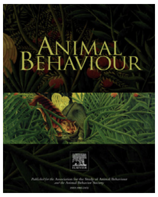
Animal Behaviour 125 (2017) 93–100

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Fish use colour to learn compound visual signals

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ARTICLE INFO

Article history:
Received 23 August 2016
Initial acceptance 26 October 2016
Final acceptance 27 December 2016

MS. number: 16-00745R

Keywords:
categorization
colour signals
colour vision
coral reefs
visual acuity

Colour patterns displayed by animals frequently comprise multiple elements, including hue, pattern, luminance and texture. Predators' perception of and learning about visual stimuli has important implications for the evolution of animal coloration, including aposematism and mimicry. This study investigated how a coral reef fish, the triggerfish *Rhinecanthus aculeatus*, learnt different elements of colour patterns. Fish trained to associate a food reward with blue, yellow and green patterns on a grey background selected novel stimuli by chromaticity, rather than pattern or luminance contrast. By comparison, when presented with small orange spots the fish appeared to learn luminance, which is consistent with findings in other animals, including bees, birds and humans, that for small objects the achromatic component of the signal is more salient than chromaticity. While internal pattern did not appear to be learnt in our first two experiments, a subsequent test showed that fish could distinguish between spotted and striped patterns over various sizes, up to the limits of their visual acuity. These results are discussed in relation to visual processing of colour patterns and the evolution of visual signals in the marine environment.

Animal visual signals can vary on multiple dimensions including colour, pattern, size, texture, shape and movement (Cott, 1940). Many studies have investigated how individual colour patches affect visually guided behaviour, especially mating and feeding (e.g. Aronsson & Gamberale-Stille, 2008; Detto, 2007; Gaudio & Snowdon, 2008; Houde & Endler, 1990; Maan & Cummings, 2008), but less is known about how animals learn and respond to different elements of compound visual stimuli. A distinction can be made here between elemental and configural theories of perception (Pearce, 1997), which propose that animals either learn individual elements in a stimulus (stimulus element learning) or alternatively learn the stimuli in its entirety (configural-cue approach; Boring, 1942; Domjan, 2003; Pearce, 1997). Elemental learning is consistent with the phenomenon of overshadowing, defined as when one element produces a stronger response than the other elements because it is more relevant or salient, which could underlie the evolution of imperfect mimicry (Kazemi, Gamberale-Stille, Tullberg, & Leimar, 2014; Ohnishi, 1991).

Although the distinction between elemental and configural perception is useful it need not be clear-cut, and, to date, studies of how animals learn and generalize colour patterns have given mixed results. In vertebrates, Aronsson and Gamberale-Stille (2008) found that domestic chicks, *Gallus gallus domesticus*, use colour over pattern when learning to avoid unpalatable food items. Similarly, blue tits, *Cyanistes caeruleus*, generalized artificial model and imperfect mimics based on colour elements, rather than pattern or shape (Kazemi et al., 2014). This does not mean that pattern is irrelevant: Ohnishi (1991) found that after appetitive training, chicks tested with familiar and novel stimuli maintained a preference for a trained colour, but preferred novel patterns with elevated achromatic contrast over training stimuli (see also Zylinski & Osorio, 2013). In water, goldfish, *Carassius auratus*, learnt both the colour and pattern elements in compound stimuli, but tended to select one element over the other, and learnt the more salient element with greater accuracy (Ohnishi, 1991).

Coral reefs are known as colourful environments, and reef fish use colour and pattern for tasks, including navigation, sexual display, territorial defence and recognition of prey. Several species

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http://dx.doi.org/10.1016/j.anbehav.2017.01.003

of coral reef fish can discriminate shapes, patterns and colours (Siebeck, Litherland, & Wallis, 2009; Siebeck, Wallis, Litherland, Ganeshina, & Vorobyev, 2014), so that understanding how they learn colour patterns will provide insights into how animals prioritize visual information in a spectrally rich environment. Our study species, the triggerfish *Rhinecanthus aculeatus*, is known to have trichromatic colour vision (Pignatelli, Champ, Marshall, & Vorobyev, 2010), but here we examined how they learn chromaticity (hue and saturation), luminance and pattern in conspicuous stimuli. The triggerfish were trained to receive a food reward by pecking at a visual stimulus in the presence of an unrewarded distractor and in tests they had to choose between novel patterns in which chromaticity, luminance and pattern elements conflicted.

METHODS

Study Species

Triggerfish ($N = 28$) were collected from shallow reef flats and sandy areas around Lizard Island, Great Barrier Reef, Australia (14°40″8′S, 145°27″34′E) using hand and barrier nets. All experiments were conducted between December 2008 and July 2015 under the approval of The University of Queensland's Animal Ethics Committee, approval numbers: SIB/181/08/ECRG, SBS/085/11/ARC and SBS/111/14/ARC. Experiments were conducted at Lizard Island Research Station in aquaria (50 × 40 cm and 30 cm deep), or fish were transported to the University of Queensland, Brisbane, Australia, where they were held in individual aquaria (60 × 40 cm and 30 cm deep) with running sea water and PVC pipes for shelter. During experiments, opaque barriers were placed between each tank to eliminate interactions between fish. We chose this species because they are abundant at our study sites and are highly trainable (Champ, Wallis, Vorobyev, Siebeck, & Marshall, 2014; Cheney, Newport, McClure, & Marshall, 2013; Pignatelli et al., 2010). *Rhinecanthus aculeatus* has three spectrally distinct cone photoreceptors ($\lambda_{max} = 413$ nm, 480 nm, 530 nm; Cheney et al., 2013; Pignatelli et al., 2010) and has a visual acuity of 1.75 cycles per degree (Champ et al., 2014), which is similar to that of other reef fish and goldfish (Collin & Pettigrew, 1989; Hester, 1968; Neumeyer, 2003).

Coloured Stimuli

Coloured stimuli (2.5 cm diameter) were created using Adobe Photoshop CS software, printed on photographic paper (Epson Photo Paper), cut out and laminated. Multiple stimuli of the same pattern were made, and use of individual stimuli was randomized throughout experiments. Reflectance spectra of the laminated stimuli were measured with an Ocean Optics USB2000 spectrophotometer (Dunedin, FL, U.S.A.), and standardized to a 99% white reflectance standard (Appendix Fig. A1). Chromaticity of stimuli was specified by the estimated excitations of triggerfish photoreceptors, and was plotted in a chromaticity diagram based on the estimated photoreceptor excitations (Kelber, Vorobyev, & Osorio, 2003; Vorobyev & Osorio, 1998; Fig. 1, Table 1). For stimuli used in experiment 2, we standardized luminance values of colour patches using receptor quantum catch (Q; as per equation (1) in Vorobyev & Osorio, 1998) of the double cone (M + L; for discussion see Marshall, Jennings, McFarland, Loew, & Losey, 2003), calculated using the spectra of ECO Lamps KR96 white LED lights used for experiment 2 (Appendix Fig. A2).

General Procedure

Operant conditioning was used to train the fish to discriminate between two stimuli (rewarded S+, unrewarded S−). Stimuli were either attached to vial caps (2.5 cm diameter) that were weighed down by a small coin placed underneath the cap (experiment 1) or attached approximately 10 cm apart to grey plastic boards with Velcro dots (experiments 2 and 3). An opaque partition was placed in the centre of the tank to contain fish at one end while stimuli were positioned at the opposite end. A trial began once this partition was removed, allowing fish to approach the stimuli. Fish selected a stimulus by flipping the vial cap over (experiment 1) or pecking on circular, laminated, grey stimuli attached to the vertical display board (experiments 2 and 3). To encourage this behaviour, initially small amounts of food were placed on the stimuli. Once fish were pecking on targets consistently, selection of the correct stimulus (S+) was rewarded with a small piece of food (chopped squid or prawn) presented either on the blade of a blunt knife or with dissecting forceps from above. This ensured fish did not choose stimuli based on olfactory cues in the water. During the training period, incorrect stimulus selection resulted in no food reward, immediate removal of the stimulus board and termination of the trial. The next trial began after a short delay of approximately

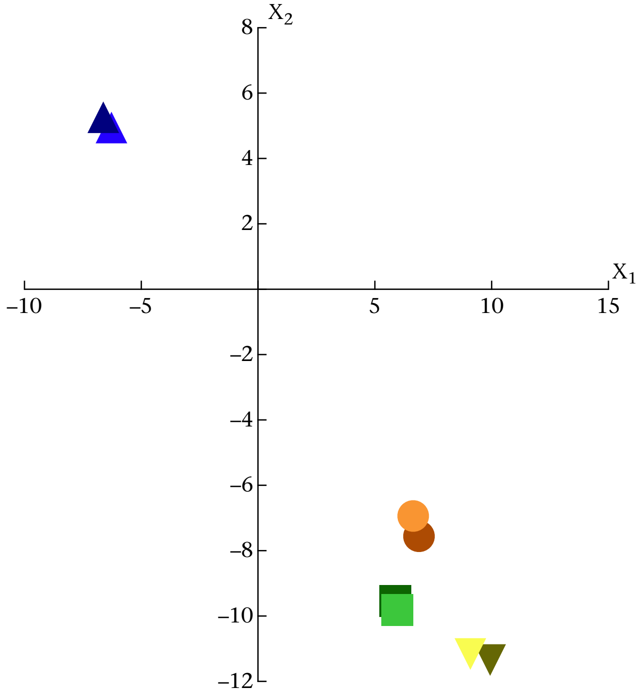

Figure 1. Chromaticity diagram corresponding to the receptor noise-limited colour opponent model (see Kelber et al. 2003; Vorobyev & Osorio, 1998). Colours of stimuli used in experiment 2 are plotted based on spectral sensitivities of triggerfish *Rhinecanthus aculeatus*. X_1 and X_2 are defined in equations B5 and B6 in Kelber et al. (2003). Discrimination thresholds between two colours are approximately 2 jnd units in triggerfish (Champ, Vorobyev, & Marshall, 2016).

Table 1
Double cone photon catch (Q) for coloured stimuli used in experiment 2

Colour	Double cone photon catch	
	High luminance	Low luminance
Blue	42.3	12.4
Yellow	40.1	14.2
Orange	28.3	9.5
Green	26.0	8.8
Grey	17.6	