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Can Colored Object Enrichment Reduce the Escape Behavior of Captive Freshwater Turtles?

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Abstract:

The effect of environmental enrichment on the behavior and welfare in captivity of reptiles and of freshwater turtles in particular, which are popular aquarium and pet species, is very little studied compared to other taxa.

We carried out a small scale case-study on the effect of colored object enrichment, with and without fish scent, on the behavior of a group of fifteen cooters (*Pseudemys sp.*) and sliders (*Trachemys scripta ssp.*) on display at a public aquarium. The new enrichment aimed to reduce the escape behavior (interaction with transparent boundaries) and increase exploration and random swimming. We used simultaneous recording of behavior at whole group level and for focal individually-marked turtles.

The escape behavior decreased on days with new enrichment before feeding at whole group level and for the focal turtles overall, in spite of the relatively low interest in the colored objects. Fish-scented objects attracted significantly more interest. Random swimming, enrichment focus, aggression and submission increased significantly and basking decreased significantly at whole group level before feeding, with smaller differences after feeding.

There were large differences between individual turtles with respect to activity budgets and changes in behavior on days with new enrichment, with both increases and decreases seen in escape behavior, aggression and levels of activity.

Our outcomes suggested that introducing new colored objects with food scent may be beneficial for reducing escape behavior in captive freshwater turtles. However, careful monitoring of effects at individual level and much larger scale investigations, including post-enrichment periods, are needed.

Keywords: olfactory cues, behavioral types, *Pseudemys sp.*, *Trachemys scripta ssp.*

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1. INTRODUCTION

A very small proportion of the peer-reviewed articles that evaluate the many types of enrichment used in everyday practice in zoos and aquaria have been carried out on reptiles (Rose, Brereton, Rowden, Lemos de Figueiredo & Riley, 2019). Global reptile populations are decreasing due to pollution, destruction of habitats, climate change and unsustainable exploitation (Maxwell et al., 2019). Reptiles have several innate behaviors, including hunting, foraging, nesting, hiding and exploration that make captive conditions unsuitable for them due to limited space and complexity and may lead to poor welfare (Burghardt, 2013). Even fewer enrichment studies have been carried out on turtles (Eagan, 2019). Of the 360 currently recognized species in the order Testudines, around 56% are threatened (Rhodin et al., 2018), placing turtles among the most threatened of the major groups of vertebrates (Lovich, Ennen, Agha & Gibbons, 2018). Therefore, improved captive conditions, as well as continued conservation and monitoring efforts are particularly important for turtles (Gibbons & Lovich, 2019).

Many species of freshwater turtles are still abundant and red-eared sliders (*T. scripta elegans*) are in fact listed among the world's 100 worst invasive species (Lowe, Browne, Boudjelas & De Poorter, 2000). However, cooters (*Pseudemys sp.*) and sliders (*Trachemys scripta ssp.*) are popular aquarium and pet species (Pasmans et al., 2017) and there are no recent peer-reviewed publications investigating the effects of enrichment on their behavior, emphasizing the need for more research to ensure good standards of welfare in zoos, aquaria and domestic settings (Moszuti, Wilkinson & Burman, 2017).

In the wild freshwater turtles spend most of their time resting, with periods of activity for food acquisition (Clavijo-Bacquet & Magnone, 2017), escaping predators (Ibanez, Martin, Gazzolla & Pellitteri-Rosa, 2018), reproduction (Rowe, Nawrot & Clark, 2017) or terrestrial migration during drought (Anthonysamy, Dreslik & Phillips, 2013). *Pseudemys sp.* and *Trachemys scripta ssp.* are more abundant in areas with slow flowing water, soft substrates, abundant vegetation and plenty of basking sites (Steen et al., 2012). In captivity, turtles actively selected enclosures enriched with vegetation, water and hiding areas (Tetzlaff, Sperry & DeGregorio, 2018). Enrichment stimulated exploratory and play behaviors (Burghardt, Ward & Rosscoe, 1996) and decreased escape behavior (Case, Lewbart & Doerr, 2005). The effectiveness of new enrichments is usually assessed based on frequency of use and behavioral changes, with decreases expected in stress indicators (Bishop, Hosey & Plowman, 2013). Individual perception may vary (Bashaw, Gibson, Schowe & Kucher, 2016) and more curious and explorative individuals may suffer more in a limited unstimulating space, while fearful ones may need more hiding places or be stressed by new objects (Richter & Hintze, 2019).

This case-study investigated the effectiveness of colored object enrichment on reducing the escape behavior of the freshwater turtles held at a public aquarium, at whole group and individual level. Colors and olfactory signals play important roles in the feeding and reproductive biology of Emydid turtles (Brejcha et al., 2019), therefore the introduction of colored objects with or without food scent was expected to attract interest by providing objects to explore and decrease escape behavior (Warwick, Arena, Lindley, Jessop & Steedman, 2013).

2. MATERIALS & METHODS

2.1. Turtles, Enclosure and Standard Enrichment

The Tynemouth Aquarium (TAQ), (England), housed 15 turtles (Table 1) in a relatively large (15m²) indoor enclosure (Figure 1). Turtles were hand-fed mid-day three times a week (whiting, bloodworms, live insects, turtle pellets, lettuce).

Table 1. Freshwater turtles housed at the Tynemouth Aquarium in November-December 2018.

Turtles ¹	Sex	Carapace Size	Code
River cooters <i>Pseudemys concinna</i> LeConte 1830	2 males 3 females	20-28 cm	Psc
Peninsula Cooter <i>Pseudemys peninsularis</i> Carr 1938	1 male	24cm	Psp
Yellow-bellied Sliders <i>Trachemys scripta scripta</i> Schoepff 1792	3 males 2 females	17-25cm	Trs
Red-eared Sliders <i>Trachemys scripta elegans</i> Wied 1839	3 females	23-25 cm	Tre
Slider <i>Trachemys scripta troostii</i> Holbrook 1836	1 male	24 cm	Trt

¹All turtles were pet rescues, captive bred. Individuals were distinguished by white or blue paint spots on different scutes of the carapace, maintained by aquarium staff. Species and subspecies identification based on van Dijk (2011a,b) and van Dijk, Harding & Hammerson (2011)

Figure 1. Turtle enclosure with various structural enrichments, according to recommendations for the species housed (Hawkins & Willemsen, 2004): Water Area 1 = 0.5 m depth, including logs, pebbles and rocks; Water Area 2 = 0.5 m depth, closer to waterfall and bright light, including lower end of ramp and hiding places under decking (shaded areas); Decking Area 3 = dry flat wooden planks and upper part of ramp used to climb out of water; Sand Area 4 = sand pit under the heat lamp and UV/VIS light (provided 8:00-18:00). The filtered recirculated pool water was maintained at 24±0.5°C. Fish kept in the turtle pool included cichlids (*Andinoacara rivulatus*, *Amphilophus citrinellus*, *Heros severus*) and catfish (*Ameiurus melas*).



2.2. New Enrichment

The new enrichment involved suspending two colored cups (Figure 2.a) with or without fish scent and feeder toy (Figure 2.b) into the water, next to the front glass boundary (Figure 2.c), in various combinations (Table 2).

Figure 2. Enrichment devices used: a) colored plastic cups (white, red, green and yellow; 65 x 65 x 45 mm; ASDA Stores Ltd. UK), chosen to be of biologically relevant colors, based on body coloration (red, yellow) and food given (green, white); b) yellow fish-shaped turtle feeder (190 x 70 x 40 mm; Zoo Med Laboratories Inc. USA); c) location of colored objects in the turtle pool: left = near the waterfall and the light source; right = near the opposite corner of the enclosure.



Table 2. Novel colored enrichment object and olfactory cues combinations presented to the turtles at the Tynemouth Aquarium.

Stimulus Type ¹	Colors Presented ²
Color & No fish scent 4 x 30min observation periods	Red – Yellow; Green - White Red – Red; Yellow –Yellow
Color & Fish scent 6 x 30min observation periods	Red – Yellow; Green – White; Red – Red; Yellow –Yellow; Green – Green; White – White
Color & Fish scent & Fish toy 6 x 30min observation periods	Red – Yellow; Green – White; Red – Red; Yellow –Yellow; Green – Green; White – White

¹The fish scent was applied with gloved hands to clean objects immediately prior to the start of each observation period by rubbing the objects twice on all sides with a piece of defrosted whiting (20 x 20 x 10 mm size), normally used for feeding. ²The same color combination was used for a whole 30 minutes observation period, swapping sides every 5 minutes. On the day when the ‘color & no fish scent’ stimulus was used, two observation periods were completed without stimulus, with the data grouped as ‘new enrichment’.

2.3. Data Collection

Data were collected on six days in November-December 2018, once a week, on feeding days, 6x30 minute periods per day, before (10:30-12:00) and after feeding (13:00-14:30).

Focal instantaneous behavior sampling every 30 seconds (Observer 1; two turtles per observation period, usually the nearest two in sight that had not already been observed during the same half-day, distinguished by paint markings) and continuous whole group scan observation (Observer 2; recording all behaviors that occurred at least once during each 1-minute interval, one-zero rule) (Martin & Bateson, 2007) were used simultaneously, according to the same ethogram (Table 3), with high inter-observer reliability (0.937 intra-class correlation coefficient; Rees, 2015). The interest in the new enrichment was quantified as the number of 1-minute intervals during which the turtles interacted with the colored objects.

Table 3. Freshwater turtle ethogram adapted from Liu et al. (2009) and Therrien et al. (2007). Behaviors marked with an asterisk (*) were categorised as ‘naturalistic’, based on similarity to behaviors described for Emydid turtles in the wild (Thomas et al., 1999)

Behaviors	Description (Category of Behavior ¹ where applicable)
Walking*	Slowly crawling or creeping forward by alternating limb movement on flat areas (Active)
Climbing*	Moving slowly up a gradient using the forelimbs, while supporting body with hind legs (Active)
Random Swimming*	Moving in the water by paddling with alternating limbs, without following the same route more than twice (Active)
Pattern Swimming ²	Swimming along the same repetitive route around the pool more than twice
Escape Behavior	Swimming continuously or repeatedly into and along the pool glass wall
Feeding Related*	Sniffing, biting, pulling and ingesting food; sniffing enclosure substrates searching for food (Active)
Social Affiliative*	Gentle active contact with another turtle, fish or keeper; courtship displays (sniffing, gentle biting, approaching, head bobbing, matching movements, foreclaw displays, mounting), mating
Aggression*	Head-butting, biting or performing a threatening display towards another turtle or fish in the enclosure
Submission* ³	Withdrawal into shell or moving backwards in response to an aggressive display from another turtle
Basking*	Stationary out of water, resting on sand under the heat lamp, or on decking (Passive)
Resting in Water*	Stationary in the water, on the bottom of the pool, or on top of another turtle (included turtles passively receiving foreclaw display) (Passive)
Digging ³	Using front flippers to move substrates on the bottom of the pool (Active)
Enrichment Focus*	Focus on existing or new enrichment items: contact, sniffing, biting, pushing, pulling, rubbing, etc. (Active)
Other*	Jumping, standing, yawning, any other uncategorised behavior (Active)
Out of Sight*	Stationary, hidden in the dark area under the decking (the only hiding place available in their enclosure). (Passive)

¹Further grouping into categories of behavior for the statistical analysis of the focal data: Active = Walking, Climbing, Random Swimming, Feeding Related, Enrichment Focus and Other; Passive = Basking, Resting in Water and Out of Sight; ²not seen at all; ³not seen for the focal turtles.

2.4. Statistical Analysis

Relative frequencies (% of all behaviors recorded) were calculated for each behavior for each observation period for the whole group scan data (36 data sets) and for each observation period and turtle for the focal data (36x2=72 data sets).

The influence of the new enrichment on whole group behaviour before and after feed was tested using the Mann-Whitney U test ($P \leq 0.05$ level of significance; data not normally distributed; Kolmogorov-Smirnov test, $P < 0.05$; SPSS V25). The focal data (actual frequencies) were grouped into five broader behavior categories (Table 3) and the two-way Pearson's chi-square test of association (Microsoft Excel, $p \leq 0.05$ level of significance) (Plowman, 2008) was used to determine whether the new enrichment influenced the category of behavior displayed (for all focal turtles, before and after feeding, and for each individual turtle), The same test was also used to analyse whether the time relative to feeding and the stimulus type influenced the turtles' interest in the new enrichment.

2.5. Ethical Considerations

The project received ethical approval from the Ethics Commission of Northumbria University and consent from the staff at the TAQ.

3. RESULTS

3.1. Interest in the New Enrichment Items

The whole group of turtles showed interest in the new enrichment during 31% of all 1-minute intervals, with significantly more than expected interest in the fish-scented cups (Table 4.a). The focal turtles showed less interest, during only 11% of the 1-minute intervals, with significantly more than expected interest in the combination of fish-scented cups and fish-shaped toy (Table 4.a). Both the whole group and the focal turtles were significantly more interested in the novel enrichment before feeding (Table 4.b).

3.2. Whole Group Behavior

Under standard enrichment conditions, the activity budgets of the whole group of turtles consisted mainly of random swimming, resting in water, basking, social affiliative interactions and feeding related behaviors, both before and after feeding (Figure 3). The escape behavior occurred relatively frequently before feeding (12%) and decreased to 7.2% after feeding. The relative frequencies of aggression, submission and enrichment focus were low before feeding (Figure 3a) and increased after feeding (Figure 3b).

On days with new enrichment, the relative frequency of escape behavior before feeding decreased to 7.9% (Figure 3a). Basking decreased significantly from 20.1% to 11.6%. Significant increases were found for random swimming (19% to 21.7%), enrichment focus (2.6% to 10.5%), aggression (0.6% to 3.5%) and submission (0.5% to 5%). After feeding, the differences relative to days with standard enrichment were of smaller magnitudes and no statistical significance (Figure 3b).

3.3 Focal Turtles Overall Behavior

Under standard enrichment conditions, around 41-44% of the activity budget of the focal turtles was represented by active behaviors and affiliative or aggressive social interactions (Table 5). Escape behavior represented 7.8% of the budget both before and after feeding. On days with new enrichment, the relative frequency of escape behaviour was significantly lower than expected before feeding, at

4.8%, with corresponding increases in the relative frequencies of aggression and active behaviours and decreases in passive behaviors.

Table 4: Turtle interest in the novel enrichment: the influence of a) type of stimuli, and b) time relative to feeding. The values represent the percentage and number (in brackets) of 1-minute intervals out of the total observation time during which “Interest” (at least one direct contact with cup) or “No Interest” (zero contacts) were recorded.

a) Type of Stimuli ¹	Whole Group		Focal Turtles	
	Interest	No Interest	Interest	No Interest
Colored Cups with No Scent	29% (35)	71% (85)	8% (10)	92% (110)
Colored Cups with Fish Scent	42% (75)	58% (105)	8% (15)	92% (165)
Colored Cups & Fish Shape with Fish Scent	22% (39)	78% (141)	16% (28)	84% (152)
b) Time Relative to Feeding ²	Interest	No Interest	Interest	No Interest
Before Feed	51% (107)	49% (103)	16% (34)	84% (176)
After Feed	16% (42)	84% (228)	7% (19)	93% (251)
Total	31% (149)	69% (331)	11% (53)	89% (427)

¹Chi-square test, $X^2_2 = 17.1$, $P = 0.0002$ for the whole group, $X^2_2 = 6.0$, $P = 0.050$ for the focal turtles;

²Chi-square test, $X^2_1 = 68.8$, $P < 0.0001$ for the whole group, $X^2_1 = 10.1$, $P = 0.002$ for the focal turtles;

3.4. Individual Turtles Behavior

Under standard enrichment conditions, the most active turtles were Psp1M (17.1% passive) and Trs3M (27.8% passive) (Table 6, see caption for turtle codes). Psp1M also displayed most social affiliative behavior (15%) and most escape behavior (26.7%) of all turtles. The least active turtles were Psc1F (84.2% passive) and Trs1F (67.2% passive).

The new enrichment appeared to have different effects on individuals, with significant changes in behavior for five turtles (Table 6). For escape behavior, there was a 2.2 times decrease for Psp1M (highest value), a 13.6 times decrease for Trt1M (third highest value) and a 3.5 times increase for Psc3M (fourth highest value). Aggression decreased for Psc3M (4.5 times) and Psc2M (7.3 times) (highest values of 5% and 4.4%, respectively, under standard conditions) and increased for Trs3M (13 times, from 0.6% to 7.8%) and for Trt1M (from 0 to 5%).

Figure 3. Effect of the new enrichment on whole group behaviour: a) before feeding (means + standard deviations; $N = 9$; Mann-Whitney U tests: $* = P \leq 0.05$; $** = P \leq 0.01$; $*** = P \leq 0.001$); b) after feeding (means + standard deviations; $N = 9$; Mann-Whitney U tests: no significant differences at $P \leq 0.05$ level of significance).

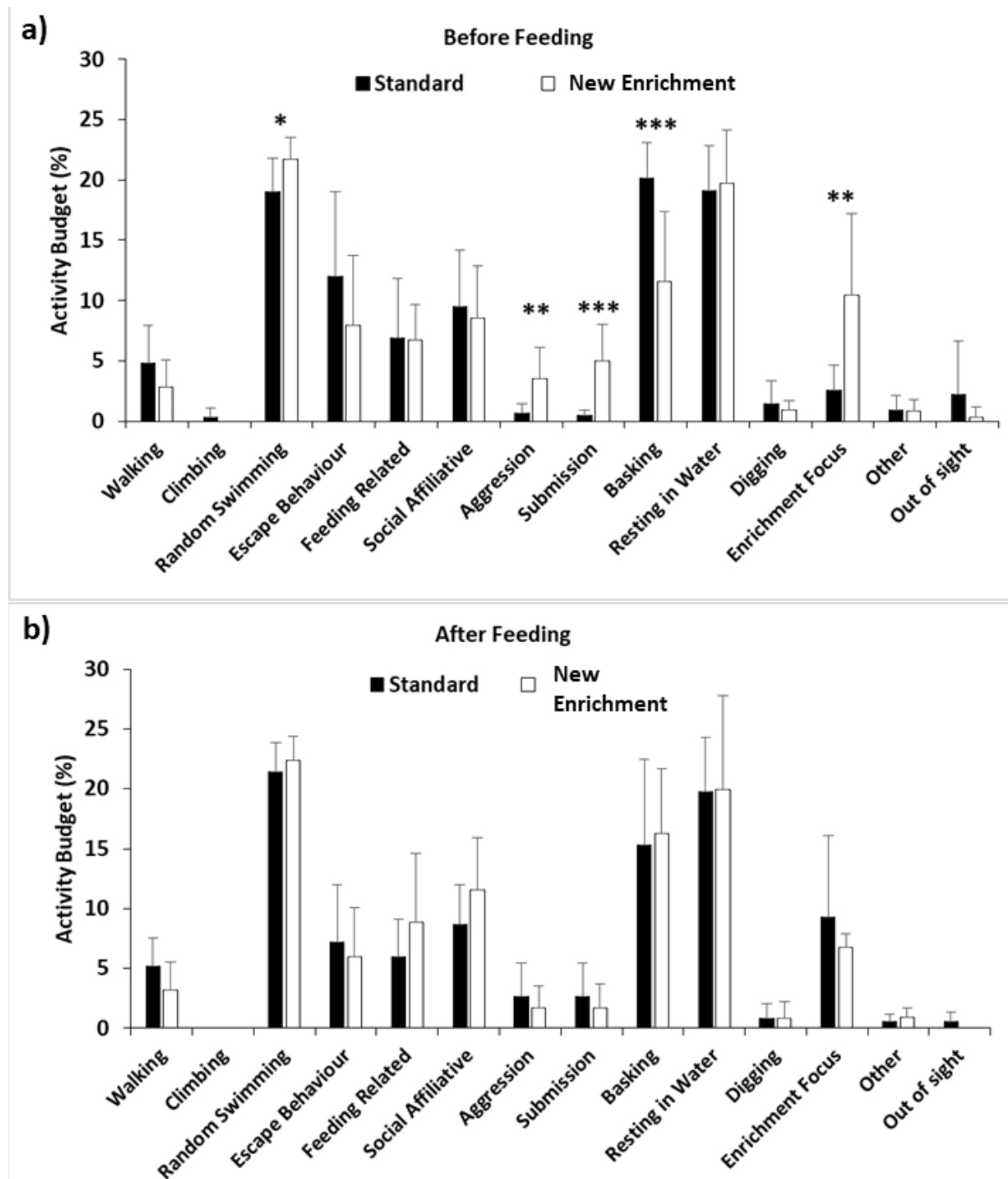


Table 5. Focal turtles overall behavioral time budgets under standard and new enrichment conditions, before (data sets: 18 under standard and 18 under new enrichment conditions) and after feeding (data sets: 18 under standard and 18 under new enrichment conditions) (NS = not significant at $P \leq 0.05$ level of significance; Active = Walking, Climbing, Random Swimming, Feeding Related, Enrichment Focus and Other; Passive = Basking, Resting in Water and Out of Sight).

Time Chi-Square Test Outcome	Enrichment Type	Category of Behaviour (Relative Frequency %)				
		Active	Aggression	Escape Behaviour	Passive	Social Affiliative
Before Feed $P = 0.0002$ $X^2_4 = 22.5$	Standard	34	1.7	7.8	51.3	5.3
	New	40	3	4.8	45.5	6.8
	<i>Total</i>	37	2.3	6.3	48.4	6
After Feed NS	Standard	40.8	0.6	7.8	48.4	2.3
	New	39	0.4	7.7	48.9	4.1
	<i>Total</i>	39.9	0.5	7.7	48.7	3.2

4. DISCUSSION

The escape behavior of the turtles at the TAQ decreased on days with new enrichment before feeding, possibly due to having more choice of items to explore. Random swimming and enrichment focus increased and there was significantly more interest in the fish-scented colored objects before feeding, suggesting a predominantly food-related motivation, rather than interest in colors, as expected based on the turtles' tetrachromatic UV/VIS vision (Ventura, Zana, De Souza & Devoe, 2001).

The escape behavior was targeted by the enrichment intervention because it represented up to 26.7% of individual turtle activity budgets under standard conditions. At whole group level, around 85-86% of the activity budgets of the turtles at the TAQ was represented by naturalistic behaviors also seen in the wild, suggesting good welfare (Warwick et al., 2013). However, recent literature recommended consideration of enrichment at individual level (Richter & Hintze, 2019), as more evidence accumulates supporting the existence of personality and individuality in many species, including reptiles (Waters, Bowers & Burghardt, 2017). Frequent escape behavior is associated with stress or low welfare caused by the unavoidable limitations in space and structural complexity (Burghardt, 2013) and may cause abrasions or wounds to the head and neck, seen in captive but not in wild reptiles (Rose, Nash & Riley, 2017). Studies on other species of freshwater turtles showed that object and structural enrichment reduced undesirable behaviors and stimulated behaviors associated with good welfare by providing behavioral choices (Burghardt et al., 1996, Case et al., 2005).

There were large differences between individual turtles with respect to changes in escape behavior, aggression and levels of activity on days with new enrichment. The turtle with the most escape behavior and social affiliative behavior (Psp1M) was a very brightly colored turtle, indicative of good health and nutritional status (Steffen, Hultberg & Drozda, 2019) and possibly stronger drive to seek mates. On days with new enrichment, Psp1M displayed significantly less escape behavior, while Psc3M displayed significantly more escape behavior, possibly associated with its higher level of interest in the colored objects than other turtles, and Trs3M and Trt1M were significantly more aggressive, suggesting food-related or color-induced sexual motivation (Brejcha & Kleisner, 2016), as both showed more interest in red and yellow objects (Thomson, unpublished observations). *Trachemys scripta* are environmentally aggressive turtles, who threaten and bite during competition for food (Polo-Cavia, Lopez & Martin, 2011). Trs3M and Trt1M were also the most melanistic of the

males at the TAQ, and melanistic males engage in aggressive courtship more frequently than non-melanistic ones (Thomas, 2002).

Table 6. Behavioral time budgets of individual turtles under standard and new enrichment conditions, with the number of data sets for each enrichment condition shown in brackets (Turtle codes: Psc1-3 = *Pseudemys concinna concinna*; Psp1 = *Pseudemys peninsularis*; Tre1 = *Trachemys scripta elegans*; Trs1-3 = *Trachemys scripta scripta*; Trt1 = *Trachemys scripta troostii*; F = Female; M = Male); (Chi-square test outcomes: number of d.f. variable 1-4, due to further grouping of aggression and/or escape behaviour within the active category and of social affiliative within the passive category where frequencies were zero or very low, to maintain validity of the Chi-square test; NS = not significant at $P \leq 0.05$ level of significance; Active = Walking, Climbing, Random Swimming, Feeding Related, Enrichment Focus and Other; Passive = Basking, Resting in Water and Out of Sight).

Turtle Code Chi-Square Test Outcome	Enrichment Type	Category of Behaviour (Relative Frequency %)				
		Active	Aggression	Escape Behaviour	Passive	Social Affiliative
Psc1F NS	Standard (2)	14.2	0.0	1.7	84.2	0.0
	New (3)	11.1	1.1	2.2	84.4	1.1
	<i>Total (5)</i>	<i>12.3</i>	<i>0.7</i>	<i>2.0</i>	<i>84.3</i>	<i>0.7</i>
Psc2M $P < 0.05$ $\chi^2_2 = 8.6$	Standard (3)	45.0	4.4	3.3	37.8	9.4
	New (3)	43.3	0.6	5.6	48.9	1.7
	<i>Total (6)</i>	<i>44.2</i>	<i>2.5</i>	<i>4.4</i>	<i>43.3</i>	<i>5.6</i>
Psc3M $P < 0.001$ $\chi^2_3 = 18.8$	Standard (2)	40.8	5.0	7.5	45.8	0.8
	New (3)	38.3	1.1	26.7	29.4	4.4
	<i>Total (5)</i>	<i>39.3</i>	<i>2.7</i>	<i>19.0</i>	<i>36.0</i>	<i>3.0</i>
Psp1M $P < 0.001$ $\chi^2_3 = 35.6$	Standard (4)	41.3	0.0	26.7	17.1	15.0
	New (4)	62.5	0.4	12.1	5.8	19.2
	<i>Total (8)</i>	<i>51.9</i>	<i>0.2</i>	<i>19.4</i>	<i>11.5</i>	<i>17.1</i>
Tre1F NS	Standard (2)	40.0	1.7	16.7	40.0	1.7
	New (3)	49.4	0.0	14.4	30.0	6.1
	<i>Total (5)</i>	<i>45.7</i>	<i>0.7</i>	<i>15.3</i>	<i>34.0</i>	<i>4.3</i>
Trs1F NS	Standard (3)	27.2	0.6	4.4	67.2	0.6
	New (4)	25.8	0.0	0.8	72.9	0.4
	<i>Total (7)</i>	<i>26.4</i>	<i>0.2</i>	<i>2.4</i>	<i>70.5</i>	<i>0.5</i>
Trs2M NS	Standard (4)	31.7	0.4	3.3	62.9	1.7
	New (3)	36.7	0.0	0.0	60.6	2.8
	<i>Total (7)</i>	<i>34.0</i>	<i>0.0</i>	<i>1.9</i>	<i>61.9</i>	<i>2.1</i>
Trs3M $P < 0.01$ $\chi^2_4 = 15.9$	Standard (3)	63.9	0.6	2.8	27.8	5.0
	New (3)	66.7	7.8	3.9	18.9	2.8
	<i>Total (6)</i>	<i>65.3</i>	<i>4.2</i>	<i>3.3</i>	<i>23.3</i>	<i>3.9</i>
Trt1M $P < 0.001$ $\chi^2_3 = 26.5$	Standard (2)	31.7	0.0	15.0	51.7	1.7
	New (3)	27.8	5.0	1.1	65.0	1.1
	<i>Total (5)</i>	<i>29.3</i>	<i>3.0</i>	<i>6.7</i>	<i>59.7</i>	<i>1.3</i>

Turtle Names: Psc1F = Bertha; Psc2M = Einstein; Psc3M = Waldorf; Psp1M = Archimedes; Tre1F = Gerty; Trs1F = Rita; Trs2M = Newton; Trs3M = Oppenheimer; Trt1M = Statler

Our results suggested four possible behavioral types: passive, evasive (reduced escape behavior), explorative (more escape behavior) and aggressive. The evasive type may relate to the ‘shy’ and the explorative and aggressive to the ‘bold’ types described for wild turtles (Ibanez et al., 2018; Kashon & Carlson, 2018). However, assigning personalities to individual turtles would require more

observations and proof of consistency through time (Ibanez et al., 2018) and may be compounded by habituation and/or changes in behavior when challenged repeatedly with the same stimulus (Davis & Burghardt, 2007).

Limitations of our case-study included the small number of replicates for individual turtles and the lack of data for a post-enrichment period, due to unexpected changes in the social group structure.

Conclusions

Escape behavior tended to decrease and active behaviors increased at whole group level before feeding on days with new enrichment.

Individual changes in behavior in the presence of the new enrichment varied, with both increases and decreases in the targeted escape behavior and in aggression. Aggression could be reduced by introducing more than two new objects at a time.

More research into individual responses to new enrichment is needed, to ensure the well-being of all turtles concerned, whether pet rescues as in this case-study, or species of conservation interest.

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REFERENCES

- Anthonyamy, W.J.B., Dreslik, M.J., & Phillips, C.A. (2013) Disruptive influences of drought on the activity of a freshwater turtle. *American Midland Naturalist*, **169**, 322-335. <https://doi.org/10.1674/0003-0031-169.2.322>
- Bashaw, M.J., Gibson, M.D., Schowe, D.M., & Kucher, A.S. (2016) Does enrichment improve welfare? Leopard geckos (*Eublepharis macularius*) respond to five types of environmental enrichment. *Applied Animal Behavior Science*, **184**, 150-160. <http://dx.doi.org/10.1016/j.applanim.2016.08.003>
- Bishop, J., Hosey, G., & Plowman, A. (2013) *Handbook of Zoo Research. Guidelines for Conducting Research in Zoos*. London. BIAZA. 231 p.
- Brejcha, J., Bataller, J.V., Bosakova, Z., Geryk, J., Havlikova, M., Kleisner, K., Marsik, P., & Font, E. (2019) Body coloration and mechanisms of color production in Archelosauria: the case of deirocheline turtles. *Royal Society Open Science*, **6**, 190319. <https://royalsocietypublishing.org/doi/10.1098/rsos.190319>
- Brejcha, J., & Kleisner, K. (2016) Turtles are not just walking stones: Conspicuous coloration and sexual selection in freshwater turtles. *Biosemiotics*, **9**, 247-266. <https://doi.org/10.1007/s12304-015-9249-9>
- Burghardt, G. M. (2013) Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. *Applied Animal Behavior Science*, **147**, 286-298. <http://dx.doi.org/10.1016/j.applanim.2013.04.013>

- Burghardt, G.M., Ward, B., & Rosscoe, R. (1996) Problems of reptile play: Environmental enrichment and play behavior in a captive Nile soft-shelled turtle, *Trinoyx triunguis*. *Zoo Biology*, **15**, 223-238. [https://doi.org/10.1002/\(SICI\)1098-2361](https://doi.org/10.1002/(SICI)1098-2361)
- Case, B.C., Lewbart, G.A., & Doerr, P.D. (2005) The physiological and behavioral impacts and preference for an enriched environment in the eastern box turtle (*Terrapene carolina carolina*). *Applied Animal Behavior Science*, **92**, 353-365. <https://doi.org/10.1016/j.applanim.2004.11.011>
- Clavijo-Baquet, S.C., & Magnone, L. (2017) Daily and seasonal basking behavior in two South American freshwater turtles, *Trachemys dorbigni* and *Phrynops hilarii*. *Chelonian Conservation and Biology*, **16**, 62-69. <https://doi.org/10.2744/CCB-1201.1>
- Davis, K.M., & Burghardt, G.M. (2007) Training and long-term memory of a novel food acquisition task in a turtle (*Pseudemys nelsoni*). *Behavioral Processes*, **75**, 225-230. <https://doi.org/10.1016/j.beproc.2007.02.021>
- Eagan, T. (2019) Evaluation of Enrichment for Reptiles in Zoos. *Journal of Applied Animal Welfare Science*, **22**, 69-77. <https://doi.org/10.1080/10888705.2018.1490182>
- Gibbons, J.W., & Lovich, J.E. (2019) Where has turtle ecology been, and where is it going? *Herpetologica*, **75**, 4. <https://doi.org/10.1655/0018-0831-75.1.4>
- Hawkins, M., & Willemsen, M. (2004) Environmental enrichment for amphibians and reptiles. ASZK Reptile Enrichment Workshop 2004.
- Ibanez, A., Martin, J., Gazzolla, A., & Pellitteri-Rosa, D. (2018) Freshwater turtles reveal personality traits in their antipredatory behavior. *Behavioral Processes*, **157**, 142-147. <https://doi.org/10.1016/j.beproc.2018.08.011>
- Kashon, E.A.F., & Carlson, B.E. (2018) Consistently bolder turtles maintain higher body temperatures in the field but may experience greater predation risk. *Behavioral Ecology and Sociobiology*, **72**, 9. <https://doi.org/10.1007/s00265-017-2428-8>
- Liu, Y.-X., Wang, J., Shi, H.-T., Murphy, R.W., Hong, M.-L., He, B., Fong, J.J., Wang, J.-C., & Fu, L.-R. (2009) Ethogram of *Sacalia quadriocellata* (Reptilia: Testudines: Geoemydidae) in captivity. *Journal of Herpetology*, **43**, 318-325. <https://doi.org/10.1670/07-277R4.1>
- Lovich, J.E., Ennen, J.R., Agha, M., & Gibbons, J.W. (2018) Where have all the turtles gone, and why does it matter? *BioScience*, **68**, 771-781. <https://doi.org/10.1093/biosci/biy095>
- Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000) *100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database*. Published by The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN), 12pp. Available at: <http://www.iucn.org/dbtw-wpd/edocs/2000-126.pdf>
- Martin, P. & Bateson, P. (2007) *Measuring behaviour. An introductory guide*. 3rd Edition. Cambridge University Press. 176 p.
- Maxwell, S.L., Butt, N., Maron, M., McAlpine, C.A., Chapman, S., Ullmann, A., Segan, D.B., & Watson, J.E.M. (2019) Conservation implications of ecological responses to extreme weather and climate events. *Diversity and Distributions*, **25**, 613-625. <https://doi.org/10.1111/ddi.12878>
- Moszuti, S.A., Wilkinson, A., & Burman, O.H.P. (2017) Response to novelty as an indicator of reptile welfare. *Applied Animal Behavior Science*, **193**, 98-103. <https://doi.org/10.1016/j.applanim.2017.03.018>

- Pasmans, F., Bogaerts, S., Braeckman, J., Cunningham, A.A., Hellebuyck, T., Griffiths, R.A., Sparreboom, M., Schmidt, B.R., & Martel, A. (2017) Future of keeping pet reptiles and amphibians: towards integrating animal welfare, human health and environmental sustainability. *Veterinary Record*, **181**, 450. <https://doi.org/10.1136/vr.104296>
- Plowman, A.B. (2008) BIAZA Statistics guidelines: Toward a common application of statistical tests for zoo research. *Zoo Biology*, **27**, 226-233. <https://onlinelibrary.wiley.com/doi/abs/10.1002/zoo.20184>
- Polo-Cavia, N., Lopez, P., & Martin, J. (2011) Aggressive interactions during feeding between native and invasive freshwater turtles. *Biological Invasions*, **13**, 1387-1396. <https://doi.org/10.1007/s10530-010-9897-2>
- Rees, P.A. (2015). *Studying Captive Animals: A Workbook of Methods in Behavior, Welfare and Ecology*. Wiley-Blackwell. 320p.
- Rhodin, A.G.J., Stanford, C.B., van Dijk, P.P., Eisemberg, C., Luiselli, L., Mittermeier, R.A., Hudson, R., Horne, B.D., Goode, E.V., Kuchling, G., Walde, A., Baard, E.H.W., Berry, K.H., Bertolero, A., Blanck, T.E.G., Bour, R., Buhlmann, K.A., Cayot, L.J., Collett, S., Currylow, A., Das, I., Diagne, T., Ennen, J.R., Forero-Medina, G., Frankel, M.G., Fritz, U., Garcia, G., Gibbons, J.W., Gibbons, P.M., Shiping, G., Guntoro, J., Hofmeyr, M.D., Iverson, J.B., Kiester, A.R., Lau, M., Lawson, D.P., Lovich, J.E., Moll, E.O., Paez, V.P., Palomo-Ramos, R., Platt, K., Platt, S.G., Pritchard, P.C.H., Quinn, H.R., Caesar Rahman, S., Tahafe Randrianjafizanaka, S., Schaffer, J., Selman, W., Shaffer, H.B., Sharma, D.S.K., Haitao, S., Singh, S., Spencer, R., Stannard, K., Sutcliffe, S., Thomson, S., & Vogt, R.C. (2018) Global conservation status of turtles and tortoises (Order Testudines). *Chelonian Conservation and Biology*, **17**, 135-161. <https://doi.org/10.2744/CCB-1348.1>
- Richter, S.H., & Hintze, S. (2019) From the individual to the population – and back again? Emphasising the role of the individual in animal welfare science. *Applied Animal Behavior Science*, **212**, 1-8. <https://doi.org/10.1016/j.applanim.2018.12.012>
- Rose, P.E., Brereton, J.E. Rowden, L.J. Lemos de Figueiredo, R. & Riley, L.M. (2019) What's new from the zoo? An analysis of ten years of zoo-themed research output. *Palgrave Communications*, **5**, 128 (2019). <https://doi.org/10.1057/s41599-019-0345-3>
- Rose, P.E., Nash, S.M., & Riley, L.M. (2017) To pace or not to pace? A review of what abnormal repetitive behavior tells us about zoo animal management. *Journal of Veterinary Behavior*, **20**, 11-21. <http://dx.doi.org/10.1016/j.jveb.2017.02.007>
- Rowe, J.W., Nawrot, M.L., & Clark, D.L. (2017) Thermoregulation in a north temperate population of Midland painted turtles (*Chrysemys picta marginata*): temporal patterns and intersexual differences. *Copeia*, **105**, 765-780. <https://doi.org/10.1643/CP-16-507>
- Steen, D.A., Gibbs, J.P., Buhlmann, K.A., Carr, J.L., Compton, B.W., Congdon, J.D., Doody, J.S., Godwin, J.C., Holcomb, K.L., Jackson, D.R., Janzen, F.J., Johnson, G., Jones, M.T., Lamer, J.T., Langen, T.A., Plummer, M.V., Rowe, J.W., Saumure, R.A., Tucker, J.K., & Wilson, D.S. (2012) Terrestrial habitat requirements of nesting freshwater turtles. *Biological Conservation*, **150**, 121-128. <http://dx.doi.org/10.1016/j.biocon.2012.03.012>
- Steffen, J.E., Hultberg, J., & Drozda, S. (2019) The effect of dietary carotenoid increase on painted turtle spot and stripe color. *Comparative Biochemistry and Biology Part B*, **229**, 10-17. <https://doi.org/10.1016/j.cbpb.2018.12.002>

- Tetzlaff, S.J., Sperry, J.H., & DeGregorio, B.A. (2018) Captive-reared juvenile box turtles innately prefer naturalistic habitat: Implications for translocation. *Applied Animal Behavior Science*, **204**, 128-133. <https://doi.org/10.1016/j.applanim.2018.03.007>
- Therrien, C.L., Gaster, L., Cunnigham-Smith, P., & Manire, C.A. (2007) Experimental evaluation of environmental enrichment of sea turtles. *Zoo Biology*, **26**, 407-416. <https://doi.org/10.1002/zoo.20145>
- Thomas, R.B. (2002) Conditional mating strategy in a long-lived vertebrate: Ontogenetic shifts in the mating tactics of male slider turtles (*Trachemys scripta*). *Copeia*, **2002**, 456-461.
- Thomas, R.B., Vogrin, N., & Altig, R. (1999) Sexual and seasonal differences in behavior of *Trachemys scripta* (Testudines: Emydidae). *Journal of Herpetology*, **33**, 511-515.
- van Dijk, P.P. (2011a) *Pseudemys concinna*. *The IUCN Red List of Threatened Species 2011*: e.T163444A97425355. <http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T163444A5606651.en>
- van Dijk, P.P. (2011b) *Pseudemys peninsularis*. *The IUCN Red List of Threatened Species 2011*: e.T170496A97427004. <http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T170496A6782626.en>
- van Dijk, P.P., Harding, J., & Hammerson, G.A. (2011) *Trachemys scripta*. *The IUCN Red List of Threatened Species 2011*: e.T22028A97429935. <http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T22028A9347395.en>
- Ventura, D.F., Zana, Y., De Souza, J.M., & Devoe, R.D. (2001) Ultraviolet color opponency in the turtle retina. *Journal of Experimental Biology*, **204**, 2527-2534.
- Warwick, C., Arena, P., Lindley, S., Jessop, M., & Steedman, C. (2013) Assessing reptile welfare using behavioral criteria. *In Practice*, **35**, 123-131. <https://doi.org/10.1136/inp.f1197>
- Waters, R.M., Bowers, B.B., & Burghardt, G.M. (2017) Personality and individuality in reptile behavior. Pp 153-184. In: *Personality in Nonhuman Animals* (J. Vonk, S.A. Kuczaj, A. Weiss, Eds). Springer International Publishing AG 2017. <https://doi.org/10.1007/978-3-319-59300-5>

Graphical Abstract: Captive freshwater turtles displayed less escape behavior and more random swimming at whole group level in the presence of colored objects in their pool. However, there were large differences between the activity budgets of individual turtles and their changes in behavior on days with new enrichment, emphasizing the importance of monitoring the effect of new enrichments at both group and individual level

