Colour vision of green turtle (*Chelonia mydas*) hatchlings: do they still prefer blue under water?

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Background. Several anatomical studies have concluded that green turtles (*Chelonia mydas)* possess the necessary anatomy for colour vision. Behavioural experiments were conducted with newly emerged hatchlings, testing their attraction towards light sources of different colours on their journey into the ocean. It was concluded that they are attracted to shorter wavelengths compared to longer ones, suggesting a possible attraction towards blue. **Methods.** Forty-one green turtles at six months of age were tested for their colour discrimination capabilities during a three-choice experiment under water. Three colours were selected for experimentation: blue, yellow, and red. Four different saturations (25, 50, 75, and 100%) of each of these colours were created, in total 12 colours were tested. The colour stimuli was printed and laminated paper colour blocks with food attached to force an interaction. Turtles were individually placed into their housing tanks with three different colours in front of them, from the same level saturation. The colour of the colour plate first approached and bitten by the turtle was noted. **Results.** The colour of the plate significantly influenced the likelihood that one food plate was selected more than another. Overall blue was selected 66.1%, yellow 18.2% and red 15.7%. There was also a significant interaction between the colour plate selected and the colour of the housing tank. **Discussion.**The findings of this study are consistent with previous research, concluding that green turtles are attracted to shorter wavelength colours, blue, compared to longer wavelength colours such as yellow or red. As the colour saturation changed and the colours became darker, turtles still chose food from the blue plates compared to the other options. These results indicate an attraction towards the colour blue, and as these research animals have never been in the wild, it is suggested that this attraction be an innate behavioural characteristic for green turtles.

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Abstract

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Discussion. The findings of this study are consistent with previous research, concluding that green turtles are attracted to shorter wavelength colours, blue, compared to longer wavelength colours such as yellow or red. As the colour saturation changed and the colours became darker, turtles still chose food from the blue plates compared to the other options. These results indicate

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Key words: marine turtle colour vision, species-specific management tools, innate behavioural attraction, colour vision management.

Introduction

Colour vision has been documented in numerous animals, and it forms an integral part of receiving crucial colour signals from other animals or the environment. Certain birds, for example, have remarkable colour displays to impress potential mates (Boughman, 2002), cephalopods are masters at adapting camouflage colouration to avoid predation (Widder, 2010), and frogs can display vibrant colours as warning signals (Cibulkova et al., 2014). Photoreceptor cells located in the retina, called the cones and rods, are responsible for processing visual information (Kramer and Davenport, 2015). Rods are mostly utilised in low light conditions as there is only one light-detection pigment, and they allow for the visualisation of movement and shapes – these are most often used at night by nocturnal animals (Bartol and Musick, 2001). The cones facilitate colour vision via multiple pigments with varying peak absorbance that function best during high light conditions (Jacobs and Rowe, 2004). Moreover, some organisms possess additional photoreceptor organelles, known as oil droplets, which filter light rays before the cone processes it. This reduces some overlap between spectral sensitivities and allows for more colours to be visible within this organism's visual spectrum (Vorobyev, 2003).

A behavioural response to varied colour stimuli suggests colour vision for that organism (Young et al. 2012). Mrosovsky and Carr (1967) investigated green turtle (*Chelonia mydas*) hatchling colour vision by assessing their attraction to blue, green, and red lights of varying intensities. It was concluded that blue was the most attractive, and red the least attractive (Mrosovsky and Carr, 1967). Moreover, Mrosovsky and Shettleworth (1968) conducted a similar experiment, and described the same conclusions (Mrosovsky and Shettleworth, 1968). Lastly, Witherington and Bjorndal (1991) updated the methodology and found again that green turtle hatchlings showed an increased preference for the shorter wavelength stimuli, specifically 360 nm to 500 nm, colours in the UV to blue visual range (Witherington and Bjorndal, 1991). These three studies used light of varied intensity to assess colour attraction within a terrestrial platform.

Colour changes as a result of the surrounding environment because it is a function of natural light from the sun (Jacobs, 1992). Light penetrates at different rates depending on the medium, for example water is much denser than air, and as a result the light cannot penetrate as deep (Gislen and Gislen, 2004). Longer wavelengths (red) are absorbed near the surface of the water, and shorter wavelengths (blue) are scattered and visible below greater depths (Widder, 2010). Furthermore, eye anatomy can change above and below water making vision vastly different within the two mediums (Land and Fernald, 1992; Gislen and Gislen, 2004). Green turtles have a spherical and rigid lens (Walls, 1941). The eye's curved shape and larger surface area allows additional light to penetrate, and the larger lens facilitates vision under water (Fernald, 1990).

Green turtles utilise terrestrial vision when breathing, nesting, and during their maiden journey into the ocean as a hatchling (Frick, 1976), and aquatic vision for foraging, mating, and navigating their immediate environment. It has been suggested that hatchlings are attracted to shorter wavelengths within the blue range, and are behaviourally attracted towards it. As green turtles spend the majority of their life in the water, it is important to understand whether this behaviour remains consistent within an aquatic environment. This current study was designed to assess green turtle hatchling's behavioural response to colour stimuli under water. The aim is to determine whether the attraction towards blue continued when the animal is fully submerged under water, and whether the saturation of blue is important.

Materials and Methods

Research Animals. Forty-one newly emerged green turtle hatchlings from the same nest were collected from Heron Island, Queensland (23°26'S, 151° 51'E) under permits from the Department of Environment and Heritage Protection (WITK15765815), Great Barrier Reef Marine Park Authority (G13/35955.1), and James Cook University Animal Ethics (A2309). The hatchlings were individually housed at the James Cook University Turtle Health Research Facility, the Caraplace, in 50L rectangular tubs maintained at 26ºC ± 1ºC, with six tubs connected to the same salt water re-circulation system. Different coloured tanks (two blue systems, two red systems, two white systems and one grey system) allowed for system and equipment colour coding to ensure biosecurity regulations. At the time of experimentation the turtles were six months of age, and the average weight was 106g, ranging between 75g and 138g. Turtles were fed a diet of 5% body weight per day of gelatine cubes containing blended human grade fish, prawns, vegetables, fish pellets, and Sea Tabs® Antioxidant Vitamins.

Colour Stimuli. Three colours were selected for experimentation: blue, yellow, and red. These colours were selected to represent the shorter (blue), middle (yellow), and longer (red) wavelengths. The colour plates were created under the Red Green Blue (RGB) colour spectrum where each of the three variables are given a value between 0 and 255, and by altering these values a vast array of pigments can be created (Liu et al., 2011). Four different shades of each colour (blue, yellow, and red) were created to represent varying levels of colour saturation, therefore, 12 different colours were tested in total. The saturation values were quantified by finding 25, 50, 75, and 100% of the RBG values for blue, yellow, and red (Table 1). Each colour plate was printed at Officeworks® on a Fuji Xerox Colour C70 printer, and laminated to maintain waterproofing throughout the duration of each experiment. The overall size of the coloured central block was 100 mm X 100 mm. Ceramic Matt White Thaicera™ tiles 47 mm X 47 mm were glued to the back of the coloured plate to add weight. Printed and laminated colour plates were examined and visually matched to colours from The Munsell Book of Colour, an internationally utilised tool that allows consistency for the discussion of colours within the field (Munsell Colour Company, 1970) (Table 1). This practise is an international standard when communicating colour within the scientific community (Luke, 1992).

Experimental Design. The formal experiment was conducted during the hours of 10.00am and 1.00pm in August 2016 over 10 days. Turtles were left to rest at least one day between tests. A GoPro HERO4® was mounted above three tanks from one system at a time, and set to record at 1080 resolution, 60 FPS, narrow lens, and all lights and sounds were disabled. Turtles were removed from their housing tanks and kept in white ice-cream containers while the colour plates were organised. One 1cm^3 gelatine cube of food was threaded onto fishing line and fastened onto the ceramic tile with masking tape. A total of three colour plates (blue, yellow and red) representing one level of saturation (25, 50, 75 or 100%) were fitted with food and evenly spaced on the floor of the housing tank (Figure 1), thereby providing a three-choice selection for each test. Once the plates were arranged the turtles were reintroduced into the tank at the opposite end, held for three seconds, and then released. The order of colour plates from left to right were randomised where six possible orders (BYR, BRY, RBY, RYB, YBR, and YRB) exist with the three different colours. The order was systematically changed, via the RANDBETWEEN(1,6) function in Microsoft Excel, so that every time a turtle was tested it was with a different colour order to counteract left/right preference bias. The test was concluded once a colour plate had been approached and the food had been bitten, or a ten-minute time limit was reached where no food was approached. The colour of the plate containing the food first engaged by the turtle was noted. Equipment was disinfected between trials. To reduce the possibility of distractions, people did not directly watch the turtles during the trials. The video footage from the GoPro HERO4® was broadcast onto an ASUS ZenFone 2® via Bluetooth®, and was watched in real time with data recorded at a later date. The depth of water within the tanks was approximately 20cm ± 5cm, therefore it would not be filtering out specific wavelengths of colour, and it is reasonable to assume that the turtle would be seeing the same shades of colour within air and water due to the same amount of natural light available. This experiment aimed to assess whether the attraction towards blue was still apparent within an aquatic environment where vison may be different due to anatomical adaptions, as opposed to testing whether colours are different above and below water with this specific set up.

Statistical Analysis. The relationships between tank colour, target colour, and shade were analysed with a fully saturated logistic regression model of the form:

target selected = target colour * shade * tank colour.

Ninety-five percent confidence limits for the proportions of the first colour selected were generated using the Agresti–Coull approximation (Jones et al., 2012). All statistical analysis involved S–Plus 8.0®.

Results

Three complete trials were conducted between August and September 2016 with changed saturation orders, the statistical results were the same irrespective of the order in which the colour saturation levels were presented, therefore only trial two is commented on in detail here as it had the most data points. Saturation percentages were tested in the following order: 100, 50, 25, and lastly 75%. Each full trial consisted of a maximum of 164 data points with each of the 41 turtles tested four times each. During trial two, all 41 turtles participated in experiment one and three with saturation levels 100%, and 25%. During experiment two with saturation level 50% only 38 turtles participated, and in experiment four with saturation level 75% 39 turtles participated. The differences in participation are due to either technical malfunctions with the GoPro HERO4®, or the ten-minute time limit was reached, and no food was approached by a turtle. Therefore, 159 points of data were analysed with this set.

Qualitative observations. Most turtles approached a colour plate and began eating food within one minute, only a small percentage reached the ten-minute time limit and did not approach anything, and this only occurred with four separate turtles once each throughout all the trials. Upon entry into the tank, the turtles could be seen assessing which food to consume by looking at each plate for several seconds before making the choice, and swimming towards one colour plate. This behaviour was consistent regardless of the saturation of colour being tested.

Quantitative observations. The colour of the plate (here called target colour) significantly influenced the probability that a particular food item was selected (logistic regression, dF = 2, Dev. = 115.08, $p < 0.0001$, Table 2). Hatchlings selected the food items on blue, yellow and red with total probabilities of 0.661, 0.182, and 0.157, respectively. A significant interaction was found between tank colour and the target colour (logistic regression, dF = 6, Dev. = 32.92, p <

0.0001, Table 2) (Figure 2). The preference for food on blue is consistent in tanks with white, grey and blue backgrounds, but becomes less in red tanks. The interaction between shade and target colour was not significant (logistic regression, df = 6, Dev. = 11.06, p = 0.086, Table 2).

Discussion

This study shows that green turtle hatchlings choose food from coloured plates in a non-random fashion, suggesting an ability to differentiate colours. Furthermore, testing multiple levels of colour saturation, and finding that food on the same colour plate was consistently selected, indicates that green turtle hatchlings have an attraction towards food on a blue background.

The turtles were housed in different coloured tanks (blue, red, white and grey) within the Caraplace. These turtles have never been in the ocean; their exposure to colour is limited to the equipment utilised within the facility in the form of their tank colours, cleaning utensils, and clothing worn by volunteers. At the time of experimentation each turtle had lived within their tank for six months and were randomly assigned a coloured tank upon entry into the facility. The analysis of the interaction between background tank colour and plate colour revealed that the tank colour has a significant influence on which colour plate the turtles selected. However, blue was selected in significantly more instances than the other choices of yellow and red, regardless of the colour of the tank. There is an exception within the red tanks where no colour was selected significantly more than the others suggesting that the environment in which an organism is raised could have an effect on their visual capabilities. Hu et al. (2011) raised guinea pigs in either violet, green, or white (control group) light facilities, and after eight weeks dissected the eyes to determine whether short-wavelength (S cones) and medium-wavelength (M cones) sensitive cones changed in density. They found that eyes reared in green light had a higher density of M cones, and eyes reared in violet light had a higher density of S cones, when compared to the control group (Hu et al. 2011). Their conclusions were that visual anatomy has developmental plasticity where animal's eyes adapt to their specific environment over time. The green turtles in the current experiment that were raised in red tanks showed less attraction towards blue compared to the turtles raised in different tank colours (Figure 2). It is reasonable to assume that the eye of a turtle raised in a red tank would express more long-wavelength (L cones) cones, compared to those raised in the white, blue, or grey tanks. This is because of developmental plasticity where the eye of the turtle becomes better adapted to the environment it lives it. As more L cones are expressed, that would leave less room for S cones or M cones, thus their ability to discriminate colours in the short-wavelength or medium-wavelength ranges would be compromised. The findings from the current experiment support the findings of Hu et al. (2011) that the environment in which an organism is raised will impact their visual capabilities.

Visual anatomy can change throughout an organism's life according to their environment, but also their lifestyle needs. The brown trout (*Salmo trutta*) relocates from living in freshwater to within the ocean as the fish matures. During this habitat shift the optical anatomy also changes to better accommodate prey within the new environment (Bowmaker and Kunz, 1987). Green turtles also shift habitats and feeding habits as they age. Hatchlings drift within the pelagic environment feeding opportunistically on molluscs and crustaceans (Boyle and Limpus, 2008). Isotopic analysis of young green turtles scutes revealed that on average the animal spends the first three to five years feeding carnivorously within open water, before shifting diets towards a more herbivorous lifestyle within coastal waters (Reich et al., 2007). The turtles used within the current experiment were six months old. It is likely that acute vision and colour differentiation may be advantageous when foraging within a pelagic environment. Schuyler et al. investigated the colours of consumed plastics in gut contents of green turtle hatchlings, and found that blue items were consumed the least. It was suggested that this be due to an inability to differentiate between the blue background of the open-ocean, and the blue objects (Schuyler et al., 2014). Although, findings from the current experiment show that hatchlings prefer food on a blue background this does not necessarily contradict Schuyler et al. findings, as this choice may be due to contrast rather than attraction. An ability to differentiate between colours is acute with hatchlings at this age, and perhaps the minimal amount of blue plastics in the gut contents was due to another factor, as opposed to an inability to find it. It is important to note however, that turtles in this experiment were not attracted to blue food, but rather food on a blue background. Furthermore, the saturation of blue was not a significant variable, indicating that various shades of blue are more attractive compared to the alternative choices. It is possible that an attraction towards blue could assist with sea-finding behaviour, or potentially foraging efforts where the ability to find contrast between the blue background of the ocean and prey items would be essential for survival.

Understanding colour vision in animals allows for previously unexplored species-specific traits to be utilised in a different way. In New Zealand, an endemic parrot, the Kea (*Nestor notabilis*), was decreasing in population due to the consumption of poisoned bait that was laid out to kill invasive species. An experiment was conducted to discover a colour that could act as a visual deterrent to prevent the bird from eating the bait, green was found to be the least attractive colour (Weser and Ross, 2012). Again, green bait on a green background could result in less contrast similar to blue plastic and the blue background and therefore, the birds may not identify it as food. The authors accepted that other variables (size, shape, smell etc.) should be accounted for as well, however, utilisation of an organism's innate attractive or deterrent behaviour towards colours opens up a new form of protective management possibilities. Green turtles (*Chelonia mydas*) have been listed as an endangered species on the International Union for the Conservation of Nature's Red List (Seminoff, 2004) since 1982 with population trends continuing to decrease internationally (Cavallo et al., 2015). The survival rate is lowest for hatchlings where only approximately one out of every one thousand hatchlings survive into adulthood (Triessnig et al., 2012). Therefore, new management techniques must be explored to increase survivorship.

In conclusion, behavioural experiments conducted with hatchling green turtles have shown that this species appear to have an innate attraction towards food on a blue background the colour blue. Evolving the ability to differentiate between background and foreground may assist in survival during this critical life stage by allowing more advanced foraging skills, as evidenced by their significant tendency to select blue regardless of the background tank colour. Utilising the species' innate behavioural response to different colours may lead to targeted strategies that could increase survivorship of green turtle hatchlings during this vulnerable life stage.

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Figure 1

Experimental design.

(A) introduction point of the turtle, (B) outflow standpipe, (C) coloured plates, (D) food attached with fishing line and masking tape, (E) inflow water pipe. All tanks had the same physical layout, the only difference between systems is the colour of the tank: white, blue, grey, or red.

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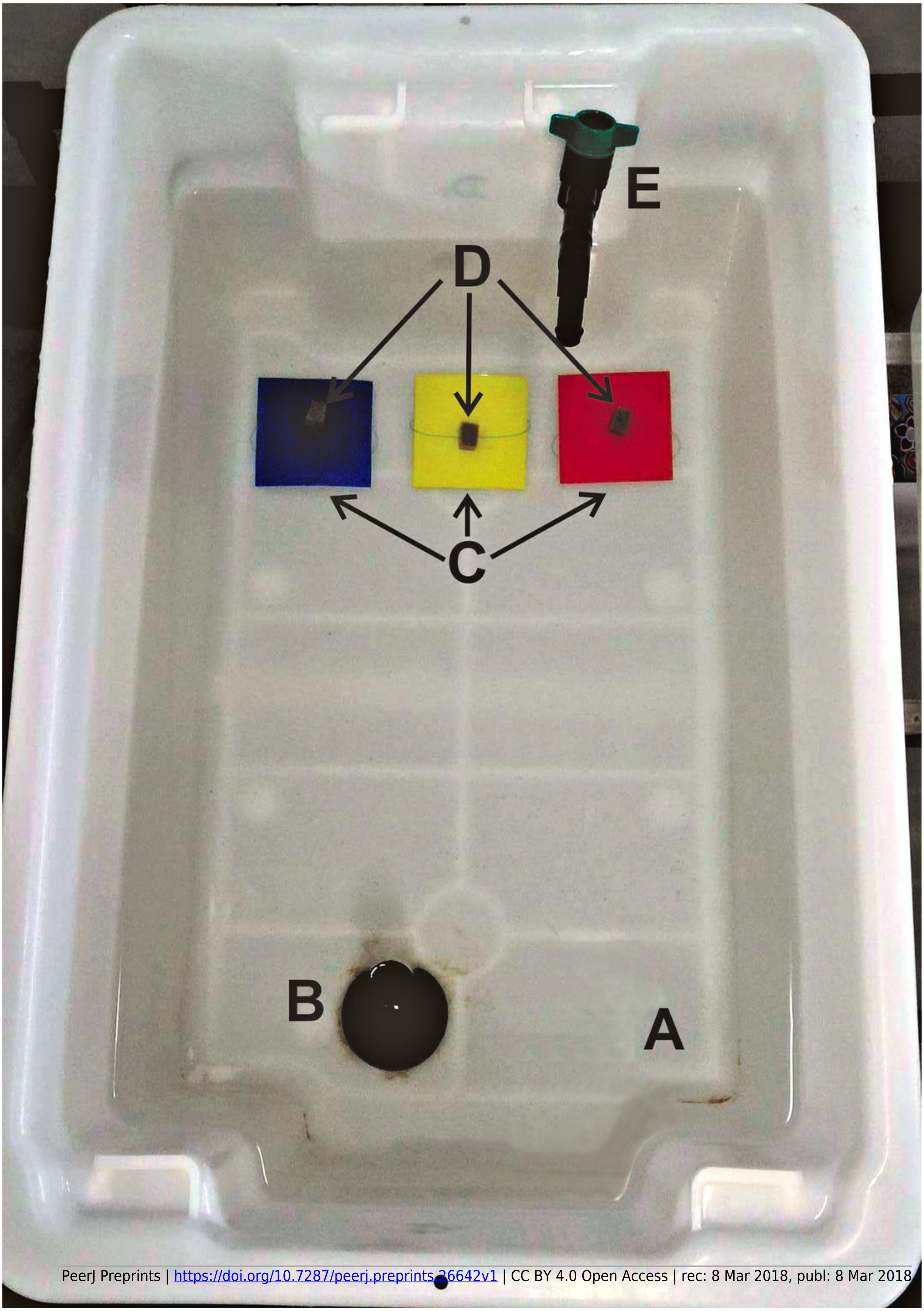
E
D
C
B
A

Figure 2

The relationships between tank colour, background colour and shading on food choice in the sea turtle Chelonia mydas.

The probability of turtle hatchlings selecting food on a particular colour background at saturation levels S1-S4 in four different coloured tanks (white, grey, blue, and red).

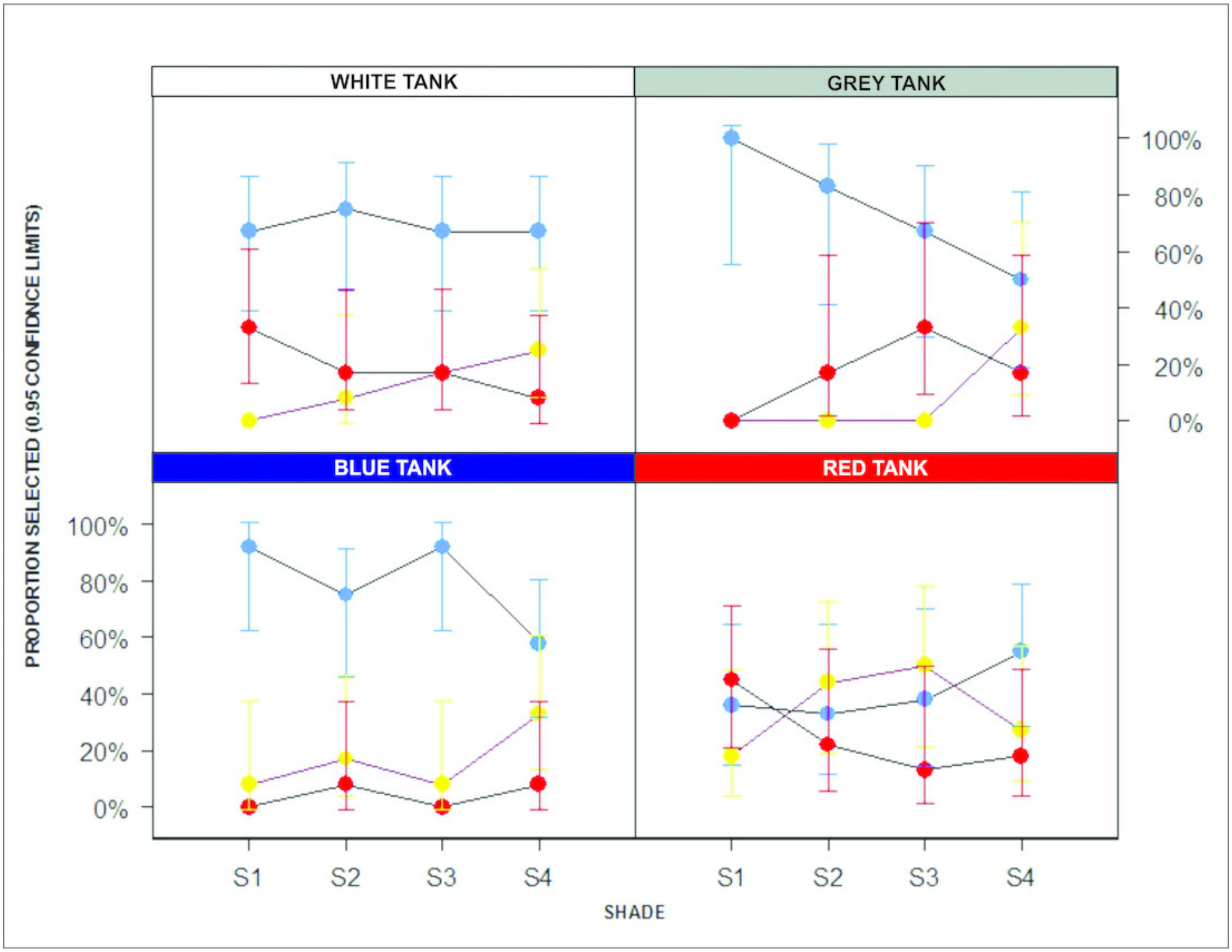

Table 1(on next page)

A breakdown of the twelve experimental colours.

The RGB values refer to the quantity of red, green, and blue within the colour. The Munsell numbers refer to the hue, value, and chroma according to The Munsell Book of Color© where PB = purple blue, Y = yellow, and R = red (Munsell Color Company 1970).

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Table 1. A breakdown of each colour. The RGB values refer to the quantity of red, green, and blue within the colour. The Munsell numbers refer to the hue, value, and chroma according to The Munsell Book of Color© where PB = purple blue, Y = yellow, and R = red (Munsell Color Company 1970).

	Blue		Yellow		Red	
Shades	RGB	Munsell	RGB	Munsell	RGB	Munsell
Shade.1	0, 0, 255	8.0PB 3.5/12	255, 255, 0	10Y 8/12	255, 0, 0	7.5R 4/16
Shade.2	0, 0, 191	7.5PB 3/12	191, 191, 0	10Y 7/10	191, 0, 0	7.5R 4/15
Shade.3	0, 0, 127	7.5PB 2/10	127, 127, 0	10Y 5/4	127, 0, 0	5R 3/10
Shade.4	0, 0, 64	7.5PB 2/3	64, 64, 0	10Y 3/4	64, 0, 0	5R 2/2

Table 2 (on next page)

The statistical output, ANOVA, of the Logistic Regression aimed at investigating the relationships between target colours (colour of the tile that the food was on), tank colour, and shade.

The results of the statistical tests showing significant p-values for the target colour, the interaction between tank colour and target colours, and the interaction between tank colour, shade, and target colour.

Table 2. The statistical output, ANOVA, of the Logistic Regression aimed at investigating the relationships between target colours (colour of the tile that the food was on), tank colour, and shade.

Variable	**Df**	**Deviance residual**	**Df residual**	**Deviance**	**P-value**
Tank colour	3	0.0013	44	189.9311	0.9999
Shade	3	0.0026	41	189.9284	0.9999
Target colour	2	115.0855	39	74.8430	< 0.0001
Tank colour and shade	9	0.0049	30	74.8380	1.0000
Tank colour and target colour	6	32.9223	24	41.9158	< 0.0001
Shade and target colour	6	11.0574	18	30.8584	0.0866
Tank colour and shade and target colour	18	30.8584	0	0.0000	0.0298

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