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Does conspicuousness scale linearly with colour distance? A test using reef fish

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To be effective, animal colour signals must attract attention—and therefore need to be conspicuous. To understand the signal function, it is useful to evaluate their conspicuousness to relevant viewers under various environmental conditions, including when visual scenes are cluttered by objects of varying colour. A widely used metric of colour difference (ΔS) is based on the receptor noise limited (RNL) model, which was originally proposed to determine when two similar colours appear different from one another, termed the discrimination threshold (or just noticeable difference). Estimates of the perceptual distances between colours that exceed this threshold—termed 'suprathreshold' colour differences—often assume that a colour's conspicuousness scales linearly with colour distance, and that this scale is independent of the direction in colour space. Currently, there is little behavioural evidence to support these assumptions. This study evaluated the relationship between ΔS and conspicuousness in suprathreshold colours using an Ishihara-style test with a coral reef fish, *Rhinecanthus aculeatus*. As our measure of conspicuousness, we tested whether fish, when presented with two colourful targets, preferred to peck at the one with a greater ΔS from the average distractor colour. We found the relationship between ΔS and conspicuousness followed a sigmoidal function, with high ΔS colours perceived as equally conspicuous. We found that the relationship between ΔS and conspicuousness varied across colour space (i.e. for different hues). The sigmoidal detectability curve was little affected by colour variation in the background or when colour distance was calculated using a model that does not incorporate receptor noise. These results suggest that the RNL model may provide accurate estimates for perceptual distance for small suprathreshold distance colours, even in complex viewing environments, but must be used with caution with perceptual distances exceeding $10\,\Delta S$.

1. Introduction

Colour displays facilitate diverse behavioural interactions with predators, prey and conspecifics throughout the animal kingdom [1]. Brightly coloured poison dart frogs signal their unprofitability to potential predators, and the peacocks' iridescent plumage broadcasts sexual fitness. Not only must such colours be detected by viewers, but the magnitude of the colour difference between the signal and the background, and between colours adjacent to one another in a pattern, is also an important aspect of the signal. There is evidence that greater chromatic contrast against the background allows an animal to rapidly identify objects and animals in cluttered visual scenes [1,2]. For example, birds are more likely to detect fruits that have greater chromatic contrast to their background [3]. Higher colour contrast is also important for the selection of mates: female

cichlids prefer males with more chromatically contrasting colour patterns [4], and golden-collared manakins alter the visual background to augment the chromatic contrast of their plumage during courtship displays [5]. However, animals may have difficulty discriminating between multiple highly contrasting colours. In the context of sexual selection, for example, females would be unable to perceive a difference between colourful traits displayed by males, thereby limiting the evolutionary processes that drive increases in colour contrast [6]. Consequently, understanding how animals detect and respond to suprathreshold colour signals is key for understanding the evolution of animal communication.

Ideally one would use direct methods to test an animal's response to a colour signal, but indirect methods such as vision models are often used to estimate colour contrast as perceived by a given viewer [7]. Colour contrast (or 'difference') can be defined by distance in colour space, such as a triangular or tetrahedral chromaticity diagram [7,8], or colour hexagon [9], as used by Chuang et al. [10], White et al. [11], Ximenes & Gawryszewski [12] and Garcia et al. [13]. However, the distance between colours in such chromaticity diagrams may misrepresent perceptual distance as they do not consider how noise in photoreceptors and other low-level visual mechanisms limit visual discrimination. In animal colour vision and visual ecology, the receptor noise limited (RNL) model [14,15] is commonly used to quantify chromatic contrast (ΔS) (e.g. [16]). This model is based on well-specified physiological principles; it disregards any achromatic (e.g. brightness) information and assumes that the ability to detect differences in colour and chromaticity is limited by intrinsic fluctuations (i.e. receptor noise and photon noise) in photoreceptor channels. The signal-to-noise ratio at which two colours become distinguishable is termed the discrimination threshold, or a just noticeable difference (JND), which is predicted to be $\Delta S \cong 1$ [14,15]. A number of authors have cautioned that predictions of perceptual distance using this model warrant careful interpretation [14,16,17]. In particular, the RNL model's relevance to the perception of 'suprathreshold' colours that exceed the discrimination threshold (i.e. greater than 1 ΔS), is not well defined and has rarely been investigated (but see [18,19]).

In spite of this, the RNL model is often used to quantify the appearance of suprathreshold colours. Studies generally assume that the conspicuousness of a colour scales linearly with ΔS (e.g. [20,21]). If the relationship is not linear, however, it is likely that the use of the RNL model within these contexts is limited. When two colours several ΔS from the background are viewable, a target colour with a higher ΔS may be selected at a higher frequency—however, it is also possible that they could be selected at an equal frequency, as both are already easily detectable. There is currently limited behavioural evidence to support either prediction, but this information is essential for understanding the ecological significance of colour signals. For example, Cortesi & Cheney [20] investigated aposematic signalling in opisthobranch nudibranchs and found a linear relationship between the level of toxicity and colour contrast of patches within the animals' colour pattern and against the background. Due to this linear relationship, they concluded that colour signals were honest indicators of chemical defence. However, if a colour patch that is 15 ΔS when viewed against its background is selected at a similar frequency than one of a lower value of 10 ΔS, then there is little benefit of displaying a colour pattern with increased colour contrast, and the conclusion that signals are honest may not

be valid. Similarly, Arenas et al. [21] quantified the relative conspicuousness of coloured ladybird models to potential predators using ΔS-based scaling and also reported a linear relationship with toxicity.

The ability for the RNL model to predict the relative conspicuousness of suprathreshold colours is limited for at least two reasons: firstly, receptor nonlinearities will become increasingly significant as colour differences increase, thereby violating the mathematical assumptions of the model; and secondly, the model does not account for post-receptoral processing including cognitive mechanisms, which are more likely to affect suprathreshold judgements than to set discrimination thresholds. Indeed, beyond the discrimination threshold, increasing salience is more likely to be influenced by higher cognitive factors such as colour preferences or colour categorization [16,17]. Although these processes are poorly understood in non-human animals, emerging evidence suggests that colour categorization influences discrimination thresholds in birds, fish and primates (reviewed in [22]). Consequently, retinal modelling may not reliably explain how suprathreshold stimuli are perceived.

To our knowledge, few behavioural studies have investigated the use of the RNL model to predict colour conspicuousness. Fleishman et al. [18] assessed model predictions by examining the response probability when coloured stimuli of increasing ΔS (defined as JND units in [18]) from the background were presented to Anolis lizards. Results suggested that ΔS (JNDs) scaled linearly with conspicuousness. Other studies, focussed on behavioural measures of discrimination thresholds ($\Delta S \cong 1$), have shown that when animals are presented with a single conspicuous stimulus at increasing colour distance from distractors, the response probability is a sigmoidal function reaching close to 100% accuracy for most (but not all) colours, once ΔS is well above the discrimination threshold [13,19,23–25].

For species where critical parameters (e.g. receptor noise) are unavailable, it may also be prudent to consider other models of colour vision. Fleishman et al. [18], for instance, demonstrated that a model that estimated perceptual distance by Euclidian distance between colours plotted in a colour space based on relative stimulation of different cone classes, without any consideration of photoreceptor noise (e.g. a triangular or tetrahedral chromaticity diagram, as described in [7]), gave predictions of relative conspicuousness that were comparable to RNL model predictions.

This study assessed the relative efficacy of the RNL ΔS-metric and Euclidean distance in a triangular chromaticity diagram (Maxwell triangle) [7] to predict the behavioural response to different levels of chromatic contrast. We tested the triggerfish, Rhinecanthus aculeatus with an Ishihara-style behavioural assay [23]. Stimuli consisted of an array of distractor dots that varied in luminance and two randomly positioned target dots of the same hue that differed in saturation (perceptual distance from a grey achromatic stimulus) from distractor dots. Hue was fixed to prevent higher-order processes, such as colour categorization, from influencing the results. Fish were trained via operant conditioning to find and peck at these target dots, with the first target dot pecked in a given test taken as a measure of which of the two appeared more conspicuous. The majority of target dots were above the discrimination threshold and should have been readily detected by fish [23]; only one stimulus for each experiment was below the threshold.

We conducted two experiments. First, we investigated how conspicuousness scales with ΔS exceeding the discrimination threshold in two areas of colour space (blue and green). Second, we tested whether this relationship was affected by chromatic background noise. If ΔS is an accurate measure of conspicuousness in suprathreshold colours, we hypothesized that fish should consistently select the target dots with higher ΔS values (between target and distractor dots) in a given trial, and that preference would scale linearly with ΔS. This would indicate that colour distance between two stimuli provides accurate estimates of the perceptual distance past the threshold. Alternatively, fish could select target dots well above the discrimination threshold at equal frequency, indicating that colour distance does not provide accurate estimates of perceptual distances.

2. Material and methods

(a) Study species

We used triggerfish, *R. aculeatus* ($n = 9$), for this study because they are easy to train and their visual system is comparably well-described [26,27]. The same fish were used for all experiments, but only seven of these fish were used for Experiment 1b. Fish remained highly motivated to perform the task for all three experiments. *R. aculeatus* has trichromatic colour vision with two distinct cone types: a single cone with a short-wavelength pigment ($\lambda_{max} = 412$ nm), and a double cone which has medium ($\lambda_{max} = 480$ nm) and long-wavelength pigments ($\lambda_{max} = 528$ nm). Their behaviourally determined achromatic spatial resolution is 1.75 cycles per degree [27]. The dots in our Ishihara-style patterns ranged from 2 to 12 mm in diameter, and therefore all dots were visible to the fish when first viewed at a distance of less than 30 cm). *R. aculeatus* is a generalist omnivore, known to feed primarily on molluscs and crustaceans in sub-tidal reef flats across the Indo-Pacific region.

Individuals, ranging in size from 60 to 160 mm (SL, standard length), were collected from shallow reefs around Lizard Island (Great Barrier Reef, 14°40′ S, 145°28′ E) using hand nets, then transported to the University of Queensland (UQ). They were collected under a QLD General Fisheries Permit (183990) and a Great Barrier Reef Marine Park Authority Permit (G12/35688). At UQ, fish were housed in individual aerated tanks (100 cm × 50 cm × 50 cm). All tanks were kept under constant illumination by KR 96–K36B, 35 W, DC 24 V overhead lights (Eco-Lamps Inc.) during the experimental testing, and the overhead fluorescent lights were on a 12 h night/day cycle. The study was conducted with approval from the University of Queensland's Animal Ethics Committee (SBS/077/17). Experiment 1a was conducted in June and July 2018, Experiment 1b in November and December 2018, and Experiment 2 in August and September 2018.

(b) Calibration of chromatic stimuli

We created Ishihara-style stimuli using MATLAB code (provided in [23]) that produced the distractor dots (2–12 mm diameter) and then randomly selected two dots of equal size (10 mm diameter) as the target dots. To calibrate and select the colours of the target and distractor dots, we used methods similar to Cheney *et al.* [23]. These colours were displayed on an iPad (iPad Air, Apple Inc., model no. A1474, 26.5 cm × 18.5 cm) that was housed in a black LifeProof NÜÜD iPad Air case to prevent water damage when submerged in the tanks. To perform screen measurements of colours, colour matrices were first saved as bitmap files (.bmp) and displayed on the screen using the iBooks application (Apple Inc., USA). All display options were kept at their default settings except for screen brightness, which was adjusted to its highest setting throughout measurement, training and testing.

We measured the spectral radiance ($\mu M\ cm^{-2}\ s^{-1}\ nm^{-1}$) of target and distractor dots, with an Ocean Optics USB4000 spectrophotometer with a 400 μm diameter UV-VIS fibre and connected to a computer running OceanView software (Ocean Optics, FL, USA). An RPA-SMA Fiber Holder Arm was used to hold the fibre 1 mm directly above the screen at a 90° angle in air. Five separate measurements were made across different areas of the iPad screen for each colour and averaged; however, very little variation between measurements was observed. Prior to each measurement, the iPad screen was briefly inspected for blemishes or marks and cleaned using KimWipes (KimTech) and water.

(c) Modelling colour contrast

In each experiment, colour contrast was measured between the average radiance of distractor dots and target dots. First, the quantum catch of a cone, q, for a visual stimulus, L, was calculated as

$$q_i = k_i \int_{300}^{700} R_i(\lambda)L(\lambda)\ d\lambda, \tag{2.1}$$

where k is a scaling coefficient for receptor adaptation to the background, L_b:

$$k_i = \frac{1}{\int_{300}^{700} R_i(\lambda)L_b(\lambda)\ d\lambda}. \tag{2.2}$$

$R_i(\lambda)$ is the normalized absorbance spectrum of the visual pigment in receptor i (i = SWS, MWS, LWS), multiplied by ocular media transmittance and λ is the wavelength in nanometres. *R. aculeatus* possess a yellow corneal pigment that increases in density over the course of the day (N.F.G. 2018, unpublished data). Model estimates for their spectral sensitivities accounted for the filtration of incident light through this corneal pigment. Data on cone sensitivities [26] and ocular media transmission are given in the electronic supplementary material. $L(\lambda)$ is the spectral radiance ($\mu M\ cm^{-2}\ s^{-1}\ nm^{-1}$) of the target and distractor dots displayed on the iPad. $L_b(\lambda)$ is the spectral radiance of the iPad displaying the white of the stimulus (in between the dots) measured from a distance of 15 cm in the experimental arena.

In the RNL model, colour discrimination is constrained by receptor noise, which can be calculated using eqn. 7 in Vorobyev & Osorio [14]. We used a standard deviation of the noise in a single receptor cell (ν) of 0.05, which has historically been chosen as a conservative measure of visual performance (as per [23,28]), being considerably less sensitive than the human LWS cone system [29]. The relative abundance of cone type per receptor field is 1 : 2 : 2 (η) (SWS : MWS : LWS) based on anatomical counts [28]. Numbers were normalized to the LWS; hence, noise values for each receptor were estimated at $e_S = 0.07$, $e_M = 0.05$ and $e_L = 0.05$.

For trichromatic animals, chromatic contrast is summarized below:

$$(\Delta S)^2 = \frac{e_S^2(\Delta f_L - \Delta f_M)^2 + e_M^2(\Delta f_L - \Delta f_S)^2 + e_L^2(\Delta f_S - \Delta f_M)^2}{(e_S e_M)^2 + (e_S e_L)^2 + (e_M e_L)^2}. \tag{2.3}$$

where ΔS describes the colour distance between two spectral stimuli, which assumes that such distances are affected by noise in photoreceptor channels, and (Δf) denotes the difference between the log-transformed quantum catch of each cone photoreceptor class (S, M and L subscripts). Colours were plotted in a noise-corrected chromaticity diagram (figure 2a) using methods outlined by Pike [30].

When plotting in a triangular chromaticity space, quantum catch of each of the photoreceptor classes are normalized:

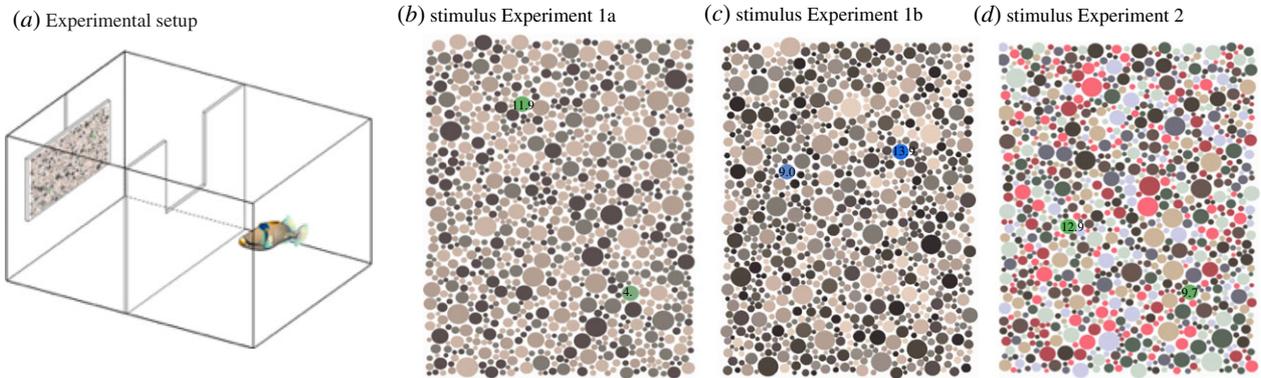$$Q_i = \frac{q_i}{q_S + q_M + q_L}$$

Figure 1. (a) Experimental tank setup: an opaque board divides the tank into halves, with a rectangular opening and door that enables fish to swim freely between both sides. (b) An example of stimulus presented to the fish from Experiment 1a: two green target dots of 1 cm diameter are randomly positioned among an array of achromatic distractor dots. Green target dots are 4.5 ΔS and 11.9 ΔS from average spectral reflectance of distractors (numbers are not present when shown to fish and are added here for illustrative purposes). (c) An example stimulus from Experiment 1b: with two blue target dots 9.0 ΔS and 13.9 ΔS from distractor dots. (d) An example of stimulus presented to the fish from Experiment 2 with two green dots randomly positioned among distractor dots that vary in both luminance and chromaticity 9.7 ΔS and 12.9 ΔS from average distractor dots.
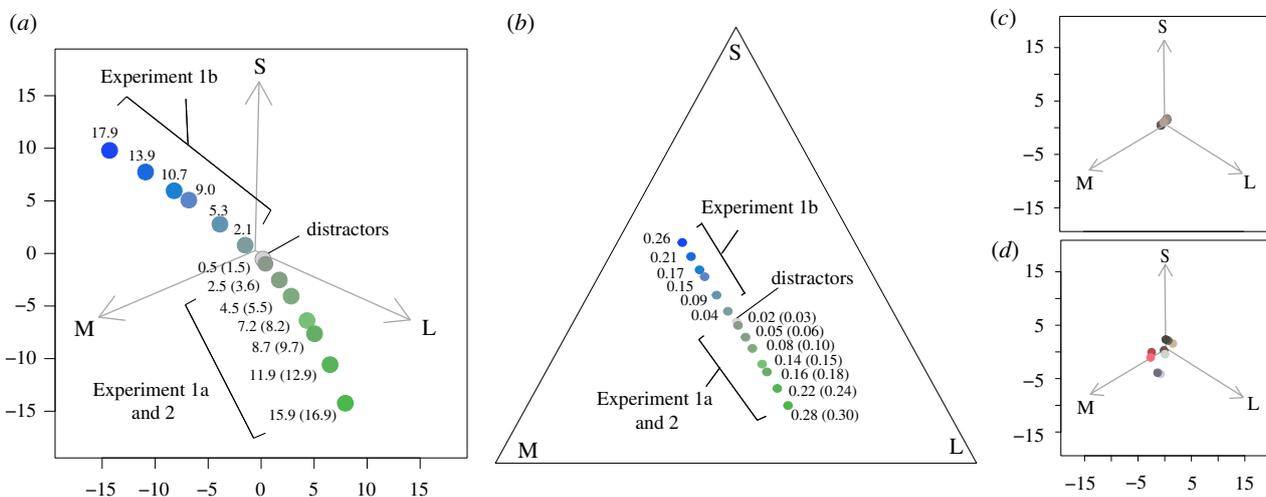

Figure 2. Colours of target and distractor dots plotted in chromaticity diagrams based on spectral sensitivities of triggerfish *Rhinecanthus aculeatus*. Target dots plotted in (a) a receptor noise-corrected chromaticity diagram (as per [30]). ΔS values are shown for each target colour from the average distractor for Experiment 1a and 1b, with values for Experiment 2 in parentheses. Arrows represent each of the cone classes contributing to chromatic discrimination, labelled S, M and L. (b) A triangular chromaticity diagram or Maxwell triangle (as per [7]), with three apices representing stimulation of S, M and L cone classes. Euclidean distances D_t are shown for each target colour. (c,d) Distractor dots for (c) Experiments 1a and 1b, and for (d) Experiment 2, plotted in receptor noise-corrected chromaticity diagram. Plots were produced using pavo 2 package [31] in R. (Online version in colour.)

These normalized quantum catches are then used to calculate the 2D Cartesian coordinates for each colour [7]:

$$x = \sqrt{\frac{1}{2}} \, (Q_M - Q_L)$$

and

$$y = \sqrt{\frac{2}{3} \left(Q_S - \frac{(Q_M + Q_L)}{2} \right)}$$

This colour space only uses information on the quantum catch of each photoreceptor type (and not, for instance, information on the noise in each photoreceptor channel). The Euclidean distances between two colours, A and B in this colour space are calculated as follows:

$$D_t = \sqrt{(x_A - x_B)^2 + (y_A - y_B)^2}$$

In Experiment 1, we used seven 'grey' distractor dots that had fixed chromaticity at the achromatic point (defined as

equal stimulation of all three photoreceptors) when modelled through the triggerfish visual system, but which varied in luminance (figure 1b,c and figure 2a). For Experiment 1a, seven 'green' target dot colours of increasing saturation were chosen. In the chromaticity diagram, this is represented by a series of points extending in a line from the achromatic point, up to a distance of 15 ΔS measured from the average radiance of distractor dots (figure 2c; increasing chroma). For Experiment 1b, six 'blue' target dot colours of increasing saturation were similarly chosen, up to a distance of 17 ΔS (figure 2c). Target dot colours were named according to their respective ΔS values (figure 2c).

In Experiment 2, we investigated the effect of introducing chromatic noise to distractor dots so we used nine distractor dots that varied in both chromaticity and luminance (figure 1d). Distractor dots ranged between 0.3 and 1.95 ΔS (mean 1.3 ΔS) in various directions from the achromatic point (figure 1b). High and low luminance pairs were selected for a given area of colour space (see electronic supplementary material). The same 'green' targets were used as per Experiment 1a, and ΔS values were calculated from the average radiance of the distractor dots (figure 2c). Stimuli with different combinations of target dots

were tested in each experiment to see whether dots were attacked based on their relative ΔS values.

(d) Experimental setup

For both experiments, tanks were divided in the centre using an opaque grey PVC partition. These partitions included a door which opened to allow fish to swim into the testing arena (figure 1a). Before the iPad was submerged, the touchscreen was deactivated through 'guided access' to prevent any further manipulation of the stimulus by the water or the fish. The iPad was then submerged landscape-oriented into the water and positioned against the end of the tank, using a modified hand net to hang the iPad roughly level with the door opening (images provided in electronic supplementary material, figure S1). This ensured fish approached the iPad in a straight path from a fixed distance to the stimuli.

(e) Training protocol

Fish were trained to approach the iPad and peck at a single training dot displayed on plain grey backgrounds of varying luminance. Five different training colours (blue, brown, green, pink and teal) were used for the training dots and were displayed in a random order. Training colours were readily discriminable from the grey backgrounds by the fish [23] and were different from colours used in testing (electronic supplementary material).

Fish were trained twice a day with a minimum of 2 h between each session. During each session, up to five stimuli were presented to each fish. After six training sessions, all fish had learned to approach and tap at the training dots on the screen. At first, squid was placed on the dot to encourage fish to approach and peck. Then the food was removed from the dots and instead given to the fish from above using tweezers after they had successfully pecked at the training dot. Fish then progressed to the final stage of training, where Ishihara-style stimuli (using distractor dot colours for Experiment 1) with two training dots were presented (electronic supplementary material, figure S2). Experimental trials commenced after fish were able to detect and tap training dots with at least 80% success rate per session, for six consecutive sessions.

(f) Trial protocol

Each trial commenced when the board covering the door opening was lifted, allowing the fish to swim into the testing arena. Fish were allowed up to 30 s to find and peck a target dot. Within this time, fish were allowed to make two errors, defined as pecking any distractor dot on the stimulus. Upon a third mistake, or if 30 s had elapsed without a target dot being pecked, the trial was terminated and fish were gently guided out of the testing arena. In either termination scenario, a reward was not provided.

In Experiment 1a, eight main combinations (M) were presented to the fish, named according to the target dots being compared (electronic supplementary material, table S1). Five different versions of each combination were generated and randomly presented to the fish to ensure that they did not learn the location of the dots. Each main combination was tested up to ten times per fish ($n = 90$ trials per combination). These were chosen as the best comparisons to generate an overall understanding of how conspicuousness operates across the region of colour space tested, and would thus benefit from increased sampling. An additional eight target dot combinations were also tested (auxiliary combinations, A; electronic supplementary material, table S1), but only five times per fish. Fish did between 76 and 120 trials in total. Overall, 1038 trials were conducted, 937 of which were successful (i.e. fish pecked one of the target dots).

In Experiment 1b, seven main combinations were presented to the fish (electronic supplementary material, table S1), which were

tested ten times for each fish. As with Experiment 1a, five auxiliary target dot combinations were also tested (electronic supplementary material, table S1), but only three times per fish. In total, 612 trials were conducted, 575 of which were successful.

In Experiment 2, the eight main combinations and eight auxiliary combinations tested in Experiment 1a were presented to this fish, this time with chromatic rather than grey distractor dots. Each main combination was tested up to 10 times per fish ($n = 90$ trials per main combination), and each auxiliary combination was tested between three and five times ($n = 45$ trials per auxiliary combination). Fish performed between 114 and 117 trials in total. Overall, 1040 trials were conducted, 926 of which were successful.

For each trial, we recorded: whether the fish were able to peck a target dot within 30 s; if successful, which target dot the fish pecked first, and the time taken. As per training, fish were tested twice a day with a minimum of 2 h between each session. During each session, up to five stimuli were presented to each fish.

(g) Statistical analyses

All statistical analyses were conducted in R v. 3.3.1 [32]. A Bradley–Terry analysis was conducted using the BradleyTerry2 package [33] to rank target dots in order of how likely they were to be pecked first. This is a probability model used in competition-type scenarios where there is a winner and loser. Given a pair of 'players' i and j from a population, it estimates the probability that the pairwise comparison of $i > j$ is true, which in this study is termed the estimated preference index. In each trial, the target dots being compared in the stimulus (e.g. 4.2v7.0) were considered the 'players', with the first target dot selected by the fish in the contest deemed the 'winner', and the other the 'loser'. An estimated preference index for each 'player' is then calculated, and compared to the target dot with the lowest ΔS in each experiment. To do this, we used the glmmPQL function: generalized mixed model using penalized quasi likelihood in which fish ID was included as a random factor.

To estimate the nature of the relationship between conspicuousness (estimated preference indices) and ΔS, we fitted different models to the data, including a linear regression, hyperbola and sigmoidal curves. A sigmoidal model had the lowest deviance value (electronic supplementary material, table S2). The ΔS at which the preference index was 80% of the asymptote was calculated for each Experiment, which we term ED_{80} (effective dose 80) after which very little change in preference indices occurred.

3. Results

In Experiment 1a, fish took between 1.0 and 30 s (mean ± s.d. = 4.42 ± 3.86) to peck a target dot in successful trials. Fish selected target dots with the higher ΔS in a given combination significantly more frequently than target dots with a lower ΔS ($t > 3.90$, $p < 0.001$), with the exception of the combination '11.6v15.5' ($t = 1.67$, $p = 0.10$) (figure 3a). Here, they selected the lower target dot '11.6' first in 43.7% of all successful trials of this combination. Fish always pecked a target dot, and made no mistakes by pecking distractor dots when the combinations of '2.3vs8.4', '2.3vs11.6' and '2.3vs15.5' were tested. The most mistakes were made when the combination of '4.2v8.6' was tested (mean ± s.d. = 0.16 ± 0.25 per trial). The ED_{80} of the slope for Experiment 1a was $\Delta S = 10.4$ (figure 3a).

In Experiment 1b, fish took between 1.02 and 30 s (mean ± s.d. = 6.4 ± 5.34) to peck a target dot in successful trials. Significant differences in target dot preference were observed in most tested combinations ($t_{141} > 2.29$, $p < 0.001$), except for the combinations of '8.7v10.2' ($t_{141} = 1.5$, $p = 0.13$) and '13.2v16.4' ($t_{141} = 1.4$, $p = 0.16$). Fish made the fewest
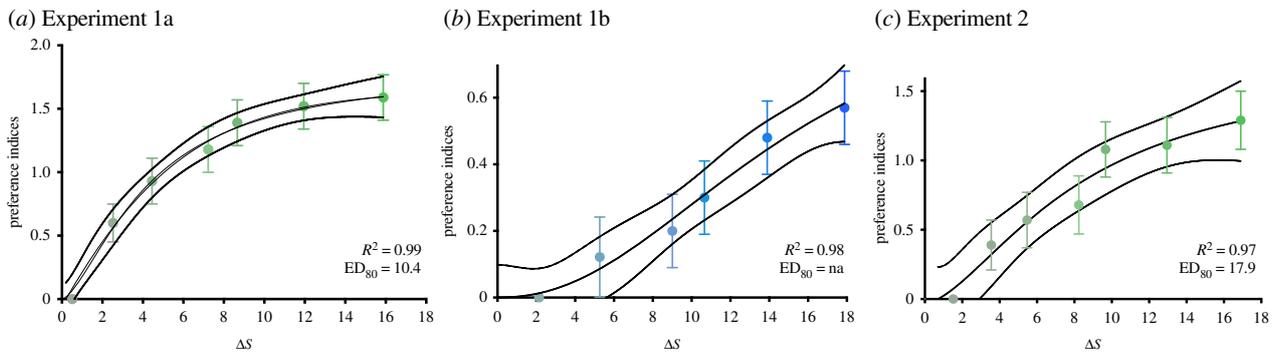
Figure 3. Estimated averaged preference indices for each target colour with different ΔS values from distractor dots for (a) Experiment 1a, (b) Experiment 1b and (c) Experiment 2. Error bars indicate quasi-standard errors, which are calculated from independent estimates of ability for each target colour. Each colour is compared to the 'reference' colour, which has the lowest ΔS value enabling comparisons between any pair of target colours. Sigmoidal curves are fitted to the data and ED_{80} values correspond to the ΔS value at which the curves reaches 80% of the estimated asymptote. (Online version in colour.)

errors when the stimulus '2.2v13.2' was tested (mean ± s.d. = 0.09 ± 0.19 per trial) and the most errors when the stimulus '2.2v5.1' was tested (mean ± s.d. = 0.72 ± 0.26 per trial). The ED_{80} of the slope was higher than Experiment 1a and was undefined (figure 3b). The preference indices in Experiment 1b were much lower than in Experiment 1a.

In Experiment 2, fish took between 1.07 and 30 s (mean ± s.d. = 4.37 ± 4.05) to peck a target dot in successful trials. Significant differences in target dot preference were observed in almost every tested combination ($t_{272} > 2.405$, $p < 0.001$) with the exception of combination '9.6v12.8' ($t_{272} = 0.28$, $p = 0.778$) and '12.8v16.7' ($t_{272} = 2.32$, $p = 0.04$) (figure 3c). The fewest mistakes were made when the stimulus '8.2v16.7' was tested (mean ± s.d. = 0.01 ± 0.03 per trial) and the most errors when the stimulus '1.5v3.5' was tested (mean ± s.d. = 0.83 ± 0.60 per trial). The ED_{80} of the slope was higher than Experiment 1a at $\Delta S = 17.9$ (figure 3c).

For all three experiments, we found that when calculating colour contrast using a non-noise model, results were very similar to the RNL model (electronic supplementary material). For Euclidean distance in the triangular chromaticity diagram, the ED_{80} of the slope for Experiment 1a and 2 was at $D_t = 0.17$ and $D_t = 0.35$, respectively, whereas for Experiment 1b, the ED_{80} was undefined (electronic supplementary material, figure S3).

4. Discussion

In this study, we provide evidence of a sigmoid-like relationship between ΔS and target dot preference across both experiments. For target dots that were a lower ΔS (less than 10) from distractor dots, fish consistently pecked the higher ΔS target dots in a given combination, and we found a linear relationship between ΔS and target preference. For target dots that had a higher ΔS, fish no longer pecked the highest ΔS target dots in a given combination and targets were selected at similar frequencies. The relationship between ΔS and preference depended on the colour being viewed, as well as the complexity of the visual background, and the sigmoidal-like relationship was strongest (in comparison to other models) in Experiment 1a. As expected, we found a similar relationship when colour contrast was measured in Euclidean distance in a triangular chromaticity diagram, which did not account for receptor noise.

This suggests that at higher ΔS, further increases in the relative magnitude of the target dots did not increase the probability

of attack. The asymptote is possibly explained by the Fechner–Weber law of proportional processing, which predicts that discrimination thresholds are greater when comparing high-magnitude stimuli [6]. Therefore, it will become difficult for an observer to detect differences between suprathreshold colours of the same or similar hue as they increase in magnitude. Fleishman et al. [18] also found that conspicuousness based on response probability in Anolis lizards scaled linearly with ΔS for distances that exceeded the threshold from 0–12 ΔS for red, 0–10 ΔS for blue and 0–4 ΔS for green; however, this study did not investigate colours with higher ΔS.

Our results indicate it is likely valid to conclude that a 8 ΔS signal is more conspicuous than one that is 4 ΔS. The relative conspicuousness of two signals that are, respectively, 20 ΔS and 24 ΔS, however, is unlikely to be different. Studies reporting high ΔS values should, therefore, be interpreted with caution. For example, Siddiqi et al. [34] modelled the conspicuousness of aposematic signals in strawberry poison frogs (Dendrobates pumilio) to an avian predator, with values of up to 24 ΔS recorded. However, Siddiqi et al. [34] did not attempt to rank species based on conspicuousness or imply potential predation pressures on different frog species based on colour distance.

Target dots in Experiment 1 were presented on a relatively simple background of achromatic distractor dots, where the relationship between ΔS and conspicuousness may not be representative of what occurs under realistic conditions. Studies often aim to understand how coloured stimuli are perceived when viewed against natural backgrounds. In these studies, the background spectrum is often simplified to an averaged measurement of various background elements. Cazetta et al. [3], for example, approximated the appearance of a natural foliage background by averaging reflectance measurements of leaves from various shrubs. However, natural environments are rarely uniform in terms of chromaticity, typically observing variation in colour and light regimes even over short distances [2]. In Experiment 2, chromatic noise was introduced to evaluate its potential effects on suprathreshold perception, and in particular to see whether it would alter the relationships observed in Experiment 1. Overall, we found a similar relationship between ΔS and target dot preference across both experiments; however, a linear relationship was also a relatively good fit in Experiment 2. This suggests that the RNL model can also be used to estimate perceptual differences between stimuli against relatively complex backgrounds, and importantly, that the detectability of the

target colours was only slightly impaired by the presence of chromatic noise.

Marked differences in the relationship between ΔS and conspicuousness were observed across the two colours (blue and green) tested in Experiment 1 for ΔS measured in both the RNL model and triangular chromaticity diagram, suggesting that predictions may not be consistent across different regions of colour space. For Cheney et al. [23], blue discrimination thresholds for R. aculeatus were also much lower than green discrimination thresholds (mean ± s.d. = blue 2.63 ± 0.72; green 1.39 ± 0.57). Similarly, in guppies, discrimination thresholds also vary depending on the colour being tested [19]. Prior associations with particular hues may impact these results. For example, triggerfish have previously demonstrated a natural aversion to blue signals, whereas they prefer to attack green coloured stimuli [26]. This may be due to the fact that blues are often used in warning signals in the marine environment, as exemplified by blue ringed octopus and many species of nudibranch molluscs.

We did not test whether ΔS can effectively predict the conspicuousness of target dots of differing hues presented simultaneously but the markedly different slopes for the two hues tested (figure 3a,b) suggests that this is unlikely. Differences may arise due to higher-order neural processes such as asymmetrical colour thresholds, pre-existing colour biases or colour categorization [35]. This describes a preferential response toward one of two stimuli based on the biological significance of a colour, and bears no correlation to their relative ability to see or discriminate colour. Outside of humans, colour categorization has been suggested to exist in birds, fish and flies (reviewed in [22]). Colour categorization might depend explicitly on the sigmoid relationship, where the point of inflection determines the category boundaries.

5. Conclusion and recommendations

This study provides evidence that the RNL model accurately estimates the conspicuousness of smaller suprathreshold colours, but should be used with caution when ΔS values are high and is unlikely to accurately predict the perceptual distance between colours that lie far beyond the threshold ($\Delta S \gg 1$). Because the model is based on well-defined and simple physiological principles, deviations from its predictions can give useful insight into colour processing beyond the retina—for example, colour categorization. It should be reiterated, however, that our understanding of suprathreshold perception still needs more work across a variety of behavioural contexts. Results may vary depending on the relevance of the task to the animal and the reward provided. That is, food-rewarded tasks may return different suprathreshold discrimination cut-offs than a fear-based or mate-choice based task. There may be also differences between detection and discrimination tasks, and responses may vary between different spatio-temporal and spatiochromatic properties of visual scenes [36]. Also, when comparing suprathreshold colours, one should be wary that the point of inflection (threshold) and asymptote will be likely to vary across different species and areas of colour space.

This study also further emphasizes the importance of behaviourally validating model predictions. Behavioural studies are imperative to the study of colour vision as they form the ultimate basis for validating theoretical predictions regarding how colours are perceived. By contrast to the wide application of the RNL model, relatively few behavioural studies have been conducted to validate its predictions (but see [18] and [13]). This is attributed to the fact that behavioural experiments are both time-consuming and difficult to design, especially in non-human animals [16]. The Ishihara-style tests we use here partly address this problem. In addition to testing the fish in naturalistic foraging behaviour, they allow much quicker training and testing of many colours than more conventional methods [23].

In particular, it would be useful to have more direct photoreceptor noise measurements for different species. Most studies rely on estimates of receptor noise for a given species, based on relative cone densities and comparison with the relatively high-quality psychophysical or physiological data available in a few species, including humans, honeybees and poultry chicks. In many cases, discrimination thresholds calculated using noise estimates deviate from behavioural observations [25,37]. Conclusions drawn from model predictions derived from noise estimates should thus only be considered hypotheses that, as aforementioned, require behavioural validation. As we show here that the curves fitted to the preference indices using Euclidean distance D_t values calculated in triangular chromaticity diagram were similar to those fitted to RNL model results (electronic supplementary material, figure S3), relative noise levels in the different receptor types appear to be of secondary importance for studies that compare/consider responses to colours that lie on a line in chromatic space (although this may not be the case for all species, especially those living in low light conditions or those with small eyes). Indeed, when photoreceptor noises are not known for a given species, a non-noise-corrected model of vision may be preferable for investigations with suprathreshold stimuli. Without critical model parameters, it is possible to generate reasonable estimates for how animals perceive colour [16]. In spite of its limitations, the RNL model remains a highly versatile tool for the study of visual ecology; however, as many have recommended, the best approach to using the RNL model is to treat its predictions only as a starting point: a solid foundation upon which stronger arguments—ideally from behavioural experiments—can be built.

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