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Interest in coloured objects and behavioural budgets of individual captive freshwater turtles

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Abstract

Recent studies showed that freshwater turtles display inter-individual differences in various behavioural traits, which may influence their health and welfare in captivity due to differences in response to husbandry and enrichment strategies and in ability to cope with the limitations of the captive environment. This study investigated a possible correlation between individual level of escape behaviour under standard enrichment conditions and level of interest in coloured objects in a group of cooters *Pseudemys* sp. and sliders *Trachemys scripta* ssp. on display at a public aquarium. Interest in different colours, colour preference and individual differences in behavioural changes in the presence of the new enrichment were also studied. Turtles categorised as 'high' and 'moderate escape behaviour' (17–34% of behavioural budget) showed more interest in coloured objects and tended to display less escape behaviour in their presence, while turtles categorised as 'low escape behaviour' (<10% of behavioural budget) were less interested in coloured objects and tended to display more escape behaviour in their presence. Overall, there was more interest in yellow than in red, white or green objects, with more contacts with coloured objects before feeding and at the start of each observation period and a preference for yellow against red objects. The individual differences in behavioural changes in the presence of the new enrichment suggested that more studies into colour preference and response to novelty in turtles would be beneficial to ensure that no individuals are unduly stressed by new enrichments.

Introduction

Freshwater turtles are popular pets and displays in public aquaria (Pasmans et al. 2017) and are often kept in enclosures with limited spatial and structural complexity in which they cannot fully perform innate behaviours such as exploring and foraging (Burghardt 2013), leading to development of behaviours indicative of stress including escape behaviour, self-mutilation and aggression (Warwick et al. 2013). Enriching the captive environment can provide different types of stimuli (Hoy et al. 2010) to encourage species-specific behaviours and provide more choices, thus potentially reducing boredom and promoting the wellbeing of captive animals (Hosey et al. 2013). Only a small proportion of the studies evaluating different types of enrichment have been carried out on reptiles (Rose et al. 2019; Riley and Rose 2020), with even fewer of these on turtles (Eagan 2019).

Turtles have excellent tetrachromatic UV/VIS vision derived from a complex visual apparatus (Loew and Govardowskii 2001; Twyman et al. 2016) and colours play important roles in the feeding and reproductive biology of turtles (Brejcha and Kleisner 2016), suggesting that coloured objects could be used to add variety to their captive environments and target undesirable behaviours.

Emydid turtles have yellow, orange and/or red patches on their head and limbs and brighter colouration has been linked to a healthier immune system (Polo-Cavia et al. 2013) and described as an 'honest signal' in sexual selection (Ibañez et al. 2014), reflecting an individual's ability to acquire sufficient carotenoids from food to sustain both their protective antioxidant function and the conspicuous skin pigmentation (Rawski et al. 2018; Steffen et al. 2021).

Novel object enrichment (Burghardt et al. 1996; Therrien et al. 2007; Mehrkham and Dorey, 2014; Bashaw et al. 2016) and

Table 1. Freshwater turtles housed at the Tynemouth Aquarium (adapted from Bannister et al. 2021). All 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. [1]Species and subspecies identification based on van Dijk (2011a,b) and van Dijk et al. (2011); *Three turtles, one from each of the groups marked with asterisk, were part of the group only during Phase 1.

Turtles[1]	Sex	Carapace size	Code
River cooters *Pseudemys concinna* (LeConte 1830)	2 males*, 2 females	20–28 cm	Psc
Peninsula cooter *Pseudemys peninsularis* (Carr 1938)	1 male*	24 cm	Psp
Yellow-bellied sliders *Trachemys scripta scripta* (Schoepff 1792)	3 males, 2 females*	17–25 cm	Trs
Red-eared sliders *Trachemys scripta elegans* (Wied 1839)	4 females	23–25 cm	Tre
Slider *Trachemys scripta troostii* (Holbrook 1836)	1 male	24 cm	Trt

structural enrichment (Case et al. 2005; Rose et al. 2014; Tetzlaff et al. 2018) reduced repetitive behaviours and increased levels of activity in turtles. Coloured object enrichment reduced the escape behaviour and increased the level of activity of a group of captive river cooters *Pseudemys* sp. and pond sliders *Trachemys scripta* before feeding, but there were differences in the direction of change in escape behaviour at individual level (Bannister et al. 2021), indicating that it is important to study the impact of any new enrichment not only at group level, but also on each individual concerned. Turtles have consistent behavioural types (Waters et al. 2017) and individual perception and preferences may strongly influence health and wellbeing (Bashaw et al. 2016). Bolder, more curious and explorative individuals may be more deprived and stressed in a limited space or with lack of novelty items, while shy, fearful ones may be more stressed by new challenges and need more places to hide (Richter and Hintze 2019).

Pseudemys sp. and *T. scripta* turtles have highly developed cognitive abilities, with long-term memory for trained tasks involving white and black objects (Davis and Burghardt 2012); but there is no study testing their interest in specific colours or colour preference. Studies on other species of Testudines showed preference for biologically relevant colours, such as red and yellow associated with carotenoid-rich food for tortoises (Pellitteri-Rosa et al. 2010; Passos et al. 2014) and blue associated with finding their way to the ocean for sea turtle hatchlings (Hall et al. 2018a).

The aim of this study was to explore further the interest in different colours of the group of turtles studied by Bannister et al. (2021), and test the hypothesis of a possible correlation between interest in coloured objects, behavioural budgets and direction of behavioural changes observed at individual level in the presence of coloured objects, with focus on escape behaviour as an indicator of stress (Moszuti et al. 2017). Escape behaviour is considered a useful measure of stress in captive turtles, reflecting a tendency to escape conditions that elicit negative emotions (Martínez Silvestre 2014). Reptiles can experience positive and negative emotional states (Lambert et al. 2019) and while they could benefit from

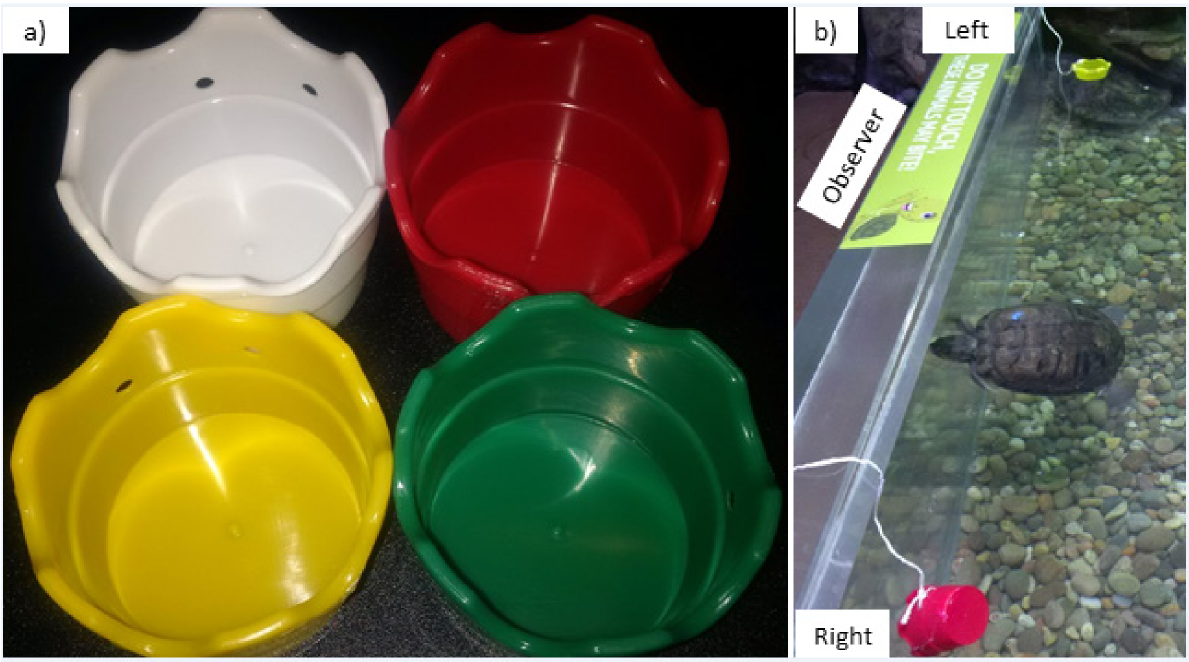

Figure 1. Enrichment devices used (adapted from Bannister et al. 2021): a) coloured plastic cups (white, red, green and yellow; 65×65×45 mm; ASDA Stores Ltd. UK) ; b) location of coloured objects in the turtle pool: left=nearer the waterfall; right=near the opposite corner of the enclosure.

Table 2. Novel coloured enrichment object and olfactory cues combinations presented to the turtles at the Tynemouth Aquarium. [1]The same colour combination was used for a whole 30-min observation period, swapping sides every 5 min; the coloured cups were fish-scented, with the exception of four observation periods on the first day of Phase 1, when clean unscented cups were used; the 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 (20×20×10 mm size), normally used for feeding; on the last day of Phase 1, a yellow fish-shaped feeder was also attached to the coloured cups, as described in Bannister et al. (2021).

Study phase	Colours presented[1] (number of observation periods)
Phase 1: November–December 2018 (15 turtles)	Red-Yellow (n=3); Green-White (n=3) Red-Red (n=3); Yellow-Yellow (n=3) Green-Green (n=2); White-White (n=2)
Phase 2: November–December 2019 and January–March 2020 (12 turtles)	Red-Yellow (n=14); Green-White (n=14) Green-Red (n=7); White-Yellow (n=8)

cognitive stimulation in captivity and more opportunities for play behaviour (Burghardt 2013), more studies on individual preferences and behavioural responses to enrichment are required to avoid unnecessary stress in some individuals (Moszuti et al. 2017) that may lead to poor health (Arena et al. 2014).

In this study, the more active exploratory individuals, displaying more escape behaviour in captivity, possibly representing the 'bold' behavioural type described for wild turtles (Ibañez et al. 2018; Kashon and Carlson 2018), were expected to display more interest in coloured objects and less escape behaviour in their presence, compared to standard enrichment conditions. The more passive individuals were expected either to ignore the coloured objects or display more escape behaviour in their presence, as a sign of novelty stress.

Methods

Turtles, enclosure and standard enrichment conditions

The Tynemouth Aquarium (TAQ), UK, housed a group of adult freshwater turtles (Table 1), in a relatively large indoor enclosure (15 m^2), with a variety of structural enrichment items, according to recommendations for the species housed (Hawkins and Willemsen 2004), including a pool (0.5 m depth, recirculated filtered water, thermostated at 24±0.5°C) with waterfall, logs, pebbles, rocks and hiding places under decking, a decking area accessed by a ramp used to climb out of water and a sand box. UV/VIS light was provided 0800–1800. Turtles were fed mid-day three times a week with pieces of whiting and scattered bloodworms, live insects, turtle pellets and lettuce.

New enrichment

The new enrichment involved placing two fish-scented coloured cups (Figure 1a) into the water, next to the front glass boundary of the turtle pool (Figure 1b), in different combinations, with a few variations during Phase 1 of the study, as described in Table 2.

Data collection, processing and statistical analysis

Data were collected on feeding days, on 6 days in November–December 2018 (2 observers), 6 days in November–December 2019 (2 observers) and 9 days in January–March 2020 (2 observers). All observers were trained by the same person and used the same ethogram with five broader categories of behaviour (Table 3) for 6×30-min periods per day, before (1030–1200) and after feeding (1300–1430), with at least one week between days of data collection, alternating standard and new enrichment conditions in Phase 2.

Instantaneous behavioural sampling every 30 sec of a selection of 2–6 identified turtles per observation period (referred to as focal turtles, as the data were recorded separately for each turtle; selected the nearest in sight that had not already been observed during the same half-day, distinguished by paint markings) and continuous observation for contacts with coloured objects of the selected focal turtles were used throughout the study (Martin and Bateson 2007).

The number of discrete contacts with coloured objects (touch, nudge, bite) was tallied per colour for individual turtles and for all focal turtles combined, then also tallied by time relative to feeding for all focal turtles combined. The data sets from 2018 and 2020 (for which the contacts had been recorded against 1-min intervals) were subsequently tallied by 10-min intervals of the respective observation periods and combined to produce a time-course of interest in coloured objects. The total number and percentages of 1-min intervals with and without contacts with coloured objects were also calculated.

A 'Colour Interest Index' (CII) was defined as the ratio between the total number of contacts with a colour and the number of observation periods during which the individual turtles or all turtles combined were exposed to that colour, to normalise for differences between numbers of observation periods with each colour. This was calculated for each colour and the sum of the CIIs for different colours was used as a cumulative measure of interest in colours. The number of observation periods in the presence of each colour varied from turtle to turtle due to the fact that some turtles remained out of sight under the decking for extended periods of time (hours) and were only occasionally available for selection as focal animals.

Colour preference was analysed using the tallies of contacts with coloured objects for individual turtles and all focal turtles combined only for the observation periods when the turtles were presented with two different colours (Table 2) (Chi-square test, Microsoft Excel, P≤0.05 level of significance) (Rees 2015).

The focal behavioural data (n=375 datasets for individual turtles) were processed as actual frequencies of recording for each category of behaviour (Table 3), for each observation period and turtle. Total frequencies (used for the Chi-square test) and behavioural budgets (used for results presentation) were calculated separately for days with standard and new enrichment for each turtle. The two-way Pearson's Chi-square test of association (Microsoft Excel, P≤0.05 level of significance) (Plowman 2008) was used to determine whether the enrichment type influenced the category of behaviour displayed.

Only the behavioural budgets of the 11 turtles that were exposed to all four colours used are presented in the Results section. The data used for the calculation of these behavioural

Table 3. Freshwater turtle ethogram adapted from Bannister et al. (2021).

Behavioural category	Behaviours included in each category and their description
Active	Locomotion in and out of water, including walking, climbing, swimming, jumping, changing position, other uncategorised movements; Sniffing food or substrates searching for food, biting, pulling and ingesting food; Interacting with existing and new enrichment items (the actual number of contacts with coloured objects was also tallied separately)
Aggression	Head-butting, biting or performing a threatening display towards another turtle in the enclosure
Escape behaviour/ Stereotypical	Swimming continuously or repeatedly into or against the pool glass wall Pattern swimming, defined as swimming along the same repetitive route more than twice around the pool rather than along the glass wall, was not seen at all and this category was referred to only as 'escape behaviour'
Passive	Basking, defined as stationary out of water, resting on sand under the heat lamp, or on decking; Stationary on the bottom of the pool, resting in the water or passively receiving courtship; Out of sight for more than 1 min, presumed to be resting in the water
Social affiliative	Gentle active contact with another individual; courtship displays (sniffing, gentle biting, approaching, head bobbing, matching movements, foreclaw displays, mounting), mating, receiving courtship and showing interest (foreclaw movement, head movements)

budgets included a selection of the 72 focal datasets from Phase 1 (presented in Bannister et al. 2021) which were analysed together with the new datasets from Phase 2 for the respective turtles to ensure full correlation with the data for interest in specific colours, which were not presented in Bannister et al. (2021). For these turtles, the individual values for percentage escape behaviour under standard enrichment conditions were tested for correlation with the individual cumulative CII (Spearman's correlation analysis, SPSS V.26) and the colour combinations used when the datasets for these 11 turtles were collected are listed in Table 4.

Ethical considerations

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

Results

Overall interest in coloured objects and colour preference

The interest in coloured objects was higher during the first 10 min after the objects were placed into the turtle pool, with 70% of all contacts being recorded during this time interval. Overall, the focal turtles showed interest in coloured objects during 9% of all 1-min intervals of observation time, with significantly more than expected interest before feeding, during 13% of all 1-min intervals, compared to 5% after feeding (Chi-square test, P<0.001, X^2_1=28.3).

The focal turtles (all data sets from Phases 1 and 2 combined) had the highest CII for yellow objects (0.9), with similar CIIs for the other three colours (0.5–0.6) (Figure 2). The CIIs for all colours were higher before feeding than after feeding.

When presented with two different colours, the focal turtles combined had a significant preference for yellow against red and no preference between green and white, red and green and yellow and white (Table 5).

Individual interest in coloured objects and behavioural budgets

At individual level, the cumulative Colour Interest Index (CII) ranged from 0.5 to 4 for the 11 turtles that had the opportunity to interact with all four colours. This range was split into three equal intervals and the turtles were categorised as showing high, moderate or low interest in coloured objects (Figure 3). All turtles showed interest in yellow and eight turtles showed interest in all four colours. The CIIs for red were generally higher for *Trachemys* than for *Pseudemys* turtles (apart from Trs5F who showed no interest in red) and the turtle with the lowest cumulative CII (Psc1F) showed interest only in yellow and white (Figure 3). No significant colour preferences were found at individual level.

There were large differences in the behavioural budgets of these 11 turtles under standard enrichment conditions, in particular with respect to escape behaviour and overall levels of activity (Table 6), with the values for escape behaviour ranging from

Figure 2. Colour Interest Indices for each colour (calculated as the ratio between the total number of contacts with a colour and the number of observation periods during which that colour was presented to the turtles), before feed, after feed and overall for the focal turtles combined (212 datasets, Phases 1 and 2).

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Table 4. Summary of the colour combinations used when data were collected for the 11 turtles that had the opportunity to interact with all four colours (turtle codes: Psc=*Pseudemys concinna*; Tre=*Trachemys scripta elegans;* Trs=*Trachemys scripta scripta*; Trt=*Trachemys scripta troostii*; F=Female; M=Male).

Turtle	Colour combinations (number of observation periods)
Psc1F	Red-Yellow (5); Green-White (5); Red-Green (3); Yellow-White (2); Yellow-Yellow (1);
Psc3M	Red-Yellow (6); Green-White (6); Red-Green (3); Yellow-White (4); Green-Green (1); Red-Red (1); White-White (1)
Psc4F	Red-Yellow (5); Green-White (7); Red-Green (3); Yellow-White (4); Yellow-Yellow (1)
Tre1F	Red-Yellow (4); Green-White (2); Red-Green (1); Yellow-White (1); Green-Green (1); Red-Red (2)
Tre2F	Red-Yellow (5); Green-White (4); Red-Green (5); Yellow-White (3)
Tre3F	Red-Yellow (5); Green-White (6); Red-Green (4); Yellow-White (3); Red-Red (1)
Trs2M	Red-Yellow (6); Green-White (8); Red-Green (3); Yellow-White (4); Red-Red (2); White-White (1)
Trs3M	Red-Yellow (9); Green-White (3); Red-Green (3); Yellow-White (4)
Trs4M	Red-Yellow (6); Green-White (6); Red-Green (2); Yellow-White (5)
Trs5F	Red-Yellow (6); Green-White (5); Red-Green (2); Yellow-White (3); Green-Green (1)
Trt1M	Red-Yellow (3); Green-White (3); Red-Green (1); Yellow-White (5); Yellow-Yellow (2); Red-Red (1)

1.9% to 34.3% and for passive behaviour from 17.9% to 75.1%. The turtles were arranged in decreasing order of the percentage of escape behaviour under standard enrichment conditions and categorised as having high (above 20%), moderate (11–19%) or low (below 10%) escape behaviour (Table 6).

There was a trend of positive correlation between the relative frequency of escape behaviour under standard enrichment conditions and the level of interest in colours (Figure 4, correlation not significant at P≤0.05 level).

Most turtles with high (e.g., Psc4F, Tre2F and Trs4M) and moderate (e.g., Tre1F and Tre3F) values for escape behaviour under standard conditions (Table 6, Figure 4) showed more interest in coloured objects (Figure 3), and a tendency of no change or significant decrease in escape behaviour in the presence of the new enrichment. Turtles with lower values for escape behaviour under standard conditions (e.g., Trt1M, Psc1F and Trs5F) tended to display low or moderate interest in colours and more escape behaviour on days with new enrichment. Trs5F and Psc1F were also among the least active turtles in the group, with passive behaviours representing 73.8–75.1% of their behavioural budgets under standard enrichment conditions. Aggression was low overall (0–2.2%) and the direction of the changes in the relative frequencies of the other categories of behaviour between days with standard and new enrichment varied from turtle to turtle, with no obvious trend.

Discussion

The level of interest in different colours, colour preference and a possible correlation between individual levels of escape behaviour and interest in novelty were investigated using fish-scented green, red, white and yellow objects as new enrichment

Table 5. Colour preference for the focal turtles combined: The values represent percentages of total number of contacts and actual numbers of direct contacts (in brackets) with objects of each colour when the turtles were presented with two objects of different colours (n.s.=not significant at P≤0.05 level, Chi-square test).

Colour combinations	Red	Yellow	Green	White
% Total contacts (number)	29% (27)	71% (65)	51% (19)	49% (18)
Chi-square test	P<0.001, χ^2= 15.7 (1d.f.) (50 focal data sets)		n.s. (41 focal data sets)	
Colour combinations	Red	Green	Yellow	White
% total contacts (number)	44% (7)	56% (9)	43% (12)	57% (16)
Chi-square test	n.s. (23 focal data sets)		n.s (30 focal data sets)	

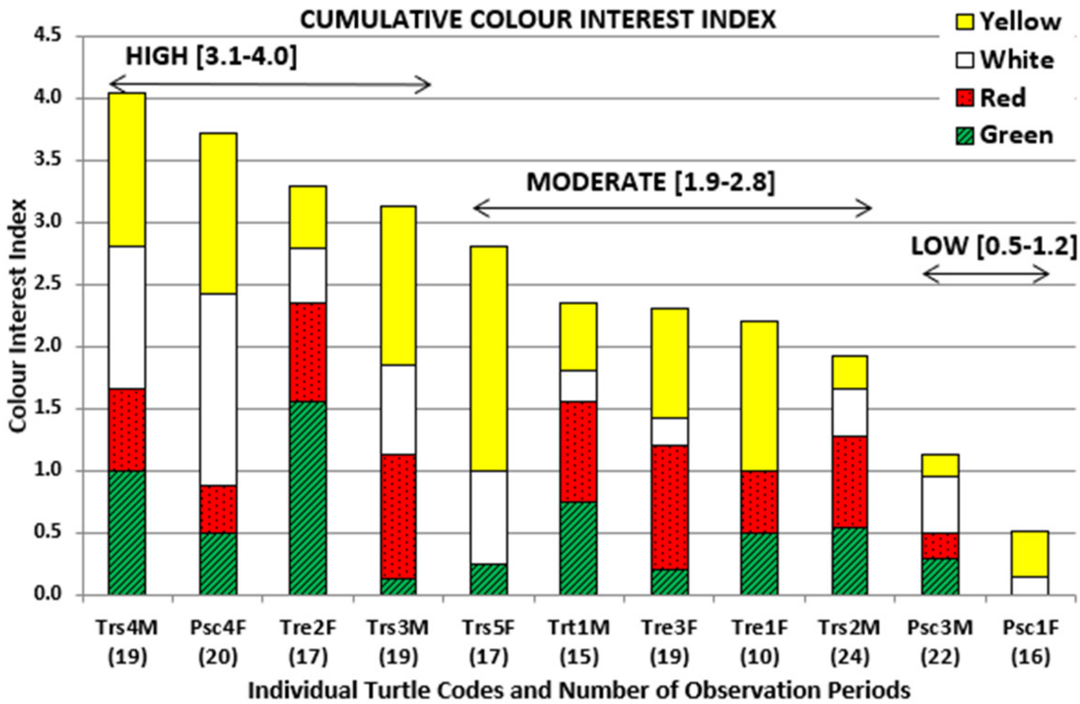

Figure 3. Colour Interest Indices for each colour for the 11 individual turtles observed in the presence of all four colours (calculated as the ratio between the total number of contacts with a colour and the number of observation periods during which that colour was presented to the respective turtle) and ranges used for ranking the cumulative interest in coloured objects (Turtle codes: Psc=*Pseudemys concinna*; Tre=*Trachemys scripta elegans*; Trs=*Trachemys scripta scripta*; Trt=*Trachemys scripta troostii*; F=Female; M=Male).

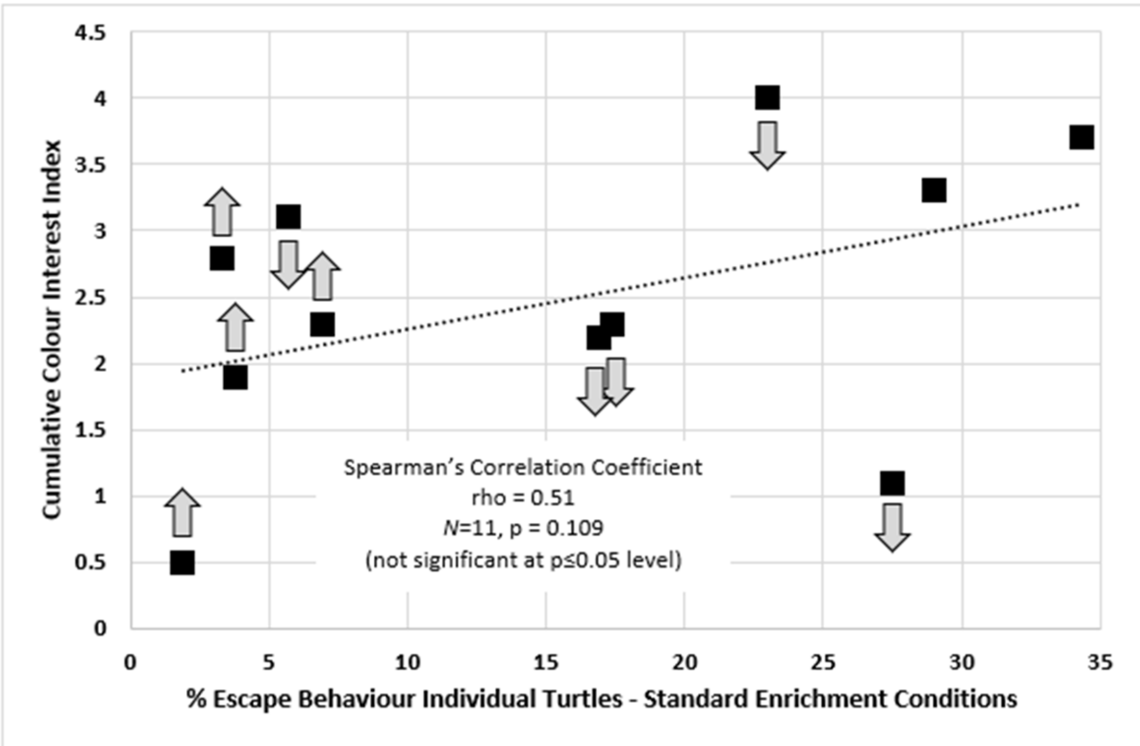

Figure 4. Cumulative Colour Interest Index plot against Escape Behaviour values under standard enrichment conditions for the 11 individual turtles observed in the presence of all four colours. The arrows indicate the direction of change in escape behaviour in the presence of the new enrichment.

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Table 6. Behavioural time budgets under standard and new enrichment conditions for individual turtles listed in decreasing order of the value for escape behaviour under standard conditions (total number of datasets for each condition shown in brackets). Turtle codes: Psc=*Pseudemys concinna*; Tre=*Trachemys scripta elegans*; Trs=*Trachemys scripta scripta*; Trt=*Trachemys scripta troostii*; F=Female; M=Male. [1]Chi-square test outcome: number of df variable 2–4, 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; n.s.=not significant at P≤0.05 level of significance

Turtle code Chi-square test outcome[1]	Enrichment type	Category of behaviour (relative frequency %)				
		Active	Aggression	Escape behaviour	Passive	Social affiliative
High escape behaviour (above 20%)						
Psc4F	Standard (16)	25.6	0.7	34.3	37.7	1.7
NS	New (20)	29.3	0.0	34.0	35.4	1.3
Tre2F	Standard (13)	48.8	0.0	29.0	17.9	4.2
P<0.001, X^2_2=143.3	New (17)	26.6	0.0	30.8	41.3	1.4
Psc3M	Standard (18)	34.6	0.6	27.5	25.7	11.5
P<0.001 , X^2_4=59.1	New (22)	46.4	0.2	18.0	20.5	14.8
Trs4M	Standard (15)	35.2	2.2	23.0	28.6	11.0
P<0.001 , X^2_4=94.0	New (19)	35.0	0.9	12.8	46.2	5.1
Moderate Escape Behaviour (11–19%)						
Tre3F	Standard (15)	15.1	0.3	17.4	64.8	17.4
P<0.001, X^2_3=48.8	New (19)	22.7	0.2	14.4	55.2	14.4
Tre1F	Standard (6)	26.7	1.7	16.9	46.1	16.9
P<0.001, X^2_4=37.3	New (10)	22.7	0.8	9.2	63.8	9.2
Lower escape behaviour (below 10%)						
Trt1M	Standard (8)	49.8	1.5	6.9	32.5	9.4
P<0.001, X^2_4=38.1	New (15)	34.6	1.1	14.1	40.6	9.7
Trs3M	Standard (15)	38.8	1.6	5.7	51.6	2.4
P<0.001, X^2_4=48.6	New (19)	41.5	1.6	3.9	43.8	9.3
Trs2M	Standard (16)	52.3	1.8	3.8	38.4	3.8
P<0.001, X^2_4=74.7	New (24)	34.7	1.9	5.1	52.6	5.8
Trs5F	Standard (13)	14.5	1.0	3.3	75.1	6.0
P<0.001, X^2_4=56.5	New (17)	25.1	0.5	7.5	63.7	3.1
Psc1F	Standard (13)	21.6	0.0	1.9	73.8	2.7
P<0.001, X^2_3=56.4	New (16)	18.3	0.2	11.4	68.3	1.8

targeted at reducing the escape behaviour of a group of pond sliders *Trachemys scripta* ssp. and river cooters *Pseudemys* sp. at Tynemouth Aquarium, England.

The turtles showed less than expected interest in the new enrichment, with the majority of the contacts with coloured objects occurring during the first 5–10 min after their placement into the turtle pool. The objects used were expected to attract more interest from the turtles studied, through both visual and olfactory cues, by association with body colouration and/or food colours and scents. Emydid turtles have highly developed tetrachromatic UV/VIS vision and colour discrimination abilities (Arnold and Neumeyer 1987), based on the most complex cone system found in vertebrates (Grotzner et al. 2020), with good ability to detect bright colours such as yellow and red in shallow water (Hall et al. 2018a). Both visual and olfactory cues play important roles in freshwater turtles' food recognition abilities (Vieyra 2011), sexual selection (Lovich et al. 1990; Liu et al. 2013) and intra and interspecific communication (Brejcha and Kleisner 2016).

In Phase 1 of the study, the turtles showed more interest in the new objects before feeding and on days when fish-scented objects were used (Bannister et al. 2021), suggesting an initial food-related interest, rapidly lost once they realised that the scented objects were not edible, similar to the decline in interest in time reported for tortoises presented with a selection of coloured panels (Simang et al. 2010). In Phase 2, only fish-scented objects were used with at least two weeks intervals between days with new enrichment, to reduce the likelihood of habituation, which is known to occur in turtles presented with novelty items (Burghardt et al. 1996). These intervals might not have been long enough, as turtles are able to discriminate between a wide range of visual stimuli (Davis and Burghardt 2007; Leighty et al. 2013) and remember learned discrimination tasks for long periods of time, up to 3 years (Davis and Burghardt, 2012). Such cognitive abilities are essential for survival, reproduction and coping with environmental changes in the wild and may underpin habituation to stimuli that are not associated with any benefit under captive conditions (Szabo et al. 2020). Another possible explanation for the turtles' low interest in the plastic objects used is the large difference in chromatic characteristics compared to natural food items and to turtle skin, which has complex spectral characteristics (Brejcha et al. 2019).

The colour interest indices calculated for specific colours revealed more interest in yellow than in red, white or green for the focal turtle data combined. The colour for which individual turtles showed the highest interest and the interest in the other colours varied from turtle to turtle. All turtles showed at least some interest in yellow and eight turtles showed interest in all four colours. The *Trachemys scripta* turtles were more interested in red than the *Pseudemys concinna* turtles, possibly related to the presence of postorbital red patches in red-eared sliders, *T. scripta elegans*, in addition to the yellow stripes and patches present in both species.

No significant colour preferences were found at individual level, due to the overall low levels of interest and the small number of observation periods per turtle with each of the four combinations of colours used. The data for all focal turtles combined showed a significant preference for yellow against red, but no preference between green and white, red and green or yellow and white. To the authors' knowledge, colour preference has not been investigated in *Pseudemys* sp. and *Trachemys* sp. turtles and these outcomes, albeit limited, provide useful insights into such preferences.

The greater interest in yellow objects may be related to an innate drive to seek food rich in carotenoids, which are the main pigments present in red, yellow and orange skin patches (Steffen et al. 2019) and are also required for important physiological functions, but can only be obtained from the diet (McGraw 2005). Freshwater turtles display conspicuous colouration on heads and limbs as 'honest signals' of fitness and health, reflecting their ability to acquire carotenoids in sufficient quantities to maintain both their physiological and signalling function (Steffen et al. 2015; Brejcha and Kleisner 2016). Tortoises were shown to prefer red and yellow against other colours and their preference was influenced by the olfactory cues associated with the coloured objects (Pellitteri-Rosa et al. 2010; Passos et al. 2014; Spiezio et al. 2017). In contrast, sea turtles showed no clear preference or avoidance of yellow, red and blue sacks at group level, but there were differences in preferred colour at individual level and there was more interest in biting the coloured sacks on offer when they contained food (Piovano et al. 2013).

Evaluating interest only at whole group level can hide differences in individual likes or dislikes, which may be important when designing training and enrichment strategies (Passos et al. 2014; Learmonth et al. 2021a).

The outcomes of the study partly supported the hypothesis of a correlation between behavioural type, level of interest in novelty items and behavioural changes in the presence of new enrichment. Individual turtles were categorised according to level of escape behaviour under standard enrichment conditions, as a possible indicator of stress in captivity or exploratory tendencies, and according to interest in coloured objects, as a measure of curiosity and found a trend of positive correlation between these behavioural traits. As expected, the overall more active turtles displayed more escape behaviour, more interest when presented with novelty items and a tendency for less escape behaviour in their presence, while the more passive turtles with lower values for escape behaviour under standard conditions were less interested in the coloured objects and tended to display more escape behaviour, which may have been associated with increased stress due to the new enrichment and/or avoidance behaviour (Martínez Silvestre 2014). The study did not involve moving or touching the turtles, which would have been an additional cause of stress (Boers et al., 2020).

Reptile behavioural types focus on traits such as shyness–boldness, exploration–avoidance, activity–passivity levels and sociability–aggression (Waters et al. 2017) and the differences in behavioural budgets of individual turtles at the Tynemouth Aquarium may relate to differences between the 'bold' (more active, exploratory) and 'shy' (more passive, fearful) behavioural types described for both wild-caught (Ibanez et al. 2018; Kashon and Carlson 2018; Pich et al. 2019; Carlson and Tetzlaff 2020) and captive-hatched freshwater turtles (Carter et al. 2016; Allard et al. 2019; Carlson and Tetzlaff 2020).

Repetitive escape behaviour is a common sign of stress in captive turtles (Warwick et al. 2013) that should be targeted by enrichment strategies (Rose et al. 2017) because the repeated friction with the enclosure boundaries often causes skin abrasions and wounds and may lead to poor health (Arena et al. 2014). Innately more active and exploratory individuals were indeed expected to be more stressed by the limited space and complexity in captivity, display more escape behaviour under standard conditions and be more interested and benefit more from the new enrichment, due to having more behavioural choices and opportunities for exploration or play (Burghardt et al. 1996; Hall et al. 2018b). Individual differences in preferences for enrichment and possible positive correlations between level of interest and impact of enrichment strategy used have been reported for tortoises (Learmonth et al. 2021a;b) and sea turtles (Therrien et al. 2007; Mehrkam and Dorey 2014).

Individuals with different behavioural types and related physiological and cognitive characteristics are likely to have different levels of fitness in the wild, which would affect their survival, growth and reproduction (Allard et al. 2019; Nichols et al. 2019), and to respond differently to captivity and enrichment (Carr, 2016). Stress in turtles is associated with increased levels of glucocorticoid hormones and may lead to decreased immune system functioning and other negative effects, such as poor growth or inhibition of reproductive function (Martínez Silvestre 2014; Richter and Hintze 2019). More studies and a consistent approach are needed to evaluate the effectiveness of enrichment strategies for freshwater turtles and other underrepresented groups of animals to improve their health and wellbeing in captivity (Lemasson et al. 2020).

Although the present study was limited to one group of turtles, the group size was comparable to those used for the few other studies on captive freshwater turtles and the much larger number of datasets per individual turtle collected during Phase 2 helped address some of the limitations of Phase 1, providing new information on colour interest and preferences and stronger evidence for the existence of inter-individual behavioural differences within the group and for the importance of evaluating the impact of any new enrichments at individual level.

Conclusion

The presence of the fish-scented coloured objects had a significant influence on behaviour at individual turtle level, with a trend of positive correlation between levels of escape behaviour under standard enrichment conditions and rates of interest in coloured objects and of reduction in escape behaviour in the presence of the new enrichment in the more active turtles.

Turtles that were more passive under standard enrichment conditions tended to display more escape behaviour in the presence of the novelty items, suggesting possible novelty stress.

The turtles displayed more interest in yellow than in red, white or green objects, with a preference for yellow against red when presented with these two colours at the same time. The overall level of interest was relatively low, with more interest before feeding and at the start of each observation period.

More studies into the colour preferences and behavioural responses to novelty of *Pseudemys concinna* and *Trachemys scripta* turtles are needed to investigate the responses to different colours and different combinations of stimuli, in order to provide more information for enrichment and husbandry strategies and ensure that no individuals are negatively affected by new enrichments.

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