

Assessing the impact of environmental enrichment on behavior in understudied armadillo species: A case study

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Abstract

The implementation of environmental enrichment (EE) can be effective in promoting zoo animal welfare by enhancing the performance of natural or species-typical behaviors. Research on the effects of EE is biased towards larger mammalian species, with less charismatic species being overlooked. Armadillos are one such overlooked example. A captive environment that results in inactivity, obesity, and associated poor health can negatively affect armadillo well-being. The aim of this study was to evaluate how the implementation of four physical object-based EEs could positively affect the behaviors of three armadillo species, housed in four similar exhibits. Behavioral data were collected both before (baseline) and during (treatment) EE periods, alongside of visitor number and environmental temperatures. The EE comprised of a plastic ball or a cardboard tube or a cardboard box, or a scatter-feed, and these were rotated each week of study until each exhibit had received them in turn. Despite the presence of different EE types, activity remained low throughout the study. However, results suggest that the plastic ball and cardboard box increased exploratory behaviors in the armadillos, but no overall increase in activity was noted during the scatter feed. Visitor presence had no effect on armadillo activity, and armadillos showed reduced activity with increasing environmental temperature. Overall, the use of physical object-based EE promoted beneficial natural behaviors in zoo-housed armadillos, but environmental conditions (i.e., temperature) also impacted armadillo activity, suggesting a complicated relationship between an enclosure's environmental variable and any behavioral husbandry measures.

KEYWORDS

animal welfare, armadillo, environmental enrichment, evidence-based husbandry, zoo behavior

1 | INTRODUCTION

An evidence-based approach supporting ecologically relevant enclosure design and husbandry routines for captive animals increases an individual's opportunities to perform natural, species-typical

behaviors and attain positive welfare states (Niemelä & Dingemans, 2014; Rose, 2018). The welfare state of an animal encompasses its physical and psychological health, in addition to its ability to adapt to the immediate environment with minimal suffering (Stafleu et al., 1996). The presence of zoo visitors can influence zoo

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animal behavior, potentially leading to stress and disrupted behavior patterns (Hosey, 2000; Wells, 2005). However, as visitor presence correlates with weather, visitor effects can be difficult to distinguish (Rose et al., 2020) and, therefore, should be evaluated alongside of changes in environmental data (Hosey et al., 2023). Observation of natural, species-typical behaviors can indicate the welfare state of an animal and may be defined as those that are exhibited in natural conditions, are considered pleasurable, and promote biological functions (Bracke & Hopster, 2006). Observing these behaviors may indicate positive welfare states (Browning, 2019), because it means the individual animal can experience satiation. However, the zoo environment may predispose captive species to higher levels of inactivity when compared to the wild due to restrictions on space and/or the predictability of daily husbandry (i.e., set feeding times) that result in specific foraging opportunities (Boccacino et al., 2020). Behavioral constraints caused by husbandry routine and enclosure size can be mitigated by providing environmental enrichment (EE); consequently, the aim of this paper was to evaluate the effect of EE on the behavior of an understudied species in a zoo.

EE describes features within the captive environment that are provided to enhance complexity, and increase psychological stimulation and physical activity for the animals (Baker et al., 2018). EE can be beneficial to the health and welfare of captive animals (Mason et al., 2007; Shyne, 2006) as its use provides a positive challenge (Meehan & Mench, 2007) to stimulate the animal physically and mentally, increase time spent on high-value behavior, and even improve reproductive success when it is implemented correctly (Cortés Duarte et al., 2016; Riley & Rose, 2020; Rose & Riley, 2019).

Although the use of EE is common and commendable in many species, we are yet to discover the full potential positive behavioral impacts of specific forms of EE due to a lack of research that evaluates its efficacy. Nocturnal species and those secretive or cryptic in movements or appearance provide extra challenges to the assessment and evaluation of their responses to husbandry (Hamilton et al., 2020). One example of an overlooked and challenging-to-observe group of species is the armadillos (Cingulata). Temperature influences behavior patterns for wild armadillos (Attias et al., 2018) and therefore, it should be measured when evaluating captive activity and the behavioral relevance of EE. Armadillos may be susceptible to the negative effects of captive living and can display reproductive failure and stereotypic behaviors, such as pacing (Cortés Duarte et al., 2016; Rideout et al., 1985; Sherwen & Hemsworth, 2019). Thus, the provision of EE could provide behavioral benefits for armadillos.

The main objective of this study was to implement and then assess how four varieties of physical object-based EE influenced the activity patterns and behavioral states exhibited by three species of armadillo: the six-banded armadillo (*Euphractus sexcinctus*), the large hairy armadillo (*ChaetophRACTUS villosus*), and the southern three-banded armadillo (*Tolypeutes matacus*). These three armadillo species are native to South America, inhabiting forest, savannah, and shrubland habitats (Abba, Lima et al., 2014; Abba, Poljak et al., 2014; Noss et al., 2014). Wild six-banded armadillos show a mix of diurnal and

Highlights

- Enrichment increased alert, exploratory, and locomotory behaviors in zoo armadillos.
- Plastic ball was the most effective enrichment type.
- Armadillo activity was influenced by temperature.

nocturnal activity, becoming increasingly active in higher temperatures (Attias et al., 2018; Maccarini et al., 2015). Whereas large hairy and southern three-banded armadillo display mostly nocturnal activity patterns, only becoming active diurnally at lower temperatures (Attias et al., 2020; Frontini & Escosteguy, 2012). In the wild, armadillos are typically highly inactive species, spending 4–6 h per day awake whilst they excavate burrows and/or forage with their claws and snouts to search for food (Ancona & Loughry, 2010; Attias et al., 2020; Rodrigues et al., 2020). Some species, such as the six-banded armadillo are dietary opportunists and will forage for carrion, plant matter, and invertebrates, adapting the diet to their immediate environment (Dalponte & Tavares-Filho, 2004), whereas many other species are predominantly insectivorous (Anacleto, 2007). For the purposes of clarity and brevity, from here on, the different armadillo species are referred to as SBA (six-banded armadillo), LHA (large hairy armadillo), and STBA (southern three-banded armadillo).

Based on their ecology (i.e., nocturnal exploration and foraging behaviors including digging and rooting), our study aimed to provide further information on how these elusive mammals respond to enrichment that could promote wider performance of ecologically relevant activity in the zoo (within natural boundaries at naturalistic times of the day) and to also define any responses to visitor presence. Ecologically relevant activities are defined as behavioral repertoires that mimic what would be observed in similar wild conditions (Rose, 2018). It was predicted that physical object-based EEs would significantly increase natural exploration and foraging behaviors that are most often seen as part of wild-type active behavior when compared to baseline conditions (no EE). To determine any impact of visitors on armadillo behavior (and potential use of EE) visitor presence was also recorded across a time of the day when more visitors were likely to be present in the zoo (e.g. late morning). Finally, due to differences in the social environment of these armadillos, we investigated any impact of housing style on behavioral outputs in the presence of EE for paired and solitary animals.

2 | MATERIALS AND METHODS

2.1 | Animals and study location

Armadillos were observed at Amazon World Zoo Park, Sandown, Isle of Wight daily from Monday, May 29, 2017 to Friday, June 23, 2017, excluding all weekends. The nocturnal area at Amazon World Zoo Park contained four exhibits, each with two nest boxes (1.0 × 1.0 m),

one at each end of the exhibits. Each nest box at the front of each exhibit contained a small viewing window (0.5 × 0.5 m) and was heated using a 150 W red lamp to allow for a basking temperature of ~28°C. The full exhibit could also be viewed from over the 1.45 m walls that surround them. Each exhibit also featured larger viewing windows (1.25 × 0.5 m) for visitors to see through. When in the nest box armadillo behavior could be categorized as resting or any other activity (see Table 2). Exhibit 1 (7.5 × 3.0 m) was one overall enclosure housing a male and female SBA. Exhibit 2 (6.6 × 2.6 m) was one overall exhibit housing one LHA. Exhibit 3 (total 6.6 × 2.6 m) was two separate enclosures of the same dimensions (3.3 × 2.6 m per armadillo) housing a male and female STBA separately. These two STBA both had a nest box each that was heated to enable basking. Exhibit 4 (6.6 × 2.6 m) was one overall enclosure that housed another female LHA.

Bark woodchip was used as the substrate in each exhibit, and permanent fixtures included natural vegetation and numerous logs to replicate key habitat areas of a natural environment (Attias et al., 2018). Each exhibit was lit with dim lighting that allowed visitors to see within the exhibit. No natural lighting was provided, and a reverse lighting system was used. Between 09:00 a.m. and 17:15 p.m., low-level lighting was used to simulate nocturnal light conditions while allowing visitors to see the armadillos. Low-level lighting was also used along the public walkway. Between 17:16 p.m. and 08:59 a.m., four large strip lights above each exhibit were used to simulate daylight and provide an opportunity for the armadillos to sleep during a “diurnal” period. Each exhibit was serviced daily between approximately 08:00 a.m. and 09:30 a.m., with one bowl of food—containing a selection of fruit, vegetables, cooked rice (apart from STBA as per the Zoo's diet plan), and cooked egg—provided to each armadillo. Diets remained the same throughout the study. All water bowls were refreshed daily during this husbandry period.

2.2 | Enrichment devices

The four types of EE used for the study are described in Table 1, and this table also explains the schedule of EE per exhibit. The types of EE

were chosen based on previous research in other armadillo species and EE usage (Clark & Melfi, 2012; Cortés Duarte et al., 2016). These papers showed that increased performance of natural foraging behaviors could be attributed to EE provision. The personal experience of the lead author with armadillo husbandry was also a contributing factor in EE design, implementation, and the use of mealworms within each EE device (due to animal preferences for these treats and mimicking of invertebrate prey that each species consumes in the wild). One EE per armadillo per enclosure was provided; SBA and LHA received 15 g mealworms per EE per day (30 g per exhibit for scatter-feed), and STBA received 10 g mealworms per EE per day (10 g per separated exhibit area). The amount of mealworms provided was taken from each animal's normal daily ration. The remainder of the armadillo's daily diet was provided during the morning servicing and thus was not considered to influence any response to the EE because there was a minimum 2 h gap between receiving their morning feed and the mealworms. EE remained in the armadillo enclosure until servicing the following day. Each EE was then refreshed with new food and placed back into each exhibit before data collection commenced. The EE was placed into each armadillo's enclosure during the morning servicing by a keeper at 09:30 a.m.

2.3 | Behavioral data collection

Due to the challenge of individual identification of each armadillo within a nocturnal house and the position of the observer outside of the enclosure during the data collection period, which meant that the animal could be seen but defining features not always reliably identified, behavioral data were grouped for animals within an enclosure to reduce any bias or pseudoreplication caused by misidentified of each individual armadillo. No invasive marking of individual armadillos was considered to prevent any change to behave that could be attributed to any tags or markings instead of from the EE.

Behavioral data were collected by the lead author using instantaneous scan sampling of animals per exhibit at 10 s intervals

TABLE 1 Description of each EE used and schedule of provision within each armadillo exhibit.

EE type	Description	Week number/exhibit number
Cardboard tube	Hollow cardboard tube of 15 cm length. Holes placed around the tube of 0.5 cm diameter to allow food to fall out when rolled; filled with straw and mealworms.	W1/E2, W2/E1, W3/E3, W4/E4
Plastic ball	Hollow ball of 10 cm diameter. Hole of 1.5 cm diameter at the top used to place food and fall out when rolled around exhibit. Straw was not placed in the plastic ball due to the small size of the holes.	W1/E1, W2/E3, W3/E4, W4/E2
Cardboard box	Cardboard box of approximately 30 × 20 × 15 cm, filled with straw and mealworms. Box closed after filling to enable armadillos to dig through the box.	W1/E4, W2/E2, W3/E1, W4/E3
Scatter feed	Mealworms scattered by hand around the exhibit. As mealworms are live, they naturally burrowed into the substrate.	W1/E3, W2/E4, W3/E2, W4/E1

Abbreviation: EE, environmental enrichment.

TABLE 2 Ethogram of armadillo state behaviors (applicable to all study species) adapted from Ancona and Loughry (2009) and Clark and Melfi (2012).

Behavior	Description
Nest box (rest)	Armadillo is stationary with head down, and eyes may be closed. May be lying on front or on back and sleeping.
Nest box (other)	Armadillo is using the nest box but specific actions cannot be identified.
Alert	Armadillo is stationary and appears aware of its surroundings with eyes fully open and may have elevated snout. May be lying down on front of body or standing on hind legs and may have elevated snout.
Locomotion	Use of limbs to maneuver around the exhibit at any speed.
Foraging	Consumption of food and/or water.
Exploratory	Using front and/or hind limbs to produce a hole within the substrate or at logs in exhibit. Using snout and claws to search within substrate or around logs within exhibit. Investigation of EE with direct contact, such as scratching and/or sniffing. Active within the nest box identifiable by dust clouds produced by the animal.

Abbreviation: EE, environmental enrichment.

for 10-min periods (Martin & Bateson, 2007) using the behavior codes (as per Table 2). Observations were conducted at each exhibit three times for a total of 30 min at each exhibit per day. Three observations were chosen to maximize the chances of animals being active and, therefore, observable. Data collection commenced at 12:00 p.m. for Exhibit 1, 12:10 p.m. for Exhibit 2, 12:20 p.m. for Exhibit 3, and 12:30 p.m. for Exhibit 4 (for 10 min behavioral sampling per exhibit). The nocturnal area that housed the armadillos was then vacated for 20 min. Then, data collection was repeated at 13:00 p.m. and at 14:00 p.m. using the same schedule of observations to allow for equivalent data collection per exhibit. Morning exhibit service took place until 11:30 a.m., and afternoon service began at 15:00 p.m., therefore, the data collection period between 12:00 p.m. and 14:40 p.m. was selected for this study.

Total visitor number within the enclosed nocturnal area, and therefore not necessarily at each armadillo exhibit, was recorded at each 10 s interval. The building containing all exhibits was not temperature controlled, so we recorded ambient exhibit temperature at 1-min intervals using digital thermometers that were placed in each exhibit. These temperatures were then averaged across each 1-h observation for subsequent analysis. Temperature and time of day were recorded because wild data suggests that armadillos are potentially sensitive to temporal and climate changes (Maccarini et al., 2015). Additionally, the time of day was included because it may correspond with different numbers of visitors during and after the lunchtime period (i.e. a higher number of visitors within the nocturnal house after lunch).

Due to the challenge of individual identification in pair-housed animals, the average scans of each behavior for the two individuals in Exhibits 1 and 3 were calculated for each 10-min sampling period for each behavior. The first week (Week 0) allowed for habituation of the armadillos to the presence of the observer and the collection of data on baseline behaviors (when no EE was present). During the baseline conditions, mealworms were presented in food bowls with the rest of their daily rations. Baseline data were then compared to the treatment periods (when EE was provided). An ethogram (Table 2)

was constructed before data collection and tested during Week 0. This ethogram was partially adapted from Ancona and Loughry (2009) and Clark and Melfi (2012). For the following 4 weeks, EE was provided to each exhibit as per the schedule in Table 1.

Ethical approval for the research was granted by the Home Office Liaison Team (HOLT) and the Animal Welfare and Ethical Review Body (AWERB) at the University of Bristol (the institution that the lead author was affiliated with at the time of the project).

2.4 | Data analysis

In total, 18,300 data points were collected over 66.7 h of observation. Data were inputted into Microsoft Excel and all inferential analysis was conducted in R v.1.4.0 (R Core Team, 2021) using RStudio v.1.4.1717 (RStudio Team, 2020). For all inferential analysis, an alpha level of 5% was used to determine statistical significance, with estimate \pm standard deviation, r^2 value, degrees of freedom, t value, and p value given. For relevant nonsignificant results, p values are given.

The average time-activity budget across the 5 day observation period for each species of armadillo was constructed to compare the performance of the state behaviors (outlined in Table 2) during baseline and treatment periods. To show if armadillos had habituated to the EE, total average (%) exploratory and foraging behaviors were compared between Day 1 and Day 5 for each EE type (Table 3).

To analyze the potential effects of time of day (time interval), EE type, average temperature, and average visitor number on armadillo behavior, a repeated measures ANOVA was run in RStudio using the "lmerTest" package (Kuznetsova et al., 2016) for group data points for each state behavior. For these repeated measures testing, day, and exhibit identifier (i.e., the name of each armadillo enclosure) were included as random factors. The "MuMin" package (Barton, 2013) was used to calculate the r^2 value for each of the models run to check for model fit. All predictors (time interval, EE type, temperature, and visitor numbers) were tested for collinearity using the "car" package (Fox & Weisberg, 2011). Predictors with a variance inflation factor (VIF) of

below 2 (signaling no collinearity) were included in the model. The model that provided the highest r^2 value and lowest VIF was chosen as the final model run. Time differences were included because the overall observation period was split into three chunks, so perhaps the armadillos were more active as the afternoon progressed, and this may have been influenced by visitor number. None of the predictors had a significant impact on foraging behaviors, and thus this behavior was not included in any further analyses.

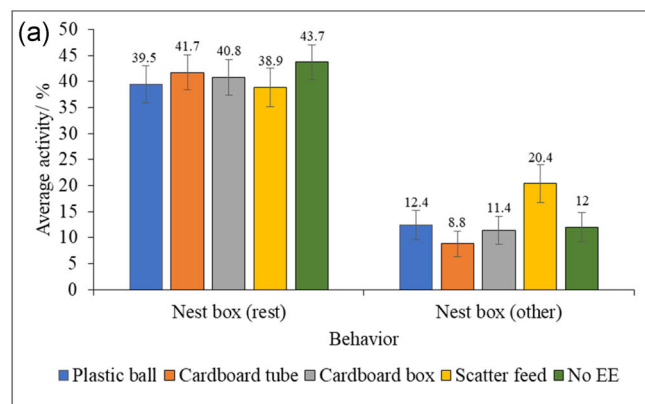
To determine the significance of specific EE types on each behavioral output, post-hoc testing was conducted using the “lsmeans” and “pbkrtest” packages (Halekoh & Højsgaard, 2014; Lenth, 2016) in RStudio. Each pairwise EE device comparison was evaluated. To remove any type 1 error (and false discovery of significance) a Benjamini–Hochberg correction (Benjamini & Hochberg, 1995) was applied to multiple comparisons of p values. A corrected level of significance was provided to identify significant predictors.

To assess any impact of social or group housing across species, a two-sample t -test was performed to analyze any differences in the performance of state behaviors across the entire study period for each housing condition.

TABLE 3 Average (\pm SD) % of time armadillos spent engaging in behavior on the first and last days that EE was presented.

EE	Day 1 exploratory/%	Day 5 exploratory/%	Day 1 foraging/%	Day 5 foraging/%
Plastic ball	1.75 \pm 1.63	1.3 \pm 1.15	0 \pm 0	0.08 \pm 0.2
Cardboard tube	2.42 \pm 2.96	3.79 \pm 2.8	0.46 \pm 1.1	0.21 \pm 0.35
Cardboard box	2.33 \pm 2.01	7.58 \pm 1	0 \pm 0	0.54 \pm 0.8
Scatter feed	0 \pm 0	0.75 \pm 1.03	0 \pm 0	0.17 \pm 0.4

Abbreviations: EE, environmental enrichment; SD, standard deviation.



3 | RESULTS

3.1 | Impacts of EE on behavior

Nest box (rest), hereafter termed “resting,” was the most common behavior across all conditions (including baseline) followed by nest box (other), as illustrated by Figure 1a. Under all EE and baseline conditions, the next commonly observed behavior was locomotion; plastic ball (3.8%), cardboard tube (3.7%), cardboard box (2.8%), scatter feed (0.42%), and baseline (2.8%). The least commonly performed behavior under all EE and baseline conditions was foraging; plastic ball (0.04%), cardboard tube (0.2%), cardboard box (0.1%), scatter feed (0.2%), and baseline (0.07%).

The armadillos did not habituate to the EE across the study period because activity levels (exploratory and foraging behavior) at the start were not higher than at the end, as shown in Table 3.

3.2 | Visitor and environmental variables

Throughout the study period, overall visitor numbers remained low. For each 10 min interval, total visitor numbers were counted and then averaged. The lowest average number of visitors was 0 and the highest average number of visitors was 15.57. For the entire study, the average number of visitors across all intervals was 1.33. Temperature was also recorded and averaged across each study interval. The lowest recorded average temperature was 22.12°C, and the highest average temperature was 30.64°C. For the entire study, the average temperature across all intervals was 25.76°C.

As Table 4 shows, EE predicted a significant change in exploratory, locomotion, nest box (other), and alert behaviors. Additionally, temperature predicted a significant change in locomotion, nest box (rest), and nest box (other) behaviors.

Different types of EE did not any predict change in performance of foraging behavior in these armadillos ($p = .59$), and no impact was found for temperature either ($p = .87$). Change in environmental

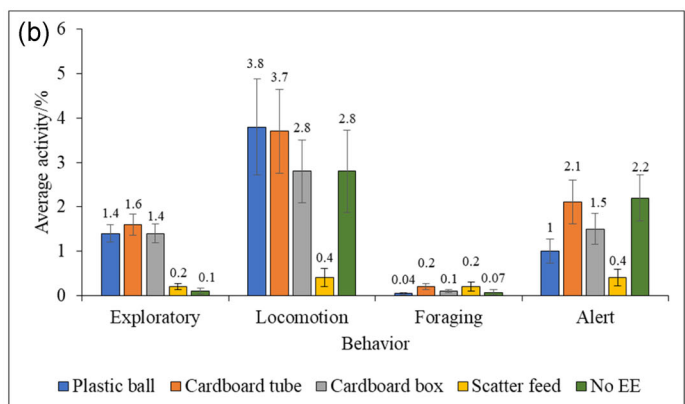


FIGURE 1 (a) Average (\pm SD) % of resting behaviors and Nest box (other) behaviors in armadillos provided with four enrichment types and under baseline conditions. (b) Average % of all remaining behaviors in armadillos provided with four enrichment types and under baseline conditions. [Color figure can be viewed at wileyonlinelibrary.com]

temperature did not significantly predict exploratory ($p = .26$) and alert ($p = .87$) behaviors in the armadillos. Provision of EE did not significantly predict resting behavior in the armadillos ($p = .12$). In addition, increasing visitor number did not predict any behavioral change, with nonsignificant findings generated for exploratory ($p = .14$), locomotion ($p = .37$), resting ($p = .54$), nest box (other) ($p = .59$), and alert ($p = .97$) behaviors.

Table 5 indicates the EE type that had a significant effect on state armadillo behaviors are highlighted with an asterisk (*) with a corrected Benjamini–Hochberg significance level of 0.043. It also shows that there was a significant positive difference between exploratory behaviors performed when the cardboard box EE was

provided compared with no EE ($p < .001$) and with scatter feed EE compared with no EE ($p < .001$), and significant negative difference when no EE was provided compared with cardboard tube EE ($p < .001$). There was also a significant positive difference in nest box (other) occurrences when scatter-feed was provided compared with the cardboard tube EE ($p < .001$). There was no significant difference in foraging behaviors performed when any of the different EE types were provided.

3.3 | Housing style and behavior

There was no significant difference in exploratory and foraging behaviors performed between paired and solitary armadillos (Table 6). In all remaining state armadillo behaviors, there was a significant difference in behaviors performed between paired and solitary armadillos.

TABLE 4 Environmental (EE and temperature) predictors that had a significant effect on state armadillo behaviors.

Behavior	Predictor	<i>F</i>	<i>df</i>	r^2	<i>p</i> -Value
Exploratory	EE	9.66	4, 284.15	.27	<.001
Locomotion	EE	3.55	4, 284.02	.33	.007
	Temperature	6.09	1, 286.26		.014
Nest box (rest)	Temperature	13.54	1, 287.44	.85	<.001
Nest box (other)	EE	7.29	4, 284.04	.75	<.001
	Temperature	25.05	1, 286.76		<.001
Alert	EE	3.9	4, 284.1	.09	.004

Abbreviations: EE, environmental enrichment; *df*, degrees of freedom.

4 | DISCUSSION

This study has identified inactivity to be a common behavioral state in these armadillos, even when housed in a reverse day–night system. When evaluating the effect of EE on the behavior of this population of captive armadillos, the use of a plastic ball influenced the amount of time spent performing a wider range of behaviors compared to the other EE types that were tested (Table 5). Plastic ball EE increased the performance of exploratory and locomotion activities, and nest box (other) occurrences. This, in part, may be due to the complexity

TABLE 5 EE types that when compared with other EE types, had a significant effect on state armadillo behaviors.

Behavior	EE types	Estimate	SE	<i>df</i>	<i>t</i> ratio	<i>p</i> -Value	<i>q</i> -Value
Exploratory	Ball-none	3.93	1.04	284	3.79	.002	.0200*
	Ball-scatter	3.43	1.03		3.34	.008	.0300*
	Box-none	4.24	1.04		4.09	<.001	.0033*
	Box-scatter	3.74	1.03		3.64	.003	.0267*
	None-tube	−4.85	1.04		−4.66	<.001	.0067*
	Scatter-tube	−0.11	1.03		−4.24	<.001	.0100*
Locomotion	Ball-scatter	3.42	1.04		3.29	.009	.0333*
	Scatter-tube	−3.35	1.04		−3.22	.012	.0400*
Resting	None-scatter	5.43	2.17		2.5	.093	.0500
Nest box (other)	Ball-scatter	−7.99	2.32		−3.45	.058	.0467
	Box-scatter	−9.1	2.32		−3.93	.001	.0167*
	None-scatter	−8.79	2.35		−3.75	.002	.0233*
	Scatter-tube	11.68	2.32		5.04	<.001	.0133*
Alert	None-scatter	1.7	0.55		3.08	.019	.0433*
	Scatter-tube	−1.78	0.54		−3.27	.011	.0367*

Note: A positive estimate indicates an increase in behavior between EE types and a negative estimate indicates a decrease in behavior between EE type. Abbreviations: EE, environmental enrichment; *df*, degrees of freedom; SE, standard error.

TABLE 6 The effect of paired and solitary housing on the performance of armadillo state behaviors.

Behavior	Average (\pm SD) % scans	t(df)	df	p-Value	Interpretation
Exploratory	Solitary 0.6 (0.07) Social 0.6 (0.1)	-0.35	604	.73	No difference
Locomotion	Solitary 3.2 (0.4) Social 1.3 (0.3)	-0.36	417	<.001*	Solitary sig. more
Nest box (rest)	Solitary 38.7 (1.5) Social 51.5 (1.6)	3.79	319	<.001*	Social sig. more
Nest box (other)	Solitary 15.2 (1.3) Social 4.9 (1.4)	-5.42	314	<.001*	Solitary sig. more
Alert	Solitary 1.8 (0.2) Social 1.1 (0.4)	-2.37	271	<.001*	Solitary sig. more
Foraging	Solitary 0.7 (0.02) Social 0.5 (0.06)	0.49	304	.63	No difference

Abbreviation: SD, standard deviation.

of manipulating the ball and moving it around to access mealworms. After EE engagement, it may have ultimately increased time spent on resting or nest box (other) as the animals were digesting or hiding their food (DeGregorio et al., 2022) in a quieter area of their exhibit as they were satiated. Evaluating increases in activity caused by EE also has implications for animal health as increased exercise caused by appropriate EE implementation may help aid individual weight maintenance (Boere, 2001). Social housing had no impact on the performance of foraging or exploratory behaviors linked to the presence of EE.

Scatter-feeding was the least effective EE in influencing armadillo behavior; this may be due to it requiring little cognitive skill, therefore having a limited impact on time spent on exploratory behavior. Exhibit size could also impact on the amount of time that armadillos spend foraging, and this is worthy of further investigation across exhibits to understand the impacts of space and degree of environmental complexity on engagement with EE. Although wild armadillos spend much of their active hours digging and foraging (Ancona & Loughry, 2009), it may be that these captive armadillos were not sufficiently motivated to forage when the reward for contra freeload (i.e., working for a reward when food is readily available) was so low (McGowan et al., 2010). Also, a decrease in alert behaviors was observed when a scatter-feed was provided, which may be attributed a the large amount of time armadillos spent in their nest boxes, thus not fully engaging with this form of EE.

Although mealworms are a favored food item, aside from when presented in EE and during baseline conditions, they are presented in their food bowl and, therefore, easier to locate and only represent a small part of their diet. Future studies could introduce unpredictability by presenting the scatter-feed at different times in the day, ideally when the armadillos are active as this may reduce habituation to the EE and subsequently promote foraging behaviors (Schneider et al., 2014). Evidence suggests that wild armadillos spend up to 90% of their waking hours foraging (Ancona & Loughry, 2009), but in this study, foraging remained low for the entire observation period. However, the large amount of time spent resting by these armadillos is concurrent with other published research (Cortés Duarte et al., 2016; Steinmeyer et al., 2010), and it was the predominant behavior in all armadillos during the baseline condition.

Assessing the use of EE to promote increased time spent on beneficial activities, such as exploration, is applicable to captive environments outside of the zoo. The nine-banded armadillo is the only reliable laboratory model for epidemiological study of leprosy (*Mycobacterium leprae*) and is used for drug trials due to the genetic similarities of its offspring (Sharma et al., 2013). Research on this species dominates existing literature on armadillo health, ecology, and physiology (Carvalho et al., 2017; Loughry et al., 2015). Laboratory animals can benefit from ecologically relevant EE because it enables animals to adapt more easily to new environments and perform better in cognitive-based tasks (Simpson & Kelly, 2011). Thus, the results from this study are applicable to the lab as well as to the zoo.

4.1 | Research extensions and potential methodological developments

Future studies into the effects of EE on zoo-housed armadillos should aim to observe these species across a range of times throughout a full 24 h period to give a better representation of their true activity patterns. The reversed day-night cycle influenced the choice of data collection time intervals (12:00–14:40 p.m.) to increase any chances of recording behavior during normal opening hours due to the typically nocturnal activity patterns of each of these species (Attias et al., 2020; Frontini & Escosteguy, 2012; Maccarini et al., 2015). It is noted that positive changes to time spent on important behaviors due to EE, can vary among species (Cortés Duarte et al., 2016), but due to the small sample size and lack of individual recognition during data collection, differences amongst species could not be further analyzed and evaluated.

The time of day should be further investigated to understand temporal effects on natural behavior patterns for captive animals. In the wild, SBA activity is considered a mix of nocturnal and diurnal patterns (Attias et al., 2018), and STBA and LHA are considered fully nocturnal (Attias et al., 2020; Frontini & Escosteguy, 2012). Although not empirically tested, during this study, SBA were as inactive as STBA and LHA during throughout the entire study period. However, this may be due to the influence of increased temperatures causing less activity in SBA (Attias et al., 2018).

Further studies should compare the behavioral repertoire of armadillos against a wider range of temperatures because species variation in responses to temperature is apparent in the wild; Inbar and Mayer (1999) found that nine-banded armadillos became more active during cooler winter months. It should also be noted that nest box use was positively influenced by scatter-feed EE, and therefore such increased time within a nest box may suggest the armadillo was satiated and comfortable. Satiation after EE is seen in other animal models, for example, Fischer et al. (2016). Active use of EE is also not considered essential for the animal to gain benefits from its presence (Decker et al., 2023), and therefore, positive outputs from the provision of EE manifest as resting and comfort behavior. However, excessive inactivity (i.e., more than is considered normal for an individual) should be investigated further to evaluate the suitability and relevance of each form of EE in promoting positive behavioral outputs at an individual animal level.

Armadillos exposed to visitors may show an increased number of stereotypical behaviors (Baird et al., 2016), however, this study showed no influence of visitor presence on armadillo behavior. Although during the observation period, visitor numbers remained consistently low, and as the number of visitors was recorded within the whole nocturnal area and not necessarily at the armadillos enclosure, they may not have been in view of the armadillos. Subsequently, this could have lessened the impact of visitors on the armadillos' behavior. Further study could explore the relationship between armadillo activity and visitor numbers in greater depth by analyzing the impact of visitors and visitor densities on armadillos during periods when they are not in their nest boxes.

Neither exploratory nor foraging behavior was influenced by the housing of armadillos alone or in pairs. Therefore, EE used to promote such important active behaviors in captive armadillos is unaffected by social group, and any difference in the housing of captive armadillos should not be considered a barrier to effective EE implementation. Overall time spent foraging behavior may appear uncommon due to the averaging of data for pair-housed animals. However, time spent foraging was also similarly uncommonly observed for solitary armadillos. Further research following the time-activity budgets of identifiable individuals is required to fully ascertain any impact of social grouping, type of EE, husbandry regime, and aspects of the physical environment on beneficial active behaviors (i.e., foraging and exploration) (Bozicovich et al., 2016). Given that solitary armadillos were more likely to be seen moving and being alert, and pair-housed animals more likely to be resting, the choice of a social partner may be an important mediator of animal comfort and restfulness and should be investigated further.

5 | CONCLUSIONS

This research has demonstrated that the implementation of physical object-based EE can significantly increase a range of natural active behaviors in zoo-housed armadillos, with a plastic ball containing

food being the most beneficial EE to use for the promotion of exploratory and locomotory behaviors. Armadillo behavior was also influenced by environmental temperature but not by visitor presence, and these data can help facilities replicate environmental conditions accordingly for their animals. Group or solitary housing did not influence the performance of foraging or exploratory behaviors performed by the armadillos, but social choice may benefit overall behavior and degree of comfort experienced. Overall, this information can be used to support guidelines on EE provided to armadillos in a range of captive environments, including those housed in laboratories, to enhance welfare and opportunities and promote natural behaviors.

AUTHOR CONTRIBUTIONS

The idea for the paper was conceived by Robert Kelly. The experiments were designed and performed by Robert Kelly. These data were analyzed by Robert Kelly and Paul Rose. The paper was written by Robert Kelly and Paul Rose.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open Research Exeter – ORE at <https://ore.exeter.ac.uk/oredeposit/auth>. Data will be made available at a link to a data-sharing repository if this paper is accepted for publication.

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REFERENCES

- Abba, A. M., Lima, E., & Superina, M. (2014). *Euphractus sexcinctus*. *The IUCN Red List of Threatened Species 2014*: e.T8306A47441708.
- Abba, A. M., Poljak, S., & Superina, M. (2014). *ChaetophRACTUS villosus*. *The IUCN Red List of Threatened Species 2014*: e.T4369A47438745.
- Anacleto, T. C. deS. (2007). Food habits of four armadillo species in the cerrado area, mato grosso, Brazil. *Zoological Studies*, 46, 529–537.
- Ancona, K. A., & Loughry, W. J. (2009). Time budgets of wild nine-banded armadillos. *Southeastern Naturalist*, 8, 587–598.
- Ancona, K. A., & Loughry, W. J. (2010). Sources of variation in the time budgets of wild nine-banded armadillos. *Mammalia*, 74, 127–134. <https://doi.org/10.1515/mamm.2010.022>
- Attias, N., Gurarie, E., Fagan, W. F., & Mourão, G. (2020). Ecology and social biology of the Southern three-banded armadillo (*Tolypeutes*

- matacus*; Cingulata: Chlamyphoridae). *Journal of Mammalogy*, 101, 1692–1705. <https://doi.org/10.1093/jmammal/gyaa117>
- Attias, N., Oliveira-Santos, L. G. R., Fagan, W. F., & Mourão, G. (2018). Effects of air temperature on habitat selection and activity patterns of two tropical imperfect homeotherms. *Animal Behaviour*, 140, 129–140. <https://doi.org/10.1016/j.anbehav.2018.04.011>
- Baird, B. A., Kuhar, C. W., Lukas, K. E., Amendolagine, L. A., Fuller, G. A., Nemet, J., Willis, M. A., & Schook, M. W. (2016). Program animal welfare: Using behavioral and physiological measures to assess the well-being of animals used for education programs in zoos. *Applied Animal Behaviour Science*, 176, 150–162. <https://doi.org/10.1016/j.applanim.2015.12.004>
- Baker, B., Taylor, S., & Montrose, V. T. (2018). The effects of olfactory stimulation on the behavior of captive ring-tailed lemurs (*Lemur catta*). *Zoo Biology*, 37, 16–22. <https://doi.org/10.1002/zoo.21392>
- Barton, K., 2013. *MuMIn: Multi-model inference*. R package version 1.9.13.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57, 289–300. <https://doi.org/10.1111/j.2517-6161.1995.tb02031.x>
- Boccacino, D., Maia, C. M., Dos Santos, E. F., & Santori, R. T. (2020). Inactivity at night: A case study of the nocturnal behaviors of two captive *Panthera onca* (Felidae) specimens. *Acta Biológica Colombiana*, 25, 359–367. <https://doi.org/10.15446/abc.v25n3.80892>
- Boere, V. (2001). Environmental enrichment for neotropical primates in captivity. *Ciencia Rural*, 31(3), 543–551. <https://doi.org/10.1590/S0103-84782001000300031>
- Bozicovich, T. F. M., Moura, A. S. A. M. T., Fernandes, S., Oliveira, A. A., & Siqueira, E. R. S. (2016). Effect of environmental enrichment and composition of the social group on the behavior, welfare, and relative brain weight of growing rabbits. *Applied Animal Behaviour Science*, 182, 72–79. <https://doi.org/10.1016/j.applanim.2016.05.025>
- Bracke, M. B. M., & Hopster, H. (2006). Assessing the importance of natural behavior for animal welfare. *Journal of Agricultural and Environmental Ethics*, 19, 77–89. <https://doi.org/10.1007/s10806-005-4493-7>
- Browning, H. (2019). The natural behavior debate: Two conceptions of animal welfare. *Journal of Applied Animal Welfare Science*, 23, 325–337. <https://doi.org/10.1080/10888705.2019.1672552>
- Carvalho, T. S. G., Saad, C. E. P., Alvarenga, R. R., Ferreira, W. M., Assis, V. D. L., Pereira, V. M., Scalón, J. D., Silva, J. P., & Zangeronimo, M. G. (2017). Use of collard green stalks as environmental enrichment for cockatiels (*Nymphicus hollandicus*) kept in captivity. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 69, 718–724. <https://doi.org/10.1590/1678-4162-8988>
- Clark, F. E., & Melfi, V. A. (2012). Environmental enrichment for a mixed-species nocturnal mammal exhibit. *Zoo Biology*, 31, 397–413. <https://doi.org/10.1002/zoo.20380>
- Cortés Duarte, A., Trujillo, F., & Superina, M. (2016). Behavioral responses of three armadillo species (Mammalia: Xenarthra) to an environmental enrichment program in Villavicencio, Colombia. *Zoo Biology*, 35, 304–312. <https://doi.org/10.1002/zoo.21305>
- Dalponde, J. C., & Tavares-Filho, J. A. (2004). Diet of the yellow armadillo, *Euphractus sexcinctus*, in South-Central Brazil. *Edentata*, 6, 37–41. <https://doi.org/10.1896/1413-4411.6.1.37>
- Decker, S., Lavery, J. M., & Mason, G. J. (2023). Don't use it? Don't lose it! Why active use is not required for stimuli, resources or "enrichments" to have welfare value. *Zoo Biology*, 42, 467–475. <https://doi.org/10.1002/zoo.21756>
- DeGregorio, B. A., Veon, J. T., & Massey, A. (2022). Wildlife associates of nine-banded armadillo (*Dasybus novemcinctus*) burrows in Arkansas. *Ecology and Evolution*, 12, e8858. <https://doi.org/10.1002/ece3.8858>
- Fischer, M. L., Aguero, W. P., Rodrigues, G. S., Simão-Silva, D. P., & Moser, A. M. (2016). Enriquecimento ambiental como princípio ético nas pesquisas com animais. *Revista Bioética*, 24, 532–541. <https://doi.org/10.1590/1983-80422016243153>
- Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage.
- Frontini, R., & Escosteguy, P. (2012). *Chaetophractus villosus*: A disturbing agent for archaeological contexts. *International Journal of Osteoarchaeology*, 22, 603–615. <https://doi.org/10.1002/oa.1278>
- Halekoh, U., & Højsgaard, S. (2014). A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models - TheRPackagepbkrtest. *Journal of Statistical Software*, 59, 1–30. <https://doi.org/10.18637/jss.v059.i09>
- Hamilton, J., Fuller, G., & Allard, S. (2020). Evaluation of the impact of behavioral opportunities on four zoo-housed aardvarks (*Orycteropus afer*). *Animals*, 10, 1433. <https://doi.org/10.3390/ani10081433>
- Hosey, G., Ward, S., & Melfi, V. (2023). The effect of visitors on the behaviour of zoo-housed primates: A test of four hypotheses. *Applied Animal Behaviour Science*, 263, 105938. <https://doi.org/10.1016/j.applanim.2023.105938>
- Hosey, G. R. (2000). Zoo animals and their human audiences: What is the visitor effect? *Animal Welfare*, 9, 343–357.
- Inbar, M., & Mayer, R. T. (1999). Spatio-temporal trends in armadillo diurnal activity and road-kills in central florida. *Wildlife Society Bulletin*, 27, 865–872.
- Kuznetsova, A., Brockhoff, P., & Christensen, R. H. B. (2016). *lmerTest: Tests in linear mixed effects models*. R package version, 2.0-33. <https://CRAN.R-project.org/package=lmerTest>
- Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, 69, 1–33. <https://doi.org/10.18637/jss.v069.i01>
- Loughry, W. J., Superina, M., McDonough, C. M., & Abba, A. M. (2015). Research on armadillos: a review and prospectus. *Journal of Mammalogy*, 96, 635–644. <https://doi.org/10.1093/jmammal/gyv005>
- Maccarini, T. B., Attias, N., Medri, Í. M., Marinho-Filho, J., & Mourão, G. (2015). Temperature influences the activity patterns of armadillo species in a large neotropical wetland. *Mammal Research*, 60, 403–409. <https://doi.org/10.1007/s13364-015-0232-2>
- Martin, P. R., & Bateson, P. P. G. (2007). *Measuring behavior: An introductory guide* (3rd ed.). Cambridge University Press.
- Mason, G., Clubb, R., Latham, N., & Vickery, S. (2007). Why and how should we use environmental enrichment to tackle stereotypic behavior? *Applied Animal Behaviour Science*, 102, 163–188. <https://doi.org/10.1016/j.applanim.2006.05.041>
- McGowan, R. T., Robbins, C. T., Alldredge, J. R., & Newberry, R. C. (2010). Contrafreeloading in grizzly bears: implications for captive foraging enrichment. *Zoo Biology*, 29, 484–502. <https://doi.org/10.1002/zoo.20282>
- Meehan, C. L., & Mench, J. A. (2007). The challenge of challenge: Can problem solving opportunities enhance animal welfare? *Applied Animal Behaviour Science*, 102, 246–261. <https://doi.org/10.1016/j.applanim.2006.05.031>
- Niemelä, P. T., & Dingemans, N. J. (2014). Artificial environments and the study of 'adaptive' personalities. *Trends in Ecology & Evolution*, 29, 245–247. <https://doi.org/10.1016/j.tree.2014.02.007>
- Noss, A., Superina, M., & Abba, A. M. (2014). *Tolypeutes matacus*. The IUCN Red List of Threatened Species 2014:e.T21974A47443233. <https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T21974A47443233.en>
- R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rideout, B. A., Gause, G. E., Benirschke, K., & Lasley, B. L. (1985). Stress-induced adrenal changes and their relation to reproductive failure in captive nine-banded armadillos (*Dasybus novemcinctus*). *Zoo Biology*, 4, 129–137. <https://doi.org/10.1002/zoo.1430040206>

- Riley, L. M., & Rose, P. E. (2020). Concepts, applications, uses and evaluation of environmental enrichment. *Journal of Zoo and Aquarium Research*, 8, 18–28. <https://doi.org/10.19227/jzar.v8i1.384>
- Rodrigues, T. F., Mantellatto, A. M. B., Superina, M., & Chiarello, A. G. (2020). Ecosystem services provided by armadillos. *Biological Reviews*, 95, 1–21. <https://doi.org/10.1111/brv.12551>
- Rose, P., & Riley, L. (2019). The use of qualitative behavioral assessment to zoo welfare measurement and animal husbandry change. *Journal of Zoo and Aquarium Research*, 7, 150–161. <https://doi.org/10.19227/jzar.v7i4.423>
- Rose, P. E. (2018). Ensuring a good quality of life in the zoo. Underpinning welfare-positive animal management with ecological evidence. In M. Berger, & S. Corbett (Eds.), *Zoo animals: Behavior, welfare and public interactions* (pp. 141–198). Nova Science Publishers Inc.
- Rose, P. E., Scales, J. S., & Brereton, J. E. (2020). Why the “visitor effect” is complicated. unraveling individual animal, visitor number, and climatic influences on behavior, space use and interactions with Keepers—A case study on captive hornbills. *Frontiers in Veterinary Science*, 7, 236. <https://doi.org/10.3389/fvets.2020.00236>
- RStudio Team. (2020). RStudio: Integrated Development for R. <http://www.rstudio.com/>
- Schneider, M., Nogge, G., & Kolter, L. (2014). Implementing unpredictability in feeding enrichment for Malayan sun bears (*Helarctos malayanus*). *Zoo Biology*, 33, 54–62. <https://doi.org/10.1002/zoo.21112>
- Sharma, R., Lahiri, R., Scollard, D. M., Pena, M., Williams, D. L., Adams, L. B., Figarola, J., & Truman, R. W. (2013). The armadillo: A model for the neuropathy of leprosy and potentially other neurodegenerative diseases. *Disease Models & Mechanisms*, 6, 19–24. <https://doi.org/10.1242/dmm.010215>
- Sherwen, S. L., & Hemsworth, P. H. (2019). The visitor effect on zoo animals: Implications and opportunities for zoo animal welfare. *Animals*, 9, 366. <https://doi.org/10.3390/ani9060366>
- Shyne, A. (2006). Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. *Zoo Biology*, 25, 317–337. <https://doi.org/10.1002/zoo.20091>
- Simpson, J., & Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats—Behavioural and neurochemical aspects. *Behavioural Brain Research*, 222, 246–264. <https://doi.org/10.1016/j.bbr.2011.04.002>
- Stafleu, F. R., Grommers, F. J., & Vorstenbosch, J. (1996). Animal welfare: Evolution and erosion of a moral concept. *Animal Welfare*, 5, 225–234.
- Steinmeyer, C., Schielzeth, H., Mueller, J. C., & Kempnaers, B. (2010). Variation in sleep behaviour in free-living blue tits, *Cyanistes caeruleus*: Effects of sex, age and environment. *Animal Behaviour*, 80, 853–864. <https://doi.org/10.1016/j.anbehav.2010.08.005>
- Wells, D. L. (2005). A note on the influence of visitors on the behaviour and welfare of zoo-housed gorillas. *Applied Animal Behaviour Science*, 93, 13–17. <https://doi.org/10.1016/j.applanim.2005.06.019>

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