Fish use colour to learn compound visual signals

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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.

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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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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
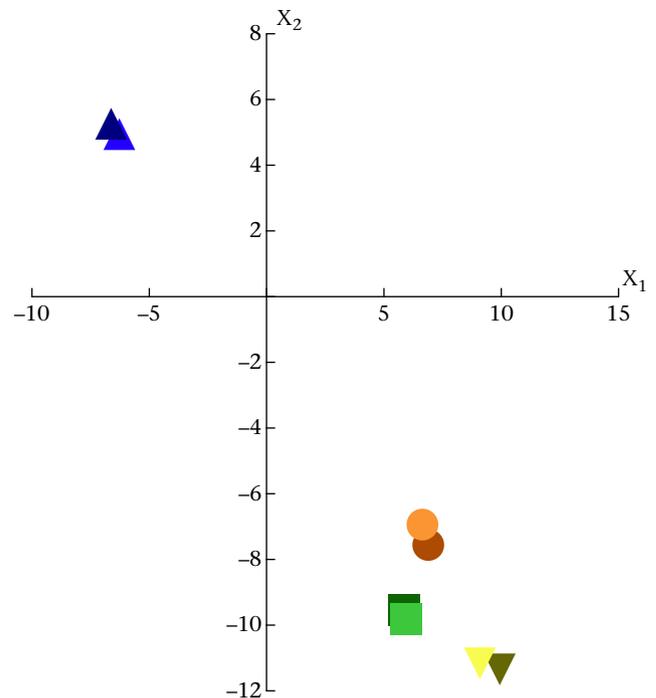

Figure 1. Chromaticity diagram corresponding to the receptor noise-limited colour opponent model (see Kelber et al. 2003; Vorobyev & Osorio, 1998). Colours of stimuli used in experiment 2 are plotted based on spectral sensitivities of triggerfish *Rhinecanthus aculeatus*. X_1 and X_2 are defined in equations 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	

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

1–3 min. The rewarded stimulus was presented in the left and right positions equally and in a pseudorandomized order, with the restriction that it was never in the same position for more than two consecutive trials. Training sessions were run until individual fish reached a statistically significant correct stimulus selection frequency based on a binomial test (see individual experimental methods for *P* values). Testing was similar to training, but the stimuli were not rewarded. During the test phase, if the fish remained in their shelter or did not complete the task within a reasonable time frame (>10 min), the test was terminated and the fish were retested in the next session.

Experiment 1: Use of Colour versus Pattern

Fish (*N* = 12) were trained to discriminate between a pair of circular stimuli featuring a contrasting pattern of either blue stripes or a yellow cross on a black background (Fig. 2). Six fish were trained to associate the blue stripes with a food reward (Fig. 2a) while the remaining six fish were trained to the yellow cross (Fig. 2b).

The colours of each pattern had similar luminance values (see Methods); however, luminance was more accurately controlled in experiment 2 (Table 1). Stimuli were placed on the bottom of the tank approximately 5 cm from the end and 10 cm apart. In training phase 1, a small piece of prawn was placed under the cap so the fish learnt to approach and turn over the cap. Once fish successfully turned the cap on five consecutive encounters, training phase 2 began, in which no food was placed beneath the vial caps and fish were fed from above with forceps. Fish moved onto the test phase once they selected the correct stimulus in six out of seven consecutive sessions.

In the test phase, fish were presented with stimuli in which the colour/pattern was reversed (e.g. if fish were trained with blue stripes and yellow crosses, they were tested with blue crosses and yellow stripes; Fig. 2a). Trials were used to determine whether the fish would select stimuli based on either the learned chromaticity or pattern, or whether the fish showed no preference. All test trials were unrewarded to preclude the fish developing further learnt associations between the test stimuli and a food reward; therefore, two reinforcement trials with the original training stimuli were conducted in between each test trial. One test session of 12 trials was conducted per fish each day for a total of 3 days (*N* = 36 trials). Once fish completed the first test phase (Fig. 2a and b), they were retrained on the alternative colour (Fig. 2c and d; e.g. if they were originally trained to blue stripes, they were retrained to yellow stripes), and the test was repeated (*N* = 36; total *N* = 72).

Experiment 2: Variable Luminance and Pattern Type

In experiment 2, we conducted a similar experiment with eight new individuals, but this time both high and low luminance stimuli were included in the test to disentangle whether the fish used chromatic or luminance cues. The range of test stimuli used was also increased to include additional patterns (circle and spots), as well as additional colours (orange and green). This allowed us to ascertain whether the learned rules generalized to novel stimuli with different colours and patterns. Each high-intensity (bright) blue and yellow and low-intensity (dim) blue and yellow (experiment 2A) and each high-intensity orange and green and low-intensity orange and green (experiment 2B) had similar luminance values (Table 1). We also altered the background to an achromatic grey, which had a luminance value mid-way between the high and low intensity colours to control for contrast (Table 1). Four fish were trained to each training stimulus. Once this experiment
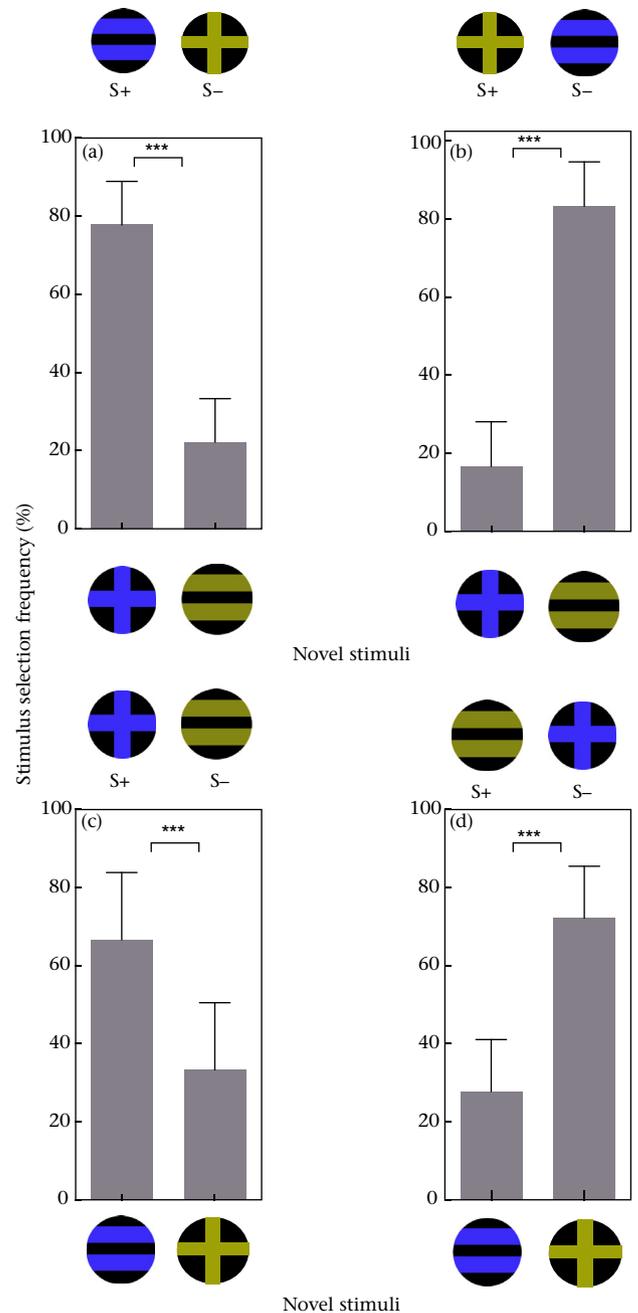

Figure 2. Stimulus selection frequency during experiment 1. For each of (a), (b), (c) and (d) fish (*N* = 6) were trained to receive a food reward from one of the stimuli shown above the graph (S+ food reward, S− no food reward), while novel stimuli used in testing are shown below the bars. Bars represent overall mean values, error bars are +1 SE. ***P < 0.001.

was completed, the same fish were randomly allocated to a new group and retrained to new colour patterns. During training, two sessions were run daily consisting of six trials each and training continued until fish achieved a correct stimulus selection frequency ≥80% across five consecutive sessions (binomial test: *N* = 30 trials, *P* < 0.001).

During the test phase, three different pairs of stimuli were presented that varied in chromaticity, luminance and pattern (Fig. 3). One session consisted of six trials: three treatment trials (one trial for each of the test pairs) and three reinforcement trials
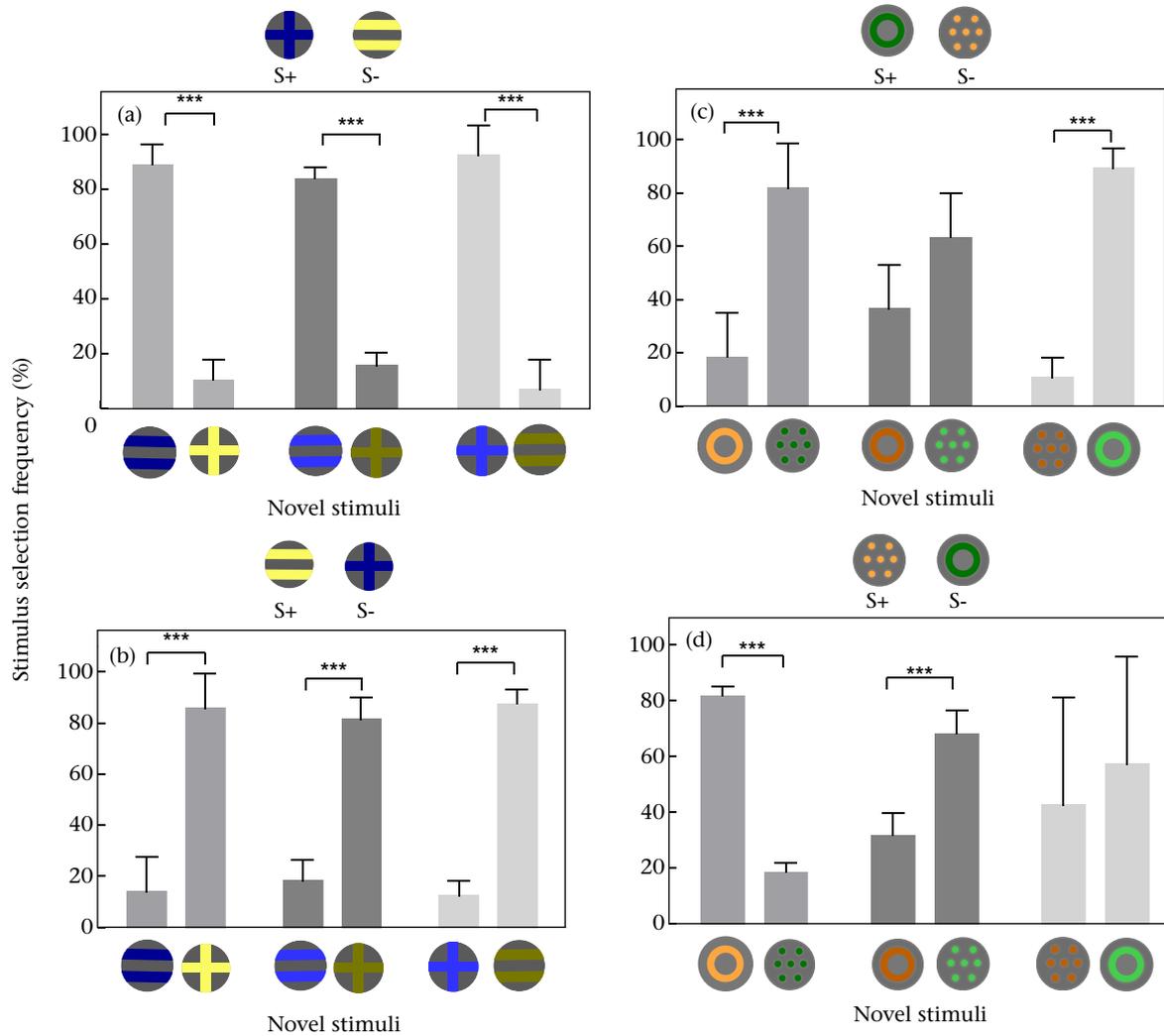
Figure 3. Stimulus selection frequency for experiment 2. For each of (a), (b), (c) and (d) fish ($N = 4$) were trained to receive a food reward from one of the stimuli shown above the graph (S+ food reward, S− no food reward), while novel stimuli used in testing are shown below the bars. Bars represent overall mean values, error bars are +1 SE. ***$P < 0.001$.

during which the original training stimuli were presented and the S+ appropriately rewarded. The order of the trial types (including reinforcement trials) was pseudorandomized so that no more than two treatment or reinforcement trials occurred consecutively. Similarly, the stimuli positions (left/right) were pseudorandomized, so that the same colour was not presented in the same position in more than two consecutive trials. Once fish had completed experiment 2A, they were rested from testing for 2 weeks before starting experiment 2B with novel stimuli.

Throughout testing, the fishes' preferences for the S+ stimulus exceeded 90% in the reinforcement trials (mean + SD %: low-luminance blue crosses: 96.2 ± 3.1; high-luminance yellow stripes: 97.0 ± 3.4; high-luminance orange spots: 94.0 ± 5.4; low-luminance green circles: 90.5 ± 7.3). Thirty testing sessions were completed for each fish; therefore test results consist of 30 choices for each treatment and 90 choices for the reinforcement trials.

Experiment 3: Variable Pattern Size

Experiments 1 and 2 showed that with our stimuli, colour was more important than pattern when learning visual signals (see Results). We also investigated whether fish ($N = 8$ new

individuals) could learn to discriminate between signals according to pattern when chromaticity and luminance remained constant, and whether fish would generalize to the same pattern when it varied in size. Fish were trained to select either dots ($N = 4$ fish) or striped patterns ($N = 4$ fish); yellow dots were 6 mm in diameter and stripes were 6 mm wide (Fig. 4), with the alternative pattern presented as a distractor. There were three or four training sessions per day (1 trial per fish/session) until fish achieved a rate of >80% correct choices in 15 consecutive sessions (binomial test: $N = 15$ trials, $P < 0.05$). During the test phase, fish were presented with patterns of varying size (4 mm, 2 mm, 1 mm diameter/wide). Test trials were unrewarded; therefore, two reinforcement trials were run in between each test trial. Each fish was presented with four to seven test trials for each spatial frequency.

Statistical Analyses

For all experiments, a generalized linear mixed model (GLMM) with a binomial distribution (log-link function) was used to test whether the selection frequencies of each stimulus type were significantly different from what would be expected due to
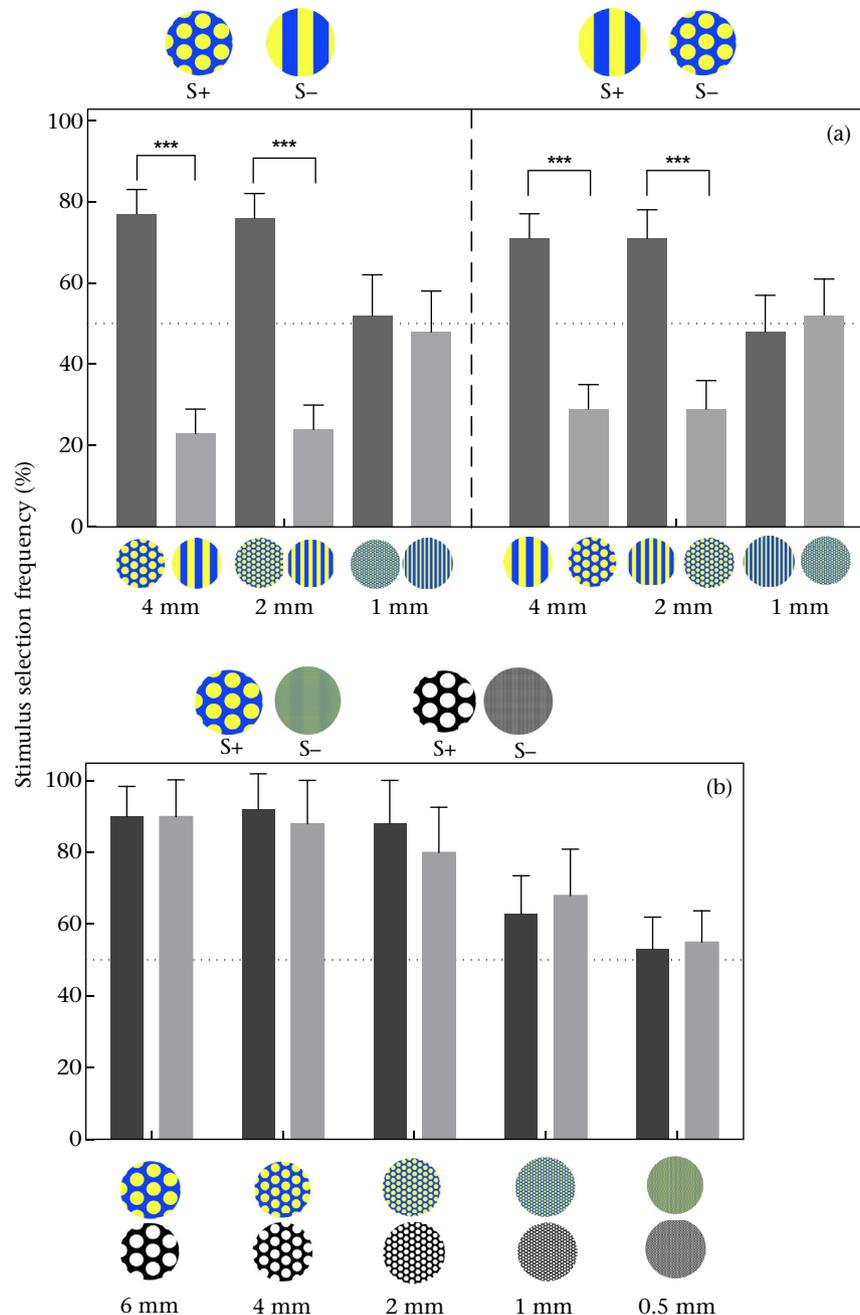
Figure 4. Stimulus selection frequency for (a) experiment 3A and (b) experiment 3B. For each of (a) and (b) fish (left/right $N = 4$; total $N = 8$) were trained to receive a food reward from one of the stimuli shown above the graph (S+ food reward, S− no food reward), while novel stimuli used in testing are shown below the bars. In experiment 3B, colour patterns shown beneath the graph were presented with a distractor stimulus (S−) in the test phase. Bars represent overall mean values, error bars are +1 SE. ***$P < 0.001$.

chance. To do this, we used the glmer function in the package lme4 (Bates, Maechler, Bolker, & Walker, 2015). Chromaticity, pattern, luminance and/or spatial frequency of test stimuli were included as fixed factors. As the same fish were tested on numerous occasions in each experiment, fish ID was included as a random factor, which resolves the nonindependence that occurs from having multiple responses from the same individual. In experiment 2, training and testing were conducted by two experimenters (C.N. and N.F.G.); therefore, the effect of experimenter was included as a fixed factor, but was found to be nonsignificant ($P > 0.25$). Time of day (0800, 1200, 1600;

experiment 3) was also nonsignificant ($P = 0.45$) and was therefore excluded from the final model.

RESULTS

Experiment 1: Use of Colour versus Pattern

In all four conditions fish relied on chromaticity of the target rather than on the pattern (Fig. 2). In 75% of all trials (mean ± -SD = 75.0 ± 7.2), fish chose the test stimuli with the same chromaticity as the rewarded stimuli (S+) they had been trained to,

irrespective of pattern (GLMM: $z = 2.67$, $N_{observations} = 72$, $N_{fish} = 12$, $P < 0.001$; Fig. 2a–d).

Experiment 2: Variable Luminance and Pattern Type

In experiment 2A, fish trained to low-luminance blue crosses (S+) chose novel stimuli according to chromaticity, rather than pattern or luminance (GLMM: all $z < -6.02$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$; Fig. 3a). Fish trained to high-luminance yellow stripes also chose stimuli based on chromaticity, rather than pattern or luminance (GLMM: $z < -3.61$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$; Fig. 3b).

In experiment 2B, fish trained to low-luminance green circles, selected low-luminance green spots significantly more frequently than high-luminance orange circles, and therefore again selected the stimulus with the same chromaticity and luminance over pattern (GLMM: $z = -5.92$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$; Fig. 3c). Fish also selected high-luminance green spots more frequently than low-luminance orange circles; however, this was not significant (GLMM: $z = -1.80$, $N_{observations} = 120$, $N_{fish} = 8$, $P = 0.07$) and fish selected high-luminance green circles over low-luminance orange spots (GLMM: $z = -6.02$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$).

The exception to the finding that chromaticity was selected over pattern or luminance was for fish trained to high-luminance orange spots. Fish selected high-luminance orange circles significantly more than green spots, and therefore again preferred colour (i.e. chromaticity and luminance) over pattern in treatment 1 (GLMM: $z = -6.57$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$; Fig. 3d). However, fish selected high-luminance green spots over low-luminance orange circles; therefore they chose the same pattern and luminance over chromaticity (GLMM: $z = 3.83$, $N_{observations} = 120$, $N_{fish} = 8$, $P < 0.001$). When presented with low-luminance orange spots and high-luminance green circles, overall there was no preference (GLMM: $z = 0.80$, $N_{observations} = 120$, $N_{fish} = 8$, $P = 0.64$); however, individual fish were highly variable (stimulus selection frequency (%) for low-luminance orange spots: Fish A = 3.3%, Fish C = 60.0%, Fish D = 50.0%, Fish K = 73.3%). This latter observation suggests that different individuals learnt different cues.

Experiment 3: Variable Pattern Changes

Fish could identify test stimuli with the same pattern (spots or stripes) as rewarded stimuli but of smaller size (GLMM: 4 mm and 2 mm diameter/wide: $z > 3.02$, $N_{observations} = 54$, $N_{fish} = 8$, $P < 0.003$; Fig. 4a) but not the smallest stripes or dots (1 mm diameter/wide: $z = 1.62$, $N_{observations} = 54$, $N_{fish} = 8$, $P = 0.32$). A further control experiment (experiment 3B) was run to determine whether this was because they no longer perceived the pattern as being the same as the trained stimuli or whether the pattern of the smallest spots simply exceeded the resolving capacity of their visual acuity. When only presented with a pattern and the control stimulus (plain green), fish were able to distinguish the control stimulus from the three largest dots (GLMM: 6 mm, 4 mm and 2 mm: $z > 3.94$, $N_{observations} = 302$, $N_{fish} = 8$, $P < 0.001$; Fig. 4b), but not from the two smallest stimuli (GLMM: 1 mm and 0.5 mm: $z = 1.10$, $N_{observations} = 92$, $N_{fish} = 8$, $P = 0.27$; Fig. 4b), suggesting that fish were unable to perceive the pattern, and were simply learning the luminance contrast in the stimulus. This final experiment was also conducted with black and white stimuli; however, there was no difference between black/white and colour trials ($P = 0.36$; Fig. 4b).

DISCUSSION

Understanding how animals use the separate components of colour patterns to find or avoid food is relevant to many questions about foraging and communication, including aposematism and mimicry. We found here that for several colour and pattern types, triggerfish selected novel stimuli according to their colour in preference to pattern, and for larger stimuli chromaticity in preference to luminance. For an S+ pattern of small orange spots, the fish chose novel stimuli that had a similar luminance rather than chromaticity. This rule can be expected to depend to some extent on the degree of change in each component (which is hard to quantify), and it did not apply for the finest patterns. The fish could sense pattern, as the final experiment showed that when the chromatic elements of the stimuli remained constant, they learnt to discriminate between two pattern types (spots and stripes), and that they generalized the same pattern type despite changes in size.

Overall, our results suggest that when generalizing to novel stimuli the fish differentiate elements of a colour pattern (consistent with stimulus element learning; Boring, 1942; Domjan, 2003; Pearce, 1997). If fish had a fully configural mechanism they would exhibit no preference for any stimuli that differed substantially in any one respect from the original learned stimuli (Mackintosh, 1976; Pearce, 1997). In a similar study, Kazemi et al. (2014) demonstrated that blue tits learned the colour of rewarded stimuli at a higher rate than pattern or shape, and when presented with mimetic variants of unrewarded stimuli, the birds continued to avoid stimuli of the same colour, but not stimuli that were only similar in pattern or shape. Colour therefore overshadowed shape and pattern, even though the latter elements were learnt when colour was not discriminative. Animals may selectively attend to certain elements that they find most relevant to the behavioural task; for example, a particular colour may indicate unprofitable food, or colour may also indicate fitness of potential mates. Furthermore, it was suggested that for poultry chicks different visual elements of a pattern have distinct roles, with luminance contrast serving mainly to attract attention, whereas colour is remembered accurately (Osorio, Miklósi, & Gonda, 1999; Zylinski & Osorio, 2013). In our experiment, it may be that fish disregarded pattern as a task-irrelevant cue. Triggerfish feed on a variety of food sources in their reef environment, including molluscs, small fish, algae and worms, and colour may be a more reliable cue than pattern in a visually heterogeneous environment.

These findings have implications for the evolution and design of animal signals, especially in aposematism and mimicry. If animals only learn one element of a visual signal, or base behavioural decisions on the most salient element, which overshadows others, then selection may be relaxed for the other elements, which can allow phenotypic variation of colour patterns and the evolution of imperfect mimics (Hunt et al., 2011; Kazemi et al., 2014). Consistent with our findings here, coral reef fish mimics closely match the colour and luminance of their models (Cheney & Marshall, 2009), whereas the accuracy of body shape and pattern is more variable. Whether colour elements are more constrained for aposematic species, such as nudibranchs, sea snakes and poison-fang blennies, remains to be investigated. In this study, we used one chromatic cue on an achromatic background, so how animals learn patterns that contain multiple colours is unclear.

The finding that fish trained to an S+ stimulus of small orange dots appeared to choose novel stimuli based on luminance contrast rather than chromaticity is not surprising. Humans, bees and birds also use chromatic cues to detect and memorize larger visual stimuli but use achromatic (or luminance) cues when presented with lower spatial frequency stimuli (de Ibarra, Giurfa & Vorobyev, 2001; Jones & Osorio, 2004; Mullen, 1985; Osorio et al., 1999).

Indeed, bees rely on colour when trained to discriminate flower-like patterns (de Ibarra et al., 2001). While bees rely on chromaticity of targets when they subtend large visual angles, the targets subtending small visual angles are detected and discriminated by lightness contrast and chromaticity is ignored (Giurfa & Vorobyev, 1997; Giurfa, Vorobyev, Brandt, Posner, & Menzel, 1997; Giurfa, Vorobyev, Kevan, & Menzel, 1996).

Our findings point to the importance of chromaticity in an appetitive learning scenario, but the significance of different components of visual stimuli is likely to be context-dependent (i.e. foraging, mate detection, predator avoidance; Osorio et al., 1999), with elemental cues producing different behavioural responses according to the situation. For example, chicks have been found to have no preference for red or green food when displayed as fruit, but prefer green prey when they resemble insects (Gamberale-Stille & Tullberg, 2001). Spottiswoode and Stevens (2010) found that the cuckoo-finch, *Anomalospiza imberbis*, when defending against brood parasitism, primarily used colour but also pattern to discriminate and reject parasitic eggs from nests. Interestingly, the birds did not use one aspect of the egg pattern, described as 'scribbled' fine lines, which are never displayed on the eggs of the brood parasites and therefore could act as an honest signal to recognize host eggs. Such pattern elements may be overshadowed by colour or other pattern elements, but it is perplexing that hosts have not evolved to use such visual cues as antiparasite defences.

Understanding how compound animal visual signals with multiple elements are transmitted and received in heterogeneous visual environments and how receivers respond to such signals (Rosenthal, 2007) is a challenging task for visual and behavioural ecologists. However, experiments such as these allow the identification of key elements that are learnt and used to generalize towards novel stimuli, providing important insights into how animals prioritize visual information such as chromatic and achromatic mechanisms, and the influence this may have on signal evolution.

Acknowledgments

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Appendix

Figure A1. Spectral reflectance curves (%) for stimuli in (a) experiments 1 and 3 and (b) experiment 2.
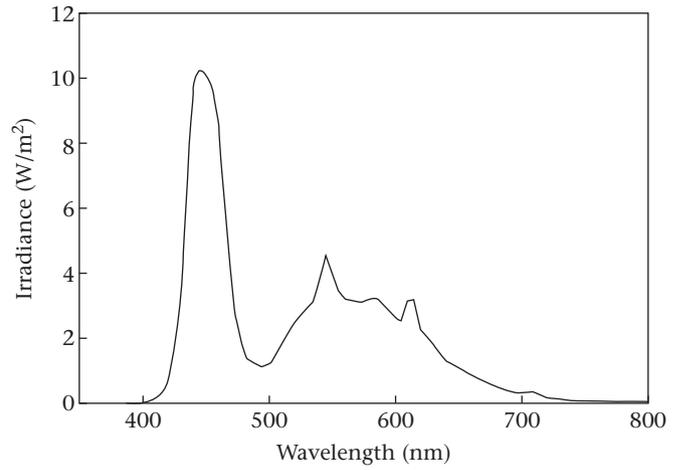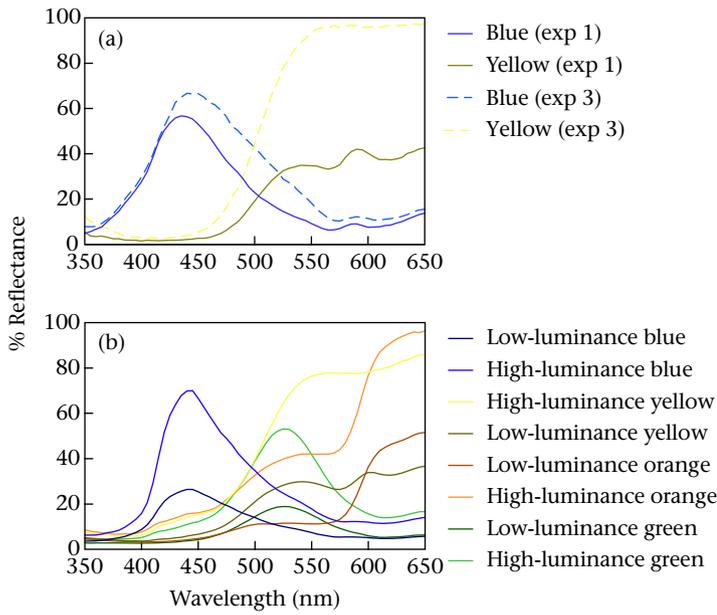

Figure A2. Spectral irradiance measurements of LED lights used in experiment 2.