shown in Table 1.

Training

A training period was conducted to allow tortoises to learn which arm would contain food and which arm would be empty. Each training trial consisted of two steps. Tortoises were placed in the start arm (arm 1) with the magnetic door closed. After 10 sec, the door was removed allowing the tortoise to access the main chamber (decision chamber) as well as either arm 2 or arm 4 (one decision arm open per training step). Tortoises were given 2 min to exit the start arm, as sometimes commencement of movement would be quite slow. Once the tortoise had fully entered the decision chamber, the start arm was closed with the magnetic door behind them. If they did not move from the start arm within 2 min, they were returned to their enclosure, and an extra training trial was attempted later the same day if the tortoise was still compliant. This protocol was the same for all phases and trials.

If the tortoises entered the decision chamber, they were given 1

Table 1. Randomised assignment of stimuli to arms for each tortoise in Phase 1 and Phase 2.

Tortoise	Phase 1		Phase 2		
	Arm 2 (Left)	Arm 4 (Right)	Arm 2 (Left)	Arm 3 (Mid)	Arm 4 (Right)
LT1	Empty	Food	Human	Food	Empty
LT2	Empty	Food	Human	Empty	Food
LT3	Food	Empty	Food	Human	Empty
LT4	Food	Empty	Food	Empty	Human
LT5	Food	Empty	Empty	Food	Human

min to explore the chamber and the open stimulus arm. If food was present, they could consume it. Once the tortoise had explored the arm or consumed the food, they were reset into arm 1 again with the magnetic door closed (for step 2), and the alternate arm was then opened (i.e., if arm 2 was open first, arm 4 was then opened and arm 2 was closed). After 10 sec, the tortoise was again given access to the decision chamber and to the alternate arm for 1 min or until food was consumed. After encountering each open stimulus arm (a two-step process), the tortoise was removed from the maze and placed back in their enclosure. Two successful training trials could be conducted per tortoise per day. Each tortoise received six training trials over a period of one week (two trials per testing day). Again, this protocol was the same for all phases and trials. Based on previous research featuring training and position discrimination in tortoise species (Pellitteri-Rosa et al. 2010; Wilkinson et al. 2010; 2013; Mueller-Paul et al. 2014; Passos et al. 2014; Gutnick et al. 2019), all five tortoises were reasonably assumed to have learned the positions of each stimulus by the end of the training period. LT3 and LT4 investigated and ate (or attempted to eat) the pawpaw on the first few training trials, but subsequently did not try to consume the food at all in any further training or trials, indicating a moderate-to-strong dislike of the offered food.

Choice trials

Once the training was completed, dichotomous choice trials commenced. Tortoises were placed in the start arm with the door closed. After 10 sec, the door was lifted, and tortoises could enter the decision chamber with arms 2 and 4 both open. Tortoises were given 2 min to make a decision (i.e., move beyond the threshold of one of the two open stimulus arms). Once tortoises had moved their whole body past the stimulus arm threshold, a magnetic door was placed behind them. Tortoises were given a maximum of 1 min in the chosen arm, then removed and placed back in their enclosure. Tortoises were removed from the arm before 1 min if they turned around and attempted to leave the arm by nudging or walking into the magnetic door that had closed behind them (a signal that they were choosing to try to leave the arm). Choice and latencies were still recorded for these trials. Tortoises were individually tested up to twice per day, with an interval of at least 30 min between first and second trials per tortoise to prevent testing fatigue. Each tortoise completed six to eight trials over four experimental days.

Preference test Phase 2: three-way competitive preference test

The three stimuli for the competitive preference test were labelled 'human interaction' – scratches and rubs on the shell by hands; 'food' – fresh pawpaw fruit segments (the same as phase 1); and an 'empty' arm. In the human interaction arm, the interactor was positioned kneeling at the end of the relevant arm, with hands extended just inside the maze arm, palm-side up and low to or resting on the floor.

Training

Phase 2 began with training trials as in Phase 1. Stimuli positions were again randomised between tortoises, but fixed for each individual for the duration of testing (Table 1). Training was consistent with the previous procedure: tortoises would be placed in start arm then allowed access to the decision chamber and one arm at a time. After entering and the arm, approaching the stimulus and interacting with it (including consuming the food, or receiving shell scratches and rubs from the human interactor for up to 1 min in the relevant arms), the tortoise would be immediately placed back into the start arm, then given access to the next arm (next stimulus), and so forth. Arm openings were randomised across training trials, so they did not always complete the same pattern of openings during training. Each tortoise completed at least six training trials; LT1 and LT3 completed seven. All five tortoises were again reasonably assumed to have learned the locations of each stimulus by the end of the training period.

Choice trials

Tortoises were placed in the start arm, then after 10 sec were given access to the decision chamber with all three choice arms open. Tortoises were given 2 min to move from the start arm into the decision chamber, and once in the decision chamber, the start arm door was closed behind them. Once in the decision chamber, tortoises were given 2 min to make a choice, by fully entering one of the three choice arms. As long as their whole body had not entered the choice arm, tortoises were allowed to retreat and return into the decision chamber to make another choice (this was counted as inspection or exploration of certain arms, but not a full choice). When tortoises were fully past the threshold of a choice arm, a magnetic door was placed behind them, and their choice was then recorded. Tortoises were allowed up to 30 sec to approach the stimulus, then given 1 min to interact with the stimulus, after which, they were returned to their enclosure. On trials that the human interaction was chosen, the interactor would wait for the tortoise to approach to within 15 cm of their hands, then move their hands slowly and calmly to scratch and rub the tortoise on the shell, for up to 1 min. This process was repeated twice per day per tortoise, with a break of at least 30 min between first and second trials per tortoise to prevent testing fatigue.

Data analysis

Video analysis was used to record key time measurements in each trial, using VLC media player with a timecode function. Key time measurements were: time spent in the decision chamber (i.e., latency to make a choice) - 'Latency to Choice' variable; time taken to approach stimulus (head within 10 cm of stimulus) - 'Latency to Stimulus' variable; and the combined total of these first two variables, labelled 'Total Choice Time' variable. All measurements were recorded in seconds and milliseconds.

Data were analysed using IBM SPSS Statistics Version 26. Natural log and square root transformations were used to normalise the distribution of the data and the residual variances. Multivariate ANOVA were performed on transformed data, including Latency to Choice, Latency to Stimulus, and Total Choice time as dependent variables; and Tortoise, Choice and Tortoise by Choice (the interaction term between Tortoise and Choice) as fixed factors. Non-parametric Fisher's exact tests were also used on variables that violated assumptions of normality or with non-normal variance distributions even after attempted transformation.

Results

Phase 1: Dichotomous choice

In Phase 1, tortoises completed at least six choice trials. Four of the five tortoises displayed a clear preference for the food arm; however, LT4 selected the food arm and the no stimulus (empty) arm four times each (Table 2).

Multivariate ANOVA (Table 3) indicated that the factor Tortoise affected Latency to Choice ($P=0.010$) and Total Choice Time ($P=0.002$), but there were no effects of Choice or Tortoise by Choice on these two variables (Choice: $P=0.328$ and $P=0.710$, respectively; Tortoise by Choice: $P=0.103$ and $P=0.211$, respectively). LSD pairwise comparisons showed that, for both dependent variables, LT3 was slower than all other tortoises ($P \leq 0.030$). There were no differences between each of the other four tortoises. Choice affected Latency to Stimulus ($P=0.048$), but no effects of Tortoise or Tortoise by Choice on Latency to Stimulus and Total Choice time ($P=0.406$ and $P=0.252$, respectively). Observations indicated that

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Table 2. Choice behaviour per tortoise in each phase of the preference test.

Choice (number of trials)					
	Phase 1		Phase 2		
Tortoise	Food	Empty	Food	Human	Empty
LT1	7	0	9	1	0
LT2	6	1	3	5	3
LT3	7	1	4	9	0
LT4	4	4	6	2	2
LT5	8	0	7	1	6

LT3 and LT4 did not consume the food on any of the test trials that they chose to enter the food arm. LT1, LT2 and LT5 consumed the entire food portion on all trials that they chose the food arm.

Phase 2: Competitive preference test

In phase 2, tortoises completed 10 to 14 trials. Three trials were abandoned (two for LT3 and one for LT4) because the tortoises did not move from the start box within the 2-min time limit.

Multivariate ANOVA (Table 4) indicated that that the factor Tortoise significantly affected Latency to Choice (P=0.025) but not Latency to Stimulus (P=0.157) nor Total Choice Time (P=0.079). For Latency to Choice, LSD pairwise comparisons showed LT4 was faster than LT3 and LT5 (P=0.006 and P=0.018, respectively), but all other tortoises did not significantly differ from each other. Choice and Tortoise by Choice interaction did not affect Latency to Choice (P=0.144 and 0.260, respectively). Choice significantly affected Latency to Stimulus (P<0.001), and Total Choice Time (P=0.039); however, there was also a significant Tortoise by Choice interaction for Latency to Stimulus (P=0.020). LSD comparisons for Total Choice Time indicated that the choice for Food was significantly faster than for Human (P=0.021), but no other choice comparisons were significantly different. Tortoise by Choice interaction did not affect Total Choice Time (P=0.206). A test for simple main effects (the interaction effects) using Fisher's LSD for the dependent variable Latency to Stimulus indicated that the effect of Choice at the level of each Tortoise was significant for only one tortoise: LT1 chose food faster than human (mean difference 20.71s, P<0.001). The simple main effects of Tortoise at the level of each Choice for Latency to Stimulus were: for food, LT1 was faster to food than LT2 (mean difference 2.15 sec, P=0.033); and for human, LT1 was slower to human than all other tortoises (mean differences LT2: 4.92 sec, P=0.048; LT3: 12.64 sec, P=0.002; LT4: 7.17 sec, P=0.034; and LT5: 8.13 sec, P=0.049), and LT2 was slower to human than LT3 (mean difference 1.78 sec, P=0.021). No other simple main effect comparisons were significant (P>0.05). As in Phase 1, LT3 and LT4 did not consume food in any of the trials that they chose to enter the food arm (6 and 4 respectively). LT1, LT2 and LT5 mostly consumed the food portion on each independent choice for the food arm, though there were a few trials that it was only partially consumed for each.

Table 3. Phase 1: Effect of tortoise and choice on the three choice behaviour variables measured, Latency to Choice, Latency to Stimulus, and Total Choice Time. [1]s.e.d. denotes standard error of difference. [2]P-values were calculated using F tests, df are presented in parentheses under each factor. [3]natural logarithmic transformation and (back-transformed) means presented. [a,b]denote LSD post-hoc groupings (unrelated groupings across variables).

Choice behaviour variable	Tortoise					Choice		s.e.d.[1]	P-value[2]		
	LT1	LT2	LT3	LT4	LT5	Food	Empty		Tortoise (4,29)	Choice (1,29)	Tortoise x Choice (2,29)
Latency to choice (s)[3]	3.12a (22.06)	3.22[a] (24.05)	3. 90[b] (40.40)	2.98[a] (19.46)	3.05[a] (20.63)	3.19 (23.39)	3.48 (29.57)	0.12	0.010	0.328	0.103
Latency to stimulus (s)[3]	2.52 (12.29)	2.40 (10.85)	2.68 (14.57)	2.39 (10.66)	2.57 (12.98)	2.61[a] (13.61)	2.32[b] (9.86)	0.09	0.406	0.048	0.252
Total choice time (s)[3]	3.54[a] (31.00)	3.56[a] (31.65)	4.22[b] (49.96)	3.39[a] (27.69)	3.51[a] (30.24)	3.62 (33.12)	3.76 (36.50)	0.10	0.002	0.710	0.211

Table 4. Phase 2: Effect of tortoise and choice on the three choice behaviour variables measured, Latency to Choice, Latency to Stimulus, and Total Choice Time. [1]s.e.d. denotes standard error of difference. [2]P-values were calculated using F tests, df are presented in parentheses under each factor. [3]square root transformation and (back-transformed) means presented. [4]natural logarithmic transformation and (back-transformed) means presented. [a,b]denote LSD post-hoc groupings (unrelated groupings across variables).

Choice behaviour variable	Tortoise					Choice			s.e.d.[1]	P-value[2]		
	LT1	LT2	LT3	LT4	LT5	Food	Human	Empty		Tortoise (4,45)	Choice (2,45)	Tortoise x Choice (6,45)
Latency to choice (s)[3]	59.55[a,b]	69.45[a,b]	92.15[a]	36.90[b]	89.39[a,b]	55.66	73.88	80.98	8.46	0.025	0.144	0.260
Latency to stimulus (s)[3]	5.35 (28.61)	4.58 (20.98)	4.02 (16.18)	4.02 (16.14)	4.06 (16.50)	3.74[a] (14.00)	5.42[b] (29.35)	3.64[a] (13.26)	0.22	0.157	<0.001	0.020
Total choice time (s)[3]	4.36 (54.60)	4.42 (56.74)	4.55 (61.40)	3.89 (40.09)	4.5 (59.58)	4.07[a] (45.34)	4.56[b] (61.99)	4.35[b] (54.50)	0.11	0.079	0.039	0.206

Discussion

The results of Phase 1 suggest that four of the tortoises had a clear preference for food over the empty arm, although LT4 made 50% of his choices each way, and hence, his choices were possibly random (not greater than chance). There were significant differences in the latencies of the tortoises, with LT3 slower than all other tortoises to make a choice, while each of the others had similar latencies. During initial training trials, both LT3 and LT4 tasted the food stimulus (pawpaw) but showed a clear disinterest in consuming it after the training trials, and neither consumed food in any of the test trials in both phases. LT5 and LT1, on the other hand, displayed a strong preference for the food. This suggests a very clear taste preference between the tortoises. LT5 is larger than the other tortoises by both weight and shell length. Zookeepers regarded LT5 as highly food-motivated, commenting that he ate for longer periods and consumed more food than his brothers (they were fed communally). LT2 showed a moderate-to-strong taste preference for the food. There is only limited research on taste preferences in reptiles, but there is evidence that taste preferences do vary between individuals, and that they can experience sensory taste pleasure and displeasure, inferred through flavour aversion learning tests (Paradis and Cabanac 2004). In all instances of food choice in Phase 1, LT1, LT2 and LT5 (who had a positive preference for the pawpaw) consumed all their food when the food arm was chosen.

Although LT3 was disinterested in consuming the food, he still chose the food arm on seven out of eight of his trials in Phase 1. There are multiple reasons why this tortoise may have chosen the non-preferred food over the empty arm, such as this tortoise having a possible side preference, or for inspective exploration (simply to check that nothing had changed) or inquisitive exploration (in which choice for either arm would itself be rewarding) (Wood-Gush and Vestergaard 1989; Keller et al. 1994; Boissy et al. 2007). Inquisitive exploration is what is often termed curiosity in animals, and affective exploration in human psychology (Keller et al. 1994). It is akin to a 'like', in that it is not to discover new survival-critical information, and it is not motivated by fear nor to reduce anxiety, but instead it is its own motivator and reward. The behaviour of inquisitive exploration satisfies the curiosity, which is hedonically rewarding, and has been linked to the release of pleasure-inducing neuro-transmitters and hormones in some species (Wood-Gush and Vestergaard 1989; Keller, et al. 1994; Inglis et al. 2001; Boissy et al. 2007).

LT4 split his trials evenly between food and empty arms, and his disinterest in consuming the food carried over successive trials. LT4 was also anecdotally considered by zookeepers and the primary observer to be bolder than the others and more curious, i.e., more interested in inspective and/or inquisitive exploration in most circumstances, such as when the zookeepers would take the tortoises outside to roam on grass lawns nearby their enclosure space. This inquisitive (curious) pattern of behaviour may explain LT4's consistent choice for the empty arm across both experimental phases. It may also have been attributable to attempts to escape the maze, although behaviours indicative of escape attempts (such as climbing or pushing over maze walls) were not observed.

In Phase 2, choices were far more varied between the tortoises. However, LT1 chose food in nine out of 10 trials, and LT3 chose human interaction in nine out of 13 trials, indicative of a clear preference directed towards one stimulus. On average, LT1 spent almost double the amount of time in the decision chamber (Latency to Choice) when his choice was for the human interaction as compared to the food arm. A similar latency pattern for arm selection occurred for other tortoises. For LT2, the average latency to select the empty arm was almost double that to select the food and human interaction arms. For LT3, the average latency to select the food arm was almost double that of selecting the human interaction arm. For LT5, the average latency of selecting the human interaction arm and the empty arm were almost triple and double (respectively) of selecting the food arm. LT4, in contrast to the others, spent a comparable amount of time in the decision chamber no matter which stimulus arm ended up being chosen, and none of LT4's average latencies were over 1 min.

In human interaction choice trials, each tortoise, except LT1, moved as close as possible to the human (right to the end of the arm) and then remained motionless; some also wiggled the rear of their shells to facilitate better scratching and rubbing, possibly indicating that these four tortoises considered the shell scratching and rubbing pleasurable and positively rewarding. LT1 only chose the human interaction arm once, but was hesitant to approach the human at the end of the arm, as shown by his near maximum approach latency for that single trial. In contrast, LT2 and LT3 showed a preference for the human interaction arm and displayed both close approach and shell wiggling behaviours. Alba et al. (2017) found that during a cognitive training task run by zookeepers, the eastern box turtles *Terrapene carolina carolina* in their study were somewhat motivated to approach and interact with the keepers during the task. This led to the keepers reporting higher degrees of bonding with the tortoises, and increased visitor interest and attraction to the tortoise enclosure during the on-display training sessions. Likewise, Mehrkam and Dorey (2014) and Learmonth et al. (2020) found that some individual giant tortoises were similarly highly motivated to interact with humans during preference tests. Therefore, it seems that individuals across many species of tortoise may indeed prefer human interaction in some circumstances.

The three tortoises that entered the empty arm at least once all spent longer than 30 sec exploring the arm on average. This may simply be inspective exploration; however, all the tortoises had previously had multiple exposures to the empty arm in both phases, so perhaps it was engaging in inquisitive exploration that was interesting or rewarding to those tortoises in an internal, imperceptible way. In any preference or behavioural demand testing, variation in the choices made by animals is expected (Duncan 1978; Hemsworth et al. 2011; Franks 2019). Inspective exploration is an evolutionary imperative that motivates an animal to keep checking whether their environment has changed, even in the absence of obvious, dramatic changes (Inglis et al. 2001; Boissy et al. 2007). Hence the variation in choices by the tortoises observed here may have simply reflected the need be aware of the maze environment. The exploration choices may also be attributable to high physiological satiety or low levels of hunger at times of testing for these tortoises. Perhaps the tortoises' choices for the empty arm were also to deliberately avoid human interaction, as they were not in a positive or affiliative state or mood. These are all extraneous factors that are difficult to control that may impact immediate choices during testing. Furthermore, the immediate level of motivation to access each of the two arms may be in conflict, causing cognitive confusion for the animal, leading it to make a less preferred choice (Franks 2019).

One of the main limitations of this current study was the extended time period that was necessary to gather sufficient choice data. This research was conducted over a nine-month period, at every available opportunity within the zoo's operating procedures and requirements. Unfortunately, as a slow-moving species, these tortoises were tested as efficiently as possible. It is suggested that future research examining tortoise preferences may be able to adjust testing periods accordingly, to be able to gather more data (i.e., conduct more choice trials).

Conclusion

This two-phase preference test attempted to determine: 1) if leopard tortoises could make choices; and 2) if they would prefer human interaction over other stimuli. To some degree this aim was achieved, as indeed LT2 and LT3 chose human interaction significantly more times than the other stimuli. However, more research is needed to demonstrate the efficacy of human interaction as a positive and desirable enrichment or reward for this (and other) tortoise species. Based on preference research with other tortoise species (Mehrkam and Dorey 2014; Learmonth et al. 2020), it may be cautiously concluded that some individuals of this species of tortoise may indeed enjoy and prefer this form of human interaction over other stimuli in certain conditions. These results may assist in informing future enrichment and husbandry plans for many tortoise species, such as providing regular human-animal interaction opportunities with both zookeepers and unfamiliar zoo visitors/guests, that the tortoises may choose to participate in. However, individual preferences must first be observed, as these results suggest that preferences were highly individual-specific, and not all tortoises were motivated to interact with a human in this experiment.

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