Does enrichment improve reptile welfare? Leopard geckos (*Eublepharis macularius*) respond to five types of environmental enrichment

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A B S T R A C T

Animal welfare is a high priority for pet owners and accredited zoos and aquariums. Current approaches to measuring welfare focus on identifying consensus among behavioral and physiological indicators of positive and negative emotions. Environmental enrichment is a common strategy used to improve the welfare of captive animals. In enrichment programs, knowledge of an animal's ecology and individual history are applied to modify the animal's current environment and management to increase environmental complexity, make the environment more functional or natural, and increase behavioral opportunities. While enrichment techniques for primates and large mammals are well-studied, reptile enrichment has received little attention to date despite a few promising studies. In this study, we monitored the responses of 16 leopard geckos to five types of enrichment (Thermal, Feeding, Olfactory, Object, and Visual) using a repeated-measures design. We measured both specific behaviors we expected to change in response to each enrichment type and four behavioral indicators of welfare: exploratory behavior, species-specific behaviors (behavioral thermoregulation and hunting), behavioral diversity, and abnormal repetitive behaviors. We found geckos interacted with all five types of enrichment at above-chance levels (i.e., no 95% CIs for engagement time overlapped with 0 s). Geckos spent more time interacting with Thermal and Feeding enrichment than the other types (F(4,60) = 49.84, p < 0.001). Thermal, Feeding, Olfactory, and Object enrichments (but not Visual enrichment) changed specific relevant behaviors (e.g., Thermal enrichment altered thermoregulatory behaviors, Wilk's lambda = 0.25, F(3,13) = 13.39, p < 0.001) and improved behavioral indicators of welfare (e.g., behavioral diversity, Wilks' lambda = 0.30, F(12,178) = 12.31, p < 0.001). These results suggest that geckos respond to environmental enrichment, that their responses are predictable based on their ecology, and that environmental enrichment improves gecko welfare. As in mammals and birds, enrichments that address behavioral needs (here: thermoregulation and feeding) appear more effective than enrichments that simply provide novel stimuli to increase exploration. The extent to which our results can be generalized to other reptile species awaits further study, but we suggest that enrichment should be more widely used to improve reptile welfare.

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1. Introduction

What is animal welfare and how can we measure it? Modern welfare concepts describe a continuum from a "Life Worth Avoiding" to a "Life Worth Living" (Yeates, 2011) based on the balance of the animal's pleasant and unpleasant emotional experiences (McMillan, 2007). Welfare is a characteristic of the animal-environment interaction, raising three key ideas for welfare science. First, welfare is determined by the individual animal's perception of the environment, which is influenced by that individual's previous experiences (Harding et al., 2004; Veissier and Boissy, 2007), temperament (Izzo et al., 2011), and evolutionary history (Clubb and Mason, 2003). This implies two individuals exposed to the same environment may not necessarily have the same welfare (Hill and Broom, 2009). Second, good welfare requires overcoming challenges presented by the environment. By this standard, animals who are not challenged (Mason et al., 2007) or who are insurmountably challenged cannot achieve good welfare (Hill and Broom, 2009). Third, welfare can be improved by changing the environment or the animal's perception of the environment. Four aspects of the environment are correlated with animal welfare: how well it functions like the natural world (Fraser, 2009; Shepherdson, 2010),

its complexity (Bassett and Buchanan-Smith, 2007), the opportunities for animals to have control and choice (Boissy et al., 2007), and how well it meets species-specific and individual needs (Kagan and Veasey, 2010). In particular, successful performance of highly-motivated, species-specific behaviors increases welfare (Duncan, 1998) by increasing coping ability (Boissy et al., 2007).

Measuring welfare has proven complex. Since animals may react differently to the same environment, measuring individual animals' responses to the environment (i.e., using animal-based measures) is preferable to measuring the environment itself. Further, no single measure directly corresponds to an animal's welfare state and different measures do not always yield the same welfare evaluation (Mason and Mendl, 1993). The primary measure of poor welfare has been the rate of abnormal repetitive behaviors (ARB), but even if welfare is improved established ARB may never be fully eliminated (Mason et al., 2007). Measures of good welfare include exploration, play, species-specific behaviors, and behavioral diversity, but these may also be affected by differences in temperament (Izzo et al., 2011). Therefore, current best practice is to assess welfare using multiple animal-based measures and to look for agreement among them (Bassett and Buchanan-Smith, 2007; Mason and Mendl, 1993; Shepherdson, 2010).

Efforts to improve captive animal welfare have primarily focused on environmental enrichment (hereafter enrichment), in which knowledge of an animal's ecology and individual history are applied to modify the animal's current environment or management to achieve a behavioral outcome (Mellen and MacPhee, 2001), e.g. adding novel stimuli to increase exploration. Using enrichment to improve captive animal welfare has been approached in two different ways (Swaisgood and Shepherdson, 2005). The first approach is to increase overall environmental complexity. For example, simultaneously introducing novel objects, shelters, and climbing devices into barren cages during rat (*Rattus norvegicus*) husbandry (Abou-Ismail et al., 2010). Alternatively, enrichment can be implemented using a goal-directed, iterative approach: first identifying behaviors to change, then implementing enrichment and assessing how well it changes the target behaviors, and subsequently modifying the enrichment to improve its effectiveness (Mellen and MacPhee, 2001). For example, presenting novel scents in different locations increased locomotion and time that zoo-housed giraffe (*Giraffa camelopardalis*) spent in underutilized parts of the exhibit (Fay and Miller, 2015).

Enrichment has been documented to improve the welfare of mammals, some birds, and turtles and tortoises (Burghardt et al., 1996; Mehrkam and Dorey, 2014; Therrien et al., 2007) but other taxa have not been well-studied (Burghardt, 2013; Swaisgood and Shepherdson, 2005). Some evidence suggests reptiles benefit from enrichment (Manrod et al., 2008). For example, living in more complex environments improved rat snakes' (*Elaphe*: Almli and Burghardt, 2006) and Eastern water skinks' (*Eulamprus quoyii*: Noble et al., 2012) performance on some cognitive tasks, and eastern box turtles (*Terrapene carolina carolina*) showed a preference for an enriched environment over a barren one (Case et al., 2005). However, other evidence shows no effect of enrichment on reptile behavior (Rosier and Langkilde, 2011). In the current study, we used leopard geckos (*Eublepharis macularius*) as a model species to determine if enrichment could improve lizard welfare. Leopard geckos are small nocturnal lizards native to deserts of the Middle East that are commonly kept in captivity both as pets and in zoos (Patula, 2009; Spiess, 2010; Woods, 2001). They are ectothermic, opportunistic carnivores who use vision and chemoreception as primary senses (Schwenk, 1993; Spiess, 2010). Captive males defend territories by scent-marking, visual display, and aggression (Indiviglio, 2007; Rhen and Crews, 2001; Sakata et al., 2002). Combining these characteristics with Swaisgood & Shepherdson's "categories of enrichment" (2005, p. 503), we provided five different enrichment types: thermal, feeding, visual, olfactory, and object.

This study aimed to answer three questions. 1. Do geckos interact with enrichment, and if so, how much? 2. How do geckos respond to enrichment? Are their responses predictable based on their ecological niche, and therefore similar to small carnivorous mammals' responses? Towards this aim, we posited specific hypotheses about each enrichment category outlined in Table 1. 3. Do any of the enrichment categories tested change gecko welfare? Non-invasive physiological measures of welfare have not yet been validated for geckos and collection of body temperature has been demonstrated to be stressful for lizards (Cabanac and Gosselin, 1993). We therefore used four behavioral indicators to assess welfare: exploration, species-specific behaviors (thermoregulation, hunting), behavioral diversity, and ARB. Improved welfare was assumed if exploration increased, time spent engaged in species-specific behavior increased, behavioral diversity increased, or the frequency of ARBs decreased in the presence of enrichment.

2. Methods

2.1. Subjects

Sixteen never-bred adult male leopard geckos (*Eublepharis macularius*) were used in the experiment. Beginning prior to sexual maturity and throughout this study, each animal was individually housed in the Vivarium at Franklin & Marshall College in a plastic tub (35.5 cm wide, 55.9 cm deep, 20.3 cm high) topped with a metal grate. A wet hide (clear polyethylene storage container (Glad Products Co., Oakland, CA, USA), with a door cut out and a wet paper towel inside) was placed beneath a heat lamp at the back corner of the tub, while an opaque dry hide was placed in the opposite corner of the tub. The geckos were maintained on a consistent 12-h reversed day/night cycle (lights on at 20:00 h and off at 08:00 h), were fed crickets or mealworms three times per week, had constant water access, and were handled weekly during cage cleaning and weighing. This project was approved by the Institutional Animal Care and Use Committee of Franklin & Marshall College (#2011-4).

2.2. Design

Each animal experienced six two-week baseline conditions (A) in which no enrichment was provided and six two-week enriched conditions (E) following an AEEA AEEA AEEA pattern. Six to ten weeks elapsed between consecutive baselines. We assigned the order of enrichments for the first 6 geckos using a Latin Square design to ensure an equal number of animals received each enrichment treatment first, second, third, etc. Counterbalancing to control for order effects within each AEEA block created twelve additional combinations, each consisting of a unique sequence of enrichment treatments. For example, if one gecko had feeding followed by olfactory in the first block, another gecko would have the opposite pairing in the same block. Because only 16 geckos were available, 10 of these 12 sequences were chosen at random and assigned to the remaining 10 geckos.

Within each two-week baseline session or enrichment treatment, each gecko was recorded three times per week using a low-light HD video camera (Flip Ultra HD, Cisco Systems, San Jose, CA, USA). Recordings occurred between 09:00 h and 16:00 when overhead lights were off; heat lamps were the only light source. During enrichment treatments, geckos were recorded for the entire time they had access to enrichment items (45 min/session) so that any unforeseen health effects of enrichment would be documented (no such effects occurred during the study). Baseline recordings were time-matched to enrichment sessions. To control for time

Table 1
Tests of Hypothesized Effects of Environmental Enrichment on Gecko Behavior.

Enrichment Category	Prediction (Based on species, source)	Supported?	Specific Results
Feeding	Increased exploratory behavior (fishing cat, *Felis viverrina*, & leopard cats, *F. bengalensis*, Shepherdson et al., 1993; bush dogs, *Speothos venaticus*, Ings et al., 1997)	Yes for all categories	Locomotor exploration: Wilk's $\Lambda = 0.24$, $F_{(2,14)} = 21.64$, $p < 0.001$, $Eta^2 = 0.76$ Increased% time walking: $F_{(1,15)} = 31.20$, $p < 0.001$, $Eta^2 = 0.67$ Increased% time climbing: $F_{(1,15)} = 16.35$, $p = 0.001$, $Eta^2 = 0.52$ No change in% time burrowing Object exploration: Wilk's $\Lambda = 0.26$, $F_{(1,15)} = 43.07$, $p < 0.001$, $Eta^2 = 0.74$ Increased% time manipulating objects Olfactory exploration: Wilk's $\Lambda = 0.18$, $F_{(3,13)} = 19.17$, $p < 0.001$, $Eta^2 = 0.82$ Increased% time sniffing: $F_{(1,15)} = 18.02$, $p = 0.001$, $Eta^2 = 0.55$ Increased lip-licking rate: $F_{(1,15)} = 15.79$, $p = 0.001$, $Eta^2 = 0.51$ Increased tongue-touching rate: $F_{(1,15)} = 28.61$, $p < 0.001$, $Eta^2 = 0.66$
	Increased behavioral diversity (fishing & leopard cats, Shepherdson et al., 1993)	Yes	Overall diversity: Wilk's $\Lambda = 0.08$, $F_{(2,14)} = 76.91$, $p < 0.001$, $Eta^2 = 0.92$ Increased $H_{duration}$: $F_{(1,15)} = 106.40$, $p < 0.001$, $Eta^2 = 0.88$ Increased H_{rate}: $F_{(1,15)} = 161.31$, $p < 0.001$, $Eta^2 = 0.92$
	Increased foraging behaviors (fishing cat, Shepherdson et al., 1993; lions, *Panthera leo*, & Sumatran tigers, *P. tigris*, Bashaw et al., 2003)	Yes	Overall foraging: Wilk's $\Lambda = 0.19$, $F_{(6,10)} = 7.35$, $p = 0.003$, $Eta^2 = 0.81$ Increased stalking rate: $F_{(1,15)} = 46.41$, $p < 0.001$, $Eta^2 = 0.76$, Increased watching rate: $F_{(1,15)} = 27.71$, $p < 0.001$, $Eta^2 = 0.65$ Increased lunging rate: $F_{(1,15)} = 20.83$, $p < 0.001$, $Eta^2 = 0.58$ Trend: increase in biting rate: $F_{(1,15)} = 8.43$, $p = 0.011$, $Eta^2 = 0.36$ Trend: increase in tail writhe rate: $F_{(1,15)} = 5.29$, $p = 0.036$, $Eta^2 = 0.26$ No significant change in rushing rate
	Decreased ARB (leopard cats, Shepherdson et al., 1993; lions & tigers, Bashaw et al., 2003)	No	Overall ARB: Wilk's $\Lambda = 0.93$, $F_{(2,14)} = 0.56$, $p = 0.58$, $Eta^2 = 0.07$ No significant change in% time pacing or in other ARB
Thermal	Altered thermoregulatory behaviors (leopard geckos, Autumn and De Nardo, 1995; Indiviglio, 2007)	Yes	Overall thermoregulatory: Wilk's $\Lambda = 0.25$, $F_{(3,13)} = 13.39$, $p < 0.001$, $Eta^2 = 0.75$ Decreased% time basking: $F_{(1,15)} = 10.32$, $p = 0.006$, $Eta^2 = 0.41$ Increased% time in shade: $F_{(1,15)} = 34.68$, $p < 0.001$, $Eta^2 = 0.70$ No change in% time contact basking
	Decreased ARB (turtles, *Caretta caretta*, *Chelonia mydas*, Therrien et al., 2007)	No	Overall ARB: Wilk's $\Lambda = 0.79$ $F_{(2,14)} = 1.85$, $p = 0.19$ $Eta^2 = 0.21$ No change in% time pacing or in other ARB
Olfactory	Increased exploratory behaviors (black-footed cat, *F. nigripes*, Wells and Egli, 2004; cheetah, *Acinonyx jubatus*, Quirke and O'Riordan, 2011)	Yes for Locomotor and Object, Trend for Olfactory	Locomotor exploration: Wilk's $\Lambda = 0.46$, $F_{(2,14)} = 8.27$, $p = 0.004$, $Eta^2 = 0.54$ Increased% time walking: $F_{(1,15)} = 9.54$, $p = 0.007$, $Eta^2 = 0.39$ No change in% time climbing or burrowing Object exploration: Wilk's $\Lambda = 0.37$, $F_{(1,15)} = 25.61$, $p < 0.001$, $Eta^2 = 0.63$ Increased% time manipulating objects Olfactory exploration: Wilk's $\Lambda = 0.52$, $F_{(3,13)} = 3.93$, $p = 0.031$, $Eta^2 = 0.48$ Increased tongue-touching rate: $F_{(1,15)} = 12.72$, $p = 0.003$, $Eta^2 = 0.46$ Trend: increased% time sniffing: $F_{(1,15)} = 5.99$, $p = 0.027$, $Eta^2 = 0.29$ Trend: increased lip-licking rate: $F_{(1,15)} = 6.81$, $p = 0.020$, $Eta^2 = 0.31$
	Decreased sedentary behavior (black-footed cat, Wells and Egli, 2004)	No	Overall sedentary: Wilk's $\Lambda = 0.41$, $F_{(5,11)} = 3.18$, $p = 0.051$, $Eta^2 = 0.59$ No change in% time resting, rest/awake, basking, shade, or contact basking
Object	Increase in exploratory behavior (lion, Powell, 1995; domestic cats, *F. catus*, Machado and Genaro, 2014), especially interaction with object (bottlenose dolphins, *Tursiops truncatus*, Delfour and Beyer, 2012)	Yes for all categories	Locomotor exploration: Wilk's $\Lambda = 0.27$, $F_{(2,14)} = 18.94$, $p < 0.001$, $Eta^2 = 0.73$ Increased% time walking: $F_{(1,15)} = 39.06$, $p < 0.001$, $Eta^2 = 0.72$ No change in% time climbing or burrowing Object exploration: Wilk's $\Lambda = 0.54$, $F_{(1,15)} = 12.91$, $p = 0.003$, $Eta^2 = 0.46$ Increased% time manipulating objects Olfactory exploration: Wilk's $\Lambda = 0.24$, $F_{(3,13)} = 13.63$, $p < 0.001$, $Eta^2 = 0.76$ Increased tongue-touching rate: $F_{(1,15)} = 26.94$, $p < 0.001$, $Eta^2 = 0.64$ Increased lip-licking rate: $F_{(1,15)} = 8.86$, $p = 0.009$, $Eta^2 = 0.37$ Trend: increased% time sniffing: $F_{(1,15)} = 6.67$, $p = 0.021$, $Eta^2 = 0.31$
Visual	If reflection results in fear, increased avoidance (leopard geckos, Indiviglio, 2007; Sakata et al., 2002)	No	Overall avoidance: Wilk's $\Lambda = 0.90$, $F_{(3,13)} = 0.47$, $p = 0.71$, $Eta^2 = 0.10$ No significant effect on flee rate or% time in wet or dry hide Running was not observed in these conditions
	If reflection viewed as conspecific, increased social behavior (leopard geckos, Indiviglio, 2007; Rhen and Crews, 2001; Sakata et al., 2002)	No	Overall social behavior: Wilk's $\Lambda = 0.71$, $F_{(2,14)} = 2.91$, $p = 0.09$, $Eta^2 = 0.29$ No effect on push-up or lateral orientation Scent mark, face off, gape, high-posture, and expansion displays not observed.

of day effects on behavior, there was at least one morning and one afternoon recording per gecko during each week and recording times were kept consistent (+/− 30 min) for each individual throughout the study.

2.3. Enrichment treatments

Two enrichment items from each of five enrichment categories (Feeding, Thermal, Olfactory, Object, and Visual) were presented to each gecko. Within each enrichment treatment, we provided enrichment items in the home cage in a way designed to minimize habituation (Tarou and Bashaw, 2007). One of the two items in each category was presented to the gecko in its home cage for 45 min and then removed. At least 15 h elapsed before the next item was presented. The two items from each enrichment category were alternated, and each item was presented three times for a total of six recording sessions/enrichment presentations per category. We also completed a sixth treatment in which geckos were removed from their home cage and gently handled by an experimenter. Because geckos could avoid enrichment items placed in their home cage but could not avoid engaging with the handler, behavioral effects of the handling condition are not comparable to the other enrichment conditions. The handling treatment was therefore excluded from this report.

In the Visual enrichment treatment, animals were presented with a mirror; on alternating presentations it was reflective-side out (so they could ostensibly see their reflection) and back-side out (no reflection). In the Olfactory enrichment treatment, animals were presented with one of two scented blocks. Scented blocks were created by muddling one teaspoon of mint or snake skin with 0.5 cup of water and brushing the mixture evenly onto a 7.5 cm by 7.5 cm piece of plywood, which we air-dried overnight. To control for the deposition of the lizards' own scent and changes in concentration over time, each scent block was used once and then discarded. In the Feeding enrichment treatment, animals were presented with one of two puzzle feeders. The first was the PetSafe FUNKitty Egg-cersizer Cat Toy (Radio Systems Corp., Knoxville, TN, USA), and the second was a 30.5 cm long and 4.4 cm inner diameter clear PVC pipe, capped on one end, with a 1.27 cm hole drilled in the side. Both toys were translucent and filled with 4 live crickets from the animals' regular diet. Any insects remaining in the puzzle feeder after the end of the recording were released into the lizards' enclosure as during a typical feeding. In the Object enrichment treatment, animals were presented with one of two rubber dog toys, either a 5.1 cm diameter ball or a 2.5 cm diameter by 7.5 cm long tube (both Kong Company, Golden, CO, USA). In the Thermal enrichment treatment, animals were presented with one of two types of perching. Branched sticks (approximately 2.5 cm in diameter and 25 cm long) or the Flexi Trail (Zoo-Max, Saint-Hyacinthe, QC, CA), a 15.0 cm by 55.9 cm flexible "bridge" made of 21 wood slats linked tightly together with bendable wire. Both thermal items were placed under the heat lamp in the corner of the home cage and provided both climbing opportunities and shade.

2.4. Data collection

Pilot observations indicated it took about 3 min following the departure of the experimenter for an interrupted behavior to resume, so we began coding every 45-min video at minute 5 and ended coding at minute 15. This 10-min segment was coded using continuous focal sampling on Observer XT software (Noldus, Wageningen, Netherlands). During coding, observers recorded the onset and offset of 23 state behaviors (e.g., walking, resting; Table 2) and noted all occurrences and targets of 14 event behaviors (e.g., tongue-touch to an object, stalk a prey item; Table 2). We also con-

tinuously recorded whether or not the gecko was engaged with an enrichment item (i.e., touching, sniffing, or looking directly at it).

The first author trained three additional coders by first coding videos together (≥eight videos, including at least one from each enrichment condition) and then coding additional videos independently and reviewing them together (≥12 videos, including at least one from each enrichment condition). Finally, each coder independently coded six videos (1 h, including at least four different conditions) for reliability assessment. Using a 3-s tolerance for duration measures, observers achieved good reliability (Kappa: Min = 0.68, Mean = 0.71, r_s: Min = 0.93, Mean = 0.96, Percent agreement: Min = 78%, Mean = 80%). To control for remaining inter-observer differences, the same coder scored every video for a particular gecko. To control for intra-observer drift, a coder watched the first video from every condition for a gecko, then the second video from every condition for the same gecko, and continued until that gecko's videos were completed before moving on to the next gecko.

2.5. Statistical analysis

2.5.1. Data summary

For state behaviors, both percent of each 10-min video engaged in that behavior and rate per min were recorded. For event behaviors, only rate per min was recorded. Because climb, autogroom, burrow, and "other" behaviors (those not described on the ethogram) could be normal or abnormal depending on their duration and successfulness (e.g., continued attempts to interact with cage barriers is an indicator of poor welfare (Warwick et al., 2013)), each instance of these behaviors lasting longer than 20 s was reviewed a second time. If the behavior was successful, e.g. a slow climb to the top of a hide, then it was left as a climb. If the behavior was unsuccessful, e.g. repeatedly attempting to climb out of the home cage and falling, then that instance of the behavior was recorded as an ARB rather than a climb. Two measures of behavioral diversity were then calculated for each 10-min video using the Shannon Index (H: (Shannon, 1948)), one based on the durations of behaviors in that video ($H_{Duration}$, included only state behaviors) and one on the rates (H_{Rate}, included all behaviors). Each behavior (and behavioral diversity) was then averaged across the six videos for each treatment, such that average duration (states) or rate (events) for each behavior for each enrichment treatment category for each gecko was the unit of analysis.

Individual behaviors were categorized for the purpose of evaluating how well enrichment met specific behavioral goals and improved welfare (Table 2). These categories were based on descriptions of welfare assessment in mammals (Whitham and Wielebnowski, 2013), welfare assessment in reptiles (Warwick et al., 2013), and the behavior of captive leopard geckos (Indiviglio, 2007; Rhen and Crews, 2001; Sakata et al., 2002).

2.5.2. Data screening

Of the 37 behaviors on the ethogram, only 29 were observed during data collection. Before comparing baseline to behavior during the enrichment treatment, we used MANOVA to determine whether any behaviors differed significantly across the six baseline periods. Only one of the 29 observed behaviors ("Wet Hide Not Visible") showed a significant difference using alpha = 0.05, a result consistent with what is expected by chance (29 tests * 0.05 = 1.45 tests expected to be significant by chance). Consequently, we averaged all 6 baseline values together for each animal to create a single baseline average. We then assessed normality for the behaviors included in our hypotheses; many of these behaviors were significantly positively skewed because of one or two outlying animals. MANOVA is robust to violations of normality (Mertler and Vannatta, 2013), repeated-measures analysis allows each animal to serve as

Table 2
Behavioral Categories Used for Analyses.

Category	Sub-category	Behavior	Type	Definition
Exploration	Locomotor	Walk	State	Moving around enclosure at slow speed in no fixed pattern.
		Climb	State	Moving (or attempting to move) up or down a vertical or sloped surface.
		Burrow	State	Animal pushes its head or body into a crevice or under an item.
	Olfactory	Sniff	State	Moving closer to and further from a surface while in/exhaling, no tongue extrusion.
		Tongue Air	Event	Extruding and retracting the tongue, not touching an object or substrate.
		Tongue Touch	Event	Extruding and retracting the tongue and touching an object or substrate.
	Object	Object Manipulate	State	Pawing, rubbing, or holding an object or item of cage furniture. Includes placing 1 or 2 feet on the object.
Species-Specific Behavior	Thermo-regulatory	Bask	State	Resting or rest/awake directly under heat lamp.
		Contact Bask	State	Flattening dorsal surface against a heated substrate, usually settling backwards.
		Shade	State	Resting or rest/awake with at least 2/3 of its body in the shadow of an object or substrate.
	Foraging	Watch	Event	Focusing eyes directly on target, body motionless, head-on posture.
		Stalk	Event	Slowly approaching target on which its attention is focused.
		Tail Writhe	Event	Moving the distal portion of its tail in quivering motion.
		Lunge	Event	Rapidly propelling the whole body < 1 body length directly at a target.
		Rush	Event	Running full speed for > one body length directly at a target.
		Bite	Event	Closing jaws forcefully on target.
Abnormal Repetitive Behavior		Pace	State	Moving around enclosure on a set path, at least two repetitions required.
		Other ARB	State	Climb, autogroom, scratch, burrow, or behavior not described on the ethogram lasting more than 20 s and unsuccessful, e.g. repeatedly attempting to climb out of the cage.
Sedentary Behavior		Rest	State	Lying with eyes closed or not visible.
		Rest/Awake	State	Lying or standing with eyes open, not engaged in any active behavior.
				Bask, Contact Bask, and Shade also included in this category; definitions under thermoregulatory.
Avoidance		Flee	State	Moving rapidly away from a stimulus.
		In Wet Hide	State	Concealed in wet hide, face not visible to observer.
		In Dry Hide	State	Concealed in dry hide, face not visible to observer.
Social Behavior		Head Bob	Event	Lifting and lowering upper body and/or head repeatedly.
		Lateral Orient/Display	State	Turning body sagittal plane to target.
				Scent mark, face off, gape, high-posture, and expansion displays originally included, but were not observed.

its own control, and we considered these outlying cases representative of how a minority of geckos responds to captivity. As a result, we elected to continue without transforming the data.

2.5.3. Hypothesis tests

To address whether geckos would attend to enrichment items in each treatment category for a significant percent of the session, confidence intervals were calculated for the percent of time spent engaged with items in the enrichment categories. We then used a repeated-measures ANOVA to compare percent of time engaged across enrichment treatment categories.

To address each specific enrichment prediction hypothesis, we used a repeated-measures MANOVA to compare the combined baseline to a single enrichment category using the rates or percent time for each behavior relevant to that hypothesis as dependent measures (Ethogram: Table 2). We considered all MANOVAs on a single enrichment category to be in the same family and accordingly used Bonferroni-corrected alphas for these MANOVAs (e.g., for object enrichment, the 3 MANOVAs were each evaluated at alpha = 0.05/3 = 0.017). Each significant MANOVA was followed by univariate ANOVAs using Bonferroni-corrected alphas. We used $p < 0.05$ throughout. When Bonferroni correction

dictated a lower alpha, we identified findings where the Bonferroni-corrected alpha $< p < 0.05$ as non-significant trends.

To determine the effects of enrichment on welfare measures, we ran a repeated-measures MANOVA for each welfare measure that compared all five enrichment treatments and the combined baseline for relevant behaviors. Each significant MANOVA was followed by univariate ANOVAs and each significant ANOVA by pairwise comparisons among conditions, using Bonferroni corrections throughout. When the sphericity assumption was met, we report Wilks' lambda. When it was violated, we report Pillai's trace and use a Greenhouse-Geisser adjustment for subsequent ANOVAs. All analyses were conducted in SPSS 20 (IBM Corp., Armonk, NY, USA). Descriptive statistics are reported as Mean ± SD.

2.5.4. Scaling for graphs

For all graphs, axes were scaled relative to baseline so durations (of state behaviors) and rates (of event behaviors) could be included in the same figure. We scaled these values by dividing the average and standard error of enrichment treatment durations or rates by the appropriate baseline average. As a result, a value of 2 on the y-axis for a behavior indicates that the behavior occurred for twice as long (states) or twice as often (events) in that enrichment treatment than in baseline observations.
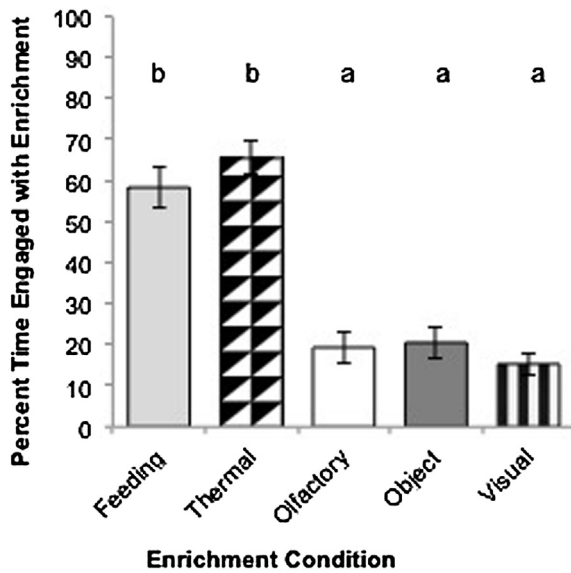
Fig. 1. Percent of observation time geckos spent engaged with enrichment items in each category. Error bars show standard errors of the mean.

3. Results

3.1. Use of enrichment

Geckos spent a significant amount of time engaged with items in every enrichment treatment category (i.e., no confidence intervals for time spent engaged with enrichment treatment included zero, Fig. 1). Excluding the baseline, repeated-measures ANOVA revealed a significant overall effect of enrichment category on time spent engaged $F(4,60) = 49.84$, $p < 0.001$, $\eta^2 = 0.77$. Bonferroni post hoc tests revealed that enrichment engagement could be divided into two tiers: geckos engaged with Feeding and Thermal items for the majority of each session, and with Olfactory, Object, and Visual enrichment for a lesser portion of the session (between tiers all p's < 0.001, within tiers all p's = 1.00, Fig. 1).

3.2. Specific predictions for each enrichment type

Most, but not all, of our specific predictions based on mammalian responses to enrichment or reptile ecology were supported (for detailed results, see Table 1). Feeding enrichment significantly increased all three types of exploratory behaviors (i.e., locomotor, olfactory, and object), foraging, and behavioral diversity. However, Feeding enrichment had no significant effect on ARB. Thermal enrichment significantly altered thermoregulatory behaviors, but like Feeding enrichment, had no significant effect on ARB. Olfactory enrichment resulted in a significant increase in locomotor and object exploration, a trend toward an increase in olfactory exploration (p = 0.031, Bonferroni corrected alpha = 0.012), but no significant effect on sedentary behavior. Object enrichment significantly increased all three types of exploratory behaviors. Visual enrichment had no significant effects on social behavior or avoidance. In case the ineffectiveness of Visual enrichment was an artifact of averaging responses to the reflective and non-reflective sides of the mirror, we used two additional repeated-measures MANOVAs to compare social behavior and avoidance among presentations of the two Visual items and Baseline. Both MANOVAs yielded no significant effect (ps ≥ 0.179).
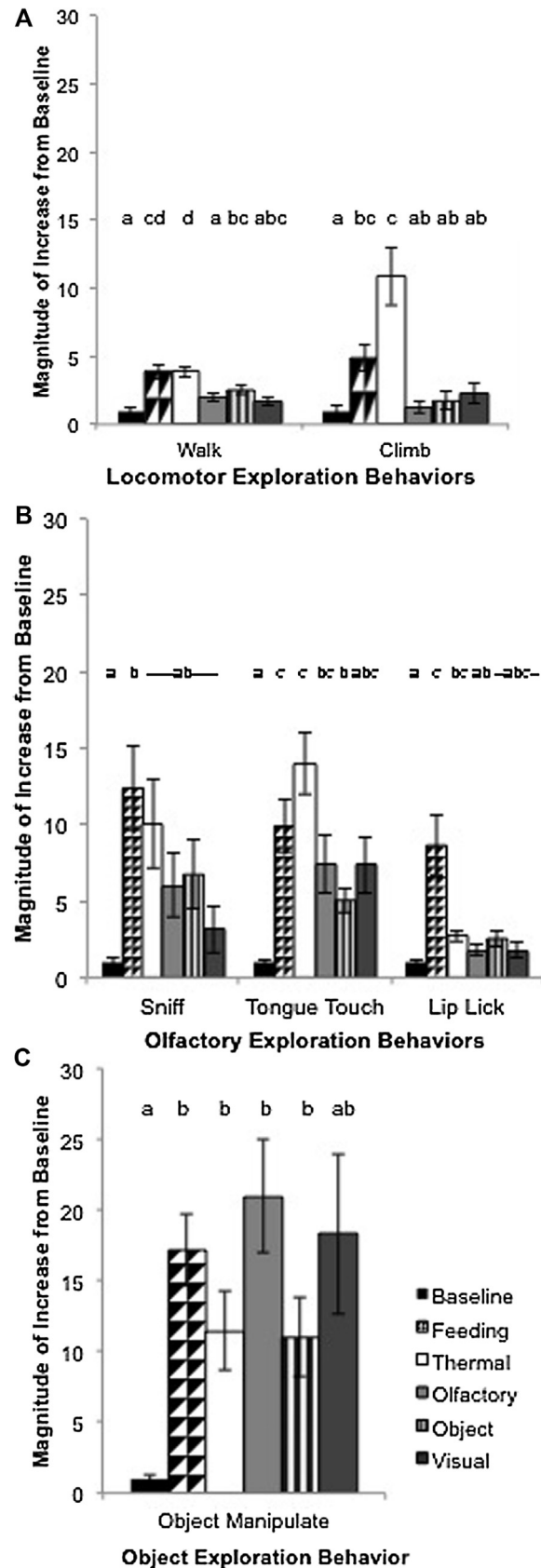

Fig. 2. (a–c) Magnitude of increase in exploratory behaviors as a function of enrichment category, calculated by dividing the average duration or rate of behavior for each enrichment condition by the baseline mean. Error bars show standard error of the mean. Bars with different letters have a significant pairwise difference at a Bonferroni-corrected alpha of 0.05.
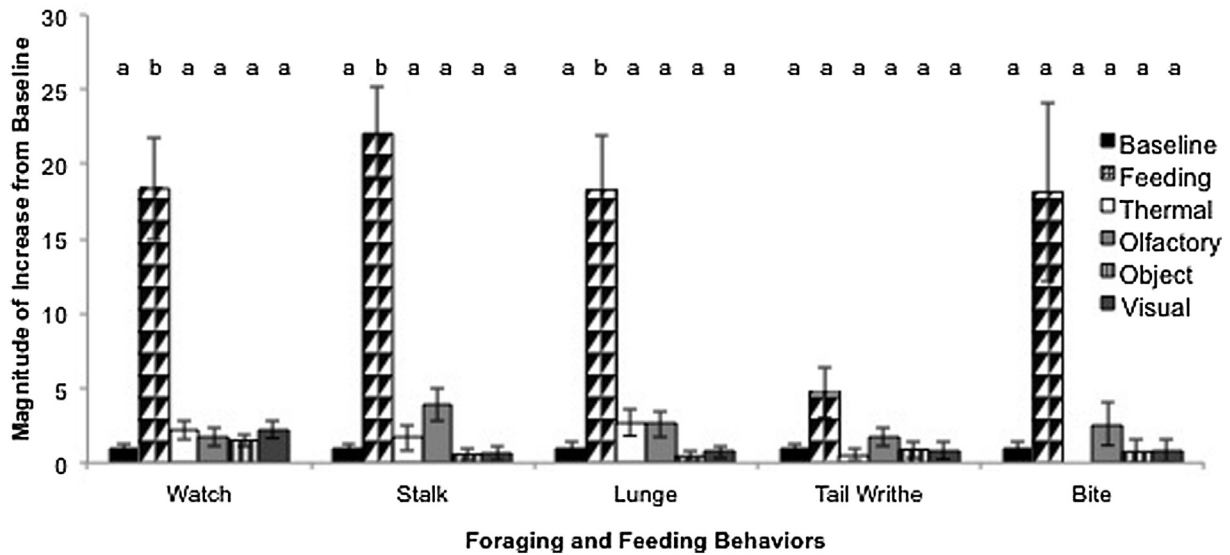
Fig. 3. Magnitude of increase in foraging behaviors as a function of enrichment category, calculated by dividing the average duration or rate of behavior for each enrichment condition by the baseline mean. Error bars show standard error of the mean. Bars with different letters have a significant pairwise difference at a Bonferroni-corrected alpha of 0.05.

3.3. Enrichment effects on welfare

To assess whether any of these enrichment categories had an effect on welfare, we evaluated four behavioral measures associated with improved welfare: increased exploration (subdivided into locomotor, olfactory, and object), increased species-specific behaviors (thermoregulatory behavior and foraging), increased behavioral diversity, and decreased ARB. All exploratory behaviors except for burrowing occurred at low frequencies in baseline observations (Walk = $2.62 \pm 2.06\%$ of the 10-min session, Climb = $0.37 \pm 0.51\%$ of session, Tongue-Touch = 0.07 ± 0.04 times/10-min session, Lip Lick = 0.15 ± 0.08 times/10-min session, Sniff = $0.30 \pm 0.35\%$ of session, Object Manipulate = $0.21 \pm 0.21\%$ of session). We found significant effects of enrichment category on all three types of exploration (locomotor: Pillai's trace = 0.84, $F(15,225) = 5.87$, $p < 0.001$, $\eta^2 = 0.28$; olfactory: Pillai's trace = 0.87, $F(15,225) = 6.09$, $p < 0.001$, $\eta^2 = 0.29$; object: Pillai's trace = 0.84, $F(5,11) = 11.12$, $p = 0.001$, $\eta^2 = 0.84$, Fig. 2). Within locomotor exploration, walking ($F(2.47,37.06) = 15.05$, $p < 0.001$, $\eta^2 = 0.50$) and climbing ($F(1.92,28.72) = 16.12$, $p < 0.001$, $\eta^2 = 0.51$) differed significantly across enrichment categories, but burrowing ($F(1,15) = 3.55$, $p = 0.079$, $\eta^2 = 0.19$) did not. Compared to baseline, Feeding and Thermal enrichment significantly increased walking and climbing and Object enrichment significantly increased walking. Within olfactory exploration, enrichment had a significant effect on tongue touching ($F(5,75) = 11.08$, $p < 0.001$, $\eta^2 = 0.43$), lip-licking ($F(1.34,20.03) = 10.49$, $p = 0.002$, $\eta^2 = 0.41$), and sniffing ($F(5,75) = 5.41$, $p < 0.001$, $\eta^2 = 0.27$). Compared to baseline, Feeding enrichment significantly increased all three of these olfactory behaviors, Thermal enrichment significantly increased tongue-touching and lip-licking, and Olfactory and Object enrichment significantly increased tongue-touching. Within object exploration, object manipulate differed significantly across enrichment categories ($F(2.45,36.69) = 5.01$, $p = 0.008$, $\eta^2 = 0.25$). Compared to baseline, Feeding, Thermal, Olfactory, and Object enrichment significantly increased object manipulation. In case the ineffectiveness of Visual enrichment was an artifact of averaging responses to the reflective and non-reflective sides of the mirror, we used three additional repeated-measures MANOVAs to compare each type of exploration among presentations of the two Visual items and Baseline. None of these MANOVAs were significant (all ps $\geq$ 0.129).

Some enrichment treatments also significantly affected species-specific behaviors. Thermoregulatory behavior was common in baseline sessions and largely consisted of basking ($24.92 \pm 3.55\%$ of the 10-min session), with a little time spent in the shade ($0.07 \pm 0.05\%$ of session) or contact basking ($0.29 \pm 0.16\%$ of session). Enrichment category significantly altered thermoregulatory behaviors (Pillai's trace = 0.78, $F(15,225) = 5.28$, $p < 0.001$, $\eta^2 = 0.26$). This effect was driven by a change in shade time ($F(1.79,26.90) = 24.94$, $p < 0.001$, $\eta^2 = 0.62$); there was a trend toward a significant difference in basking ($F(3.43,51.40) = 2.78$, $p = 0.044$, $\eta^2 = 0.16$, Bonferroni-corrected alpha = 0.017), and no significant change in contact basking ($F(1.85,27.69) = 0.93$, $p = 0.399$, $\eta^2 = 0.058$). Compared to baseline, Thermal enrichment significantly increased percent time in the shade. All foraging behaviors except Rush were observed during baseline observations (Watch = 0.008 ± 0.009 times/10-min session, Stalk = 0.006 ± 0.004 times/10-min session, Lunge = 0.005 ± 0.006 times/10-min session, Tail Writhe = 0.008 ± 0.009 times/10-min session, Bite = 0.001 ± 0.002 times/10-min session). Enrichment category also significantly altered foraging behaviors (Pillai's trace = 0.88, $F(30,370) = 2.62$, $p < 0.001$, $\eta^2 = 0.18$, Fig. 3), particularly watching ($F(1.20,17.99) = 22.93$, $p < 0.001$, $\eta^2 = 0.61$), stalking ($F(1.40,20.98) = 36.20$, $p < 0.001$, $\eta^2 = 0.71$), and lunging ($F(1.26,18.95) = 18.51$, $p < 0.001$, $\eta^2 = 0.55$). Non-significant trends were observed in tail writhing ($F(1.61,24.21) = 4.33$, $p = 0.032$, $\eta^2 = 0.22$, Bonferroni-corrected alpha = 0.008), and biting ($F(1.17,17.52) = 7.84$, $p = 0.010$, $\eta^2 = 0.34$, Bonferroni-corrected alpha = 0.008). There was no significant change in rush ($p = 0.220$). Compared to baseline, Feeding enrichment significantly increased watching, stalking, and lunging.

Behavioral diversity was quite low in baseline observations ($H_{duration} = 0.41 \pm 0.16$, $H_{rate} = 0.75 \pm 0.21$), but increased during enrichment treatments (Wilks' lambda = 0.31, $F(10,148) = 11.84$, $p < 0.001$, $\eta^2 = 0.45$, Fig. 4) for both $H_{duration}$ ($F(5,75) = 21.29$, $p < 0.001$, $\eta^2 = 0.59$) and H_{rate} ($F(5,75) = 25.97$, $p < 0.001$, $\eta^2 = 0.63$). Handling, Feeding, Thermal, Olfactory, and Object enrichment, but not Visual enrichment, significantly increased both behavioral diversity indices compared to baseline. Again, we used an additional repeated-measures MANOVA to compare behavioral diversity among presentations of the two Visual items and Baseline, but still found no significant effect ($p = 0.48$).
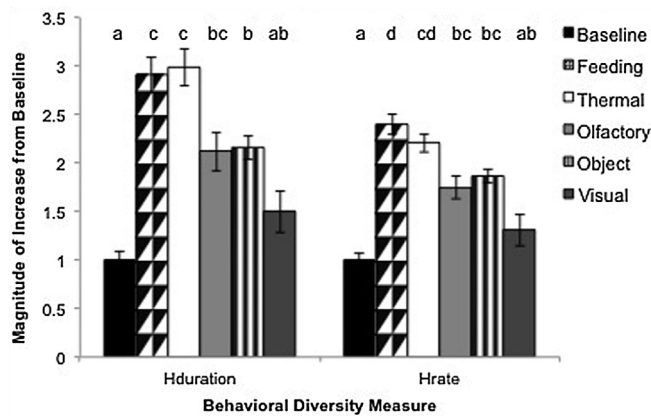
Fig. 4. Magnitude of increase in duration- and rate-based behavioral diversity, calculated by dividing the mean Shannon index of behavioral diversity for each enrichment condition by the baseline mean. Error bars show standard error of the mean. Bars with different letters have a significant pairwise difference at a Bonferroni-corrected alpha of 0.05.

Pacing and other ARB were performed at low rates by 12 of the 16 animals in this study (for these 12 animals, $M_{duration} = 0.24\% \pm 1.22\%$ of the 10-min session). Pacing was observed in two animals, typically moving in tight circles on the floor of the cage ($M_{duration} = 1.21\%$ and 2.14% of the 10-min session). Twelve animals performed some other ARB (for each of these animals, $0.05\% \leq M_{duration} \leq 0.50\%$ of the 10-min session), with the most common being unsuccessful attempts to climb onto an object or up the cage wall. We found no effect of enrichment category on pacing and other ARB ($p = 0.11$).

4. Discussion

By recording responses to short enrichment exposures in the absence of humans using low-light cameras, we were able to detect goal-related behavioral changes associated with enrichment in leopard geckos. Leopard geckos engaged with all five categories of enrichment, but spent significantly more time with Feeding and Thermal than with Olfactory, Object, and Visual enrichment. Geckos responded to Feeding, Object, and Olfactory enrichment like carnivorous mammals by increasing foraging behaviors, exploratory behaviors, and behavioral diversity. Geckos also responded to Thermal enrichment by altering thermoregulatory behaviors. One notable difference between geckos' and mammals' responses to enrichment was that sedentary behaviors and ARB were similar in baseline and enriched conditions. At least two behavioral measures of good welfare increased in every enrichment category except the Visual enrichment treatment.

4.1. Responses to specific enrichment categories

Leopard geckos engaged with Feeding and Thermal enrichment items for about 60% of each session and these enrichments increased all three indicators of good welfare. Neither of these enrichments provided unique behavioral opportunities: the same food items (live crickets) and thermal opportunities (platform under heat lamp, shade) were present throughout the study. Both hunting and thermal behaviors were observed during baseline, but increased significantly with these enrichment treatments. Feeding and Thermal enrichments may have altered behavior by providing a greater number and variety of opportunities to perform target behaviors. During Feeding enrichment, crickets were provided both at regular mealtimes and during enrichment treatment, though the total number of crickets was the same. For captive mammals that naturally eat frequent small meals, feeding more frequently is asso-

ciated with decreased ARB (e.g., in giraffe, *Giraffa camelopardalis*, Bashaw et al., 2001), greater activity, and foraging (e.g., in brown bears, *Ursus arctos*, Grandia et al., 2001 and chimpanzees, *Pan trogolodytes*, Morimura and Ueno, 1999). Similarly, Thermal enrichment provided a greater number of warm and shaded spots in the geckos' home cages. Alternatively, the enrichment items may have allowed the animals to perform target behaviors more effectively or enjoyably, for example by allowing geckos to work for food when free food was available (i.e., to contrafreeload: Inglis et al., 1997). Feeding devices were transparent or translucent, allowing the geckos to see the crickets inside. Geckos often sat outside the device and watched or stalked crickets inside. Thermal items were wood, differing from the other exhibit furniture in thermal properties, coefficients of friction, opacity, odor, and being a natural (non-plastic) material. These properties may be attractive to geckos. Behaviors critical for survival, like foraging, are intrinsically reinforcing, in that performing such a behavior increases the probability that the same behavior will recur regardless of the external consequences (Mench, 1998; Tarou and Bashaw, 2007). Animals seek out opportunities to perform intrinsically reinforcing behaviors, and several authors have suggested their welfare may be compromised if provided insufficient opportunities for these behaviors (Duncan, 1998; Tarou and Bashaw, 2007). For carnivorous reptiles like leopard geckos, both hunting and thermoregulatory behaviors are likely intrinsically reinforcing (Warwick et al., 2013).

Leopard geckos spent one-fifth of each session engaged with Olfactory and Object enrichment items, and these enrichments increased exploration and behavioral diversity by significantly increasing walking, object manipulation, and tongue-touching. Geckos may also have been engaged with these items at greater distances than we recorded (Clark and King, 2008; Gronqvist et al., 2013); leopard geckos visual and olfactory detection thresholds are unknown (Schwenk, 1993). We designed Object and Olfactory items to stimulate different sensory modalities, but the similar responses suggest they provided the same affordances (Gibson, 1979) to the geckos, rendering them effectively equivalent stimuli (Heyser and Chemero, 2012). We used large moveable items for both of these conditions (see Fig. 5) to minimize the chance the geckos could ingest the items (Duncan, 1997). The Olfactory items may have been perceived as Objects if not sufficiently scented, or the Objects may have had the novel scent of rubber and therefore acted as Olfactory enrichment. Novel objects, scented or unscented, increase exploration and interaction similarly in geckos (current study), carnivorous mammals (Clark and King, 2008; Kuczaj et al., 2002; Machado and Genaro, 2014; Pearson, 2002; Powell, 1995; Schuett and Frase, 2001; Wells and Egli, 2004), and turtles (Burghardt et al., 1996; Therrien et al., 2007). We conclude that providing novel objects improves welfare in leopard geckos by increasing their propensity for exploration; these items should be provided, but preference given to enrichment like puzzle feeders, climbing and shade structures, and warm or cool substrates that address strongly motivated behaviors like feeding and thermoregulation (Mench, 1998).

Geckos interacted with Visual enrichment items for the same duration as Object and Olfactory enrichment items. As observed in African wild dogs, *Lycaon pictus*, mirrors caused no significant changes in behavior and responses to the painted and reflective sides of the mirror were the same (Packard et al., 2010). This lack of response may have been a result of the size or material of the mirror we used, but a follow-up study with a larger glass mirror also produced no significant change in behavior (Gibson, 2015). Leopard geckos perform territorial displays (Indiviglio, 2007; Rhen and Crews, 2001), but these may rely on multimodal signals (Brattstrom, 1974). We have no evidence that reflections were per-
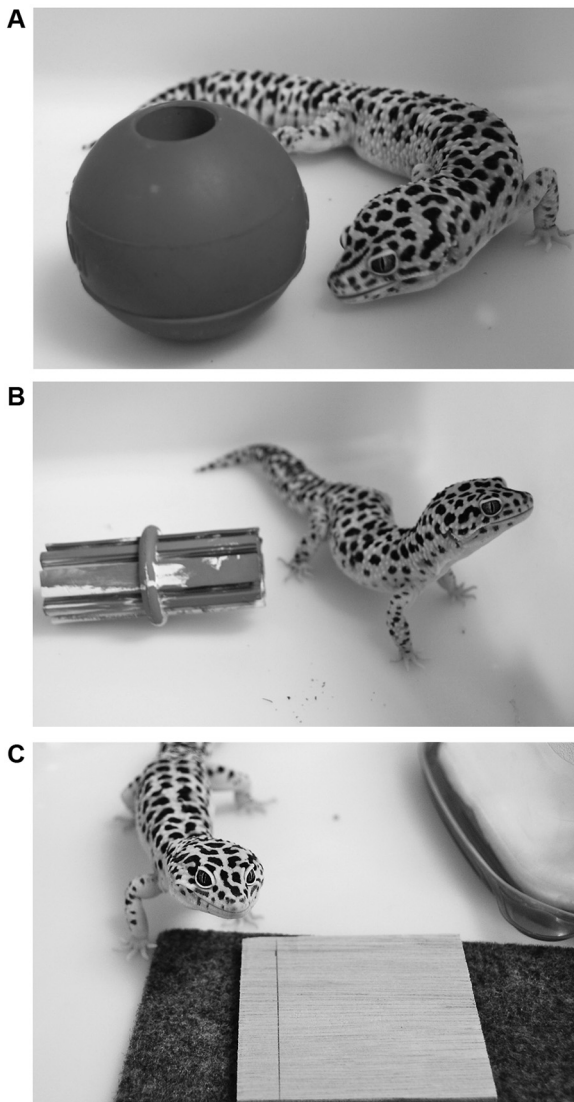
Fig. 5. (a–c) Geckos with Object (a and b) and Olfactory (c) environmental enrichment items. Photos: M. Gibson.

ceived as social stimuli by the leopard geckos or that provision of a mirror or painted flat surface improves welfare in leopard geckos.

4.2. Enrichment and reptile welfare

Using leopard geckos, we demonstrated that it is possible to combine enrichment principles developed for mammals with knowledge of reptiles' species-specific needs to generate effective enrichment for captive reptiles. Our enrichment items enabled the geckos to perform a greater variety of species-specific behaviors (Fraser, 2009; Shepherdson, 2010) and allowed them to choose different ways to perform those behaviors (Kagan and Veasey, 2010). There is a growing literature suggesting turtles and tortoises benefit from enrichment, including novel objects (Burghardt, 2013; Burghardt et al., 1996; Mehrkam and Dorey, 2014; Therrien et al., 2007) and feeding enrichment (Therrien et al., 2007), and that these species prefer complex environments (Case et al., 2005). Burghardt (2013) suggests enrichment improves the cognitive performance of reptiles and is likely to improve their welfare. Our study supports this conclusion and accords with findings that reptiles will use vertical space more when enrichment increases access to such space (Rose et al., 2014). However, the provision of raised bask-

ing platforms to eastern fence lizards (*Sceloporus undulates*) had no effect on welfare (Rosier and Langkilde, 2011), and our enrichment treatments were not all equally effective, so the type of enrichment provided appears to affect welfare outcomes. Small reptiles may exhibit less behavioral response to enrichment because their innate fear of humans may suppress active responding in our presence (Hosey, 2008) or because their lower metabolic rates make active responses to enrichment more energetically costly (Bowers and Burghardt, 1992; Burghardt et al., 1996), but this study demonstrates that they do respond measurably. As has been reported for mammals (Highfill, 2008; Izzo et al., 2011) and Galapagos tortoises (Mehrkam and Dorey, 2014), we also saw substantial differences between individual geckos in pacing, engagement with, and behavioral responses to enrichment. While we used a repeated-measures design to control for these effects in our analyses, future studies should explore the degree to which individual responses to enrichment can be predicted by specific previous experiences (Harding et al., 2004; Veissier and Boissy, 2007) or temperament (Highfill, 2008; Izzo et al., 2011).

We used best practices in measuring welfare by seeking concordance among behaviors reflecting good and poor welfare. We found remarkable agreement among behavioral measures of good welfare: no enrichment category improved only a single measure of welfare and in no case did measures conflict. However, we found no change in our single measure of poor welfare. There were two major weaknesses in our welfare assessment. First, like previous reptile enrichment studies (Burghardt et al., 1996; Mehrkam and Dorey, 2014; Therrien et al., 2007), we based our conclusions about gecko welfare on the assumption that welfare measures developed for mammals and birds can be extended to reptiles by simply incorporating taxon-specific behaviors. For example, Warwick et al. (2013) suggest persistent barrier-directed behavior (one of our ARB) and inflation of the body and hissing (in our ethogram as aggression, but not observed) indicate poor reptile welfare, while calmly smelling or tasting objects or air (our tongue-touch and tongue-air) and unhurried body movements (our walk/explore) reflect good reptile welfare. However, Cannon et al. (2002) have suggested "behavior alone may not necessarily provide a useful indication of stress in reptiles" (p. 8). Empirical tests of the relationship between behavior and welfare like comparing behavioral indicators to physiological stress responses measured using glucocorticoids will be critical to validate behavioral measures. Second, we used ARB as our only measure of poor welfare, but found no significant changes in ARB when any enrichment category was added. ARB have been reduced by enrichment in many studies of mammals and birds and in two studies of turtles (Burghardt et al., 1996; Therrien et al., 2007). In this study, the lack of a significant decrease in ARB likely occurred because ARB were observed at such low rates.

4.3. Conclusions & implications

The leopard geckos benefitted from three types of environmental enrichment: thermal, feeding, and novel objects (with and without scent). Geckos attended to each of these, but engaged more with enrichment that added complexity to meeting their basic physiological or behavioral needs than with novel objects. This implies geckos have behavioral priorities, will use novel stimuli to perform highly-motivated behaviors like hunting and behavioral thermoregulation, and are motivated by information gathering, as has been suggested for mammals and birds (Mench, 1998). Previous studies have demonstrated that enrichment is beneficial to turtles and tortoises (*Testudines*) and some squamates (e.g., corn snakes, rat snakes, and western chuckwallas). This study extends their findings to leopard geckos and suggests different types of enrichment have different effects on reptile behavior and welfare. The degree to which these results can be generalized to other

snakes and lizards awaits comparative study, but our results support Brattstrom's (1974) suggestion that the behavioral complexity of captive reptiles has been underestimated. Given accredited zoos and aquariums have committed to improving the welfare of their animals (e.g., Association of Zoos and Aquariums, 2015), pet owners want to do the best job caring for their animals, and some types of environmental enrichment have the potential to improve reptile welfare, environmental enrichment of captive reptile housing should be more fully explored.

Conflict of interest

None.

Acknowledgements

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