

## RESEARCH ARTICLE

### Colour vision and response bias in a coral reef fish

Karen L. Cheney<sup>1,\*</sup>, Cait Newport<sup>1</sup>, Eva C. McClure<sup>1,2</sup> and N. Justin Marshall<sup>2</sup>

<sup>1</sup>School of Biological Sciences, The University of Queensland, St Lucia, QLD 4072, Australia and <sup>2</sup>Queensland Brain Institute, The University of Queensland, St Lucia, QLD 4072, Australia

\*Author for correspondence (k.cheney@uq.edu.au)

#### SUMMARY

Animals use coloured signals for a variety of communication purposes, including to attract potential mates, recognize individuals, defend territories and warn predators of secondary defences (aposematism). To understand the mechanisms that drive the evolution and design of such visual signals, it is important to understand the visual systems and potential response biases of signal receivers. Here, we provide raw data on the spectral capabilities of a coral reef fish, the Picasso triggerfish *Rhinecanthus aculeatus*, which is potentially trichromatic with three cone sensitivities of 413 nm (single cone), 480 nm (double cone, medium sensitivity) and 528 nm (double cone, long sensitivity), and a rod sensitivity of 498 nm. The ocular media have a 50% transmission cut off at 405 nm. Behavioural experiments confirmed colour vision over their spectral range; triggerfish were significantly more likely to choose coloured stimuli over grey distractors, irrespective of luminance. We then examined whether response biases existed towards coloured and patterned stimuli to provide insight into how visual signals – in particular, aposematic colouration – may evolve. Triggerfish showed a preferential foraging response bias to red and green stimuli, in contrast to blue and yellow, irrespective of pattern. There was no response bias to patterned over monochromatic non-patterned stimuli. A foraging response bias towards red in fish differs from that of avian predators, who often avoid red food items. Red is frequently associated with warning colouration in terrestrial environments (ladybirds, snakes, frogs), whilst blue is used in aquatic environments (blue-ringed octopus, nudibranchs); whether the design of warning (aposematic) displays is a cause or consequence of response biases is unclear.

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#### INTRODUCTION

Colour patterns are used by animals for a variety of communication purposes, such as attracting potential mates, exhibiting dominance or avoiding predation (Cott, 1940; Espmark et al., 2000). How animals respond to such visual signals can depend on the detectability of signals against the background habitat, the light environment in which signals are viewed (Lythgoe, 1979; Hailman, 1977; Hailman, 1979; Endler, 1993) and the visual capabilities of signal receivers (Endler, 1992), which can vary considerably between species (Kelber et al., 2003). Fish range from a having a single visual pigment-bearing cone (monochromatic) to having four different types of cone cell with different absorption spectra (tetrachromatic) (Losey et al., 2003; Bowmaker and Loew, 2007; Neumeyer, 1992). However, rather like mammals (Jacobs, 1981), fish most often have two or three spectral sensitivities (dichromatic or trichromatic) (Lythgoe and Partridge, 1989; Lythgoe, 1979; Losey et al., 2003).

Psychological processes in the perceptual or cognitive system of the signal receiver and previous experience with similar stimuli may also dictate how animals respond to stimuli (Rowe and Guilford, 1996; Schuler and Hesse, 1985; Roper and Cook, 1989; Guilford and Dawkins, 1991; Mackintosh, 1974). Pre-existing (unlearned) response biases have been investigated with regards to mate choice (Basolo, 1990; Dawkins and Guilford, 1996; Ryan, 1998; Fuller et al., 2005), prey attraction and predator avoidance (Bruce et al., 2001),

foraging (Raine and Chittka, 2007; Smith et al., 2004), and warning colouration (Schuler and Roper, 1992; Lindstrom et al., 1999). For example, flower naive honey bees show an unlearned preference towards bee–UV–blue and bee–green colour, the adaptive significance of which is explained with an increase in nectar reward in flowers exhibiting these colours (Giurfa et al., 1995). However, pre-existing sensory biases can be quickly extinguished by learning-based processes (ten Cate and Rowe, 2007); bumblebees override unlearned preferences when colouration and pattern of the most profitable flowers change (Lynn et al., 2005). Understanding whether pre-existing or learned biases exist in animals may give us insights into how visual signals evolve, particularly those used in aposematic colouration and sexual signaling.

In this study, we investigated the visual capabilities and psychological processes of a coral reef fish to help us understand the function and evolution of colours and patterns in one of the most spectrally diverse environments. The spectral sensitivity of over 70 species of coral reef fish have been measured using microspectrophotometry (MSP) (Losey et al., 2003; Marshall et al., 2006) and limited behavioural evidence supports the fact that coral reef fish can see colour (Siebeck et al., 2008; Pignatelli et al., 2010). To address these issues, we: (1) used MSP to assess the spectral capabilities of a coral reef fish, the Picasso triggerfish *Rhinecanthus aculeatus*; (2) conducted a behavioural experiment to determine whether triggerfish could distinguish between coloured and grey

stimuli of varying luminance; (3) assessed whether a response bias to particular colours in a foraging scenario exists in this species; and (4) determined whether patterns affected any response bias found.

## MATERIALS AND METHODS

### Study species

Picasso triggerfish, *R. aculeatus* (Linnaeus 1758), are a common reef fish found throughout the IndoPacific and usually inhabit shallow reef flats on rubble and sandy areas (Witte and Mahaney, 2001). They feed on a variety of organisms including: algae, invertebrates, detritus, molluscs, crustaceans, worms, sea urchins, fishes, corals, tunicates, forams and eggs (Randall, 1985). Picasso triggerfish are territorial, use holes as shelter and possess intricate colour patterns including yellow lips, and blue and yellow stripes along the head (supplementary material Fig.S1). We used this species as they are easy to keep in aquaria, trainable and relatively abundant at our study sites. Individual fish ranged in size from 7 to 21 cm standard length (SL; mean  $\pm$  s.e.m. 13.0 $\pm$ 2.3 cm).

### MSP and ocular media absorbance measurements

The spectral sensitivity of cones found in Picasso triggerfish has previously been reported (Pignatelli et al., 2010); however, here we report raw data to show how spectral sensitivity curves were generated and further information on rod sensitivities and ocular media measurements. MSP was conducted as described previously using a single beam instrument (Levine and MacNichol, 1979; Losey et al., 2003; Hart et al., 2004; Hart et al., 1998; Cheney et al., 2009b). Each photoreceptor was also bleached and a difference spectrum calculated to confirm the spectral absorbance of the visual pigments (Levine and MacNichol, 1979). In order to calculate the spectral sensitivity of the photoreceptors, the spectral absorbance of the ocular media, cornea, lens and vitreous humour that lie in the light path before the photoreceptors must be measured. This procedure also followed established methods (Douglas and Thorpe, 1992; Thorpe et al., 1993; Siebeck and Marshall, 2001) and in this case we used an Ocean Optics (Dunedin, FL, USA) USB2000 spectrometer running OOIBASE32 software on a laptop computer and an Ocean Optics PX-2 pulsed xenon light source. Spectral sensitivity of each photoreceptor type was then calculated as the normalized product of its visual pigment absorbance nomogram and the transmission of the ocular media (Siebeck and Marshall, 2001).

### Behavioural experiments

We conducted behavioural experiments between April 2004 and August 2011 at the Lizard Island Research Station and One Tree Island Research Station, Great Barrier Reef, or at the University of Queensland (UQ, Brisbane, Australia). Fish were collected from reefs around the islands using hand and barrier nets and transported back to the research stations or to aquaria at UQ. Fish were held in aquarium tanks that ranged in size depending on location (Lizard: 50 $\times$ 25 $\times$ 25 cm; One Tree: 45 $\times$ 30 $\times$ 30 cm; UQ: 50 $\times$ 30 $\times$ 30 cm), given shelter and fed pieces of shrimp or squid daily. Fish were left for at least 7 days to acclimatize before they were used in experiments.

To assess the visual capabilities of fish (experiment 1), coloured stimuli were made using 'gel' filters from LEE Filters (Andover, Hants, UK) glued to the top of vial caps internally weighed down with a coin or paper clip. Fish were then trained to associate a particular colour stimulus with a food reward. To assess response biases (experiments 2–4), coloured stimuli were made so that they were edible. Stimuli were thus made into agar agar models flavoured

with chopped prawn, which are attractive and tasteful to foraging fish. To do this, stimuli were custom made using Adobe Photoshop CS and a H470 ink-jet printer (Canon, Sydney, Australia), printed on photographic paper and laminated. We then dissolved 5 g of agar agar powder (Lotus Foods, Kilsyth, Victoria, Australia) in seawater that had almost reached its boiling point. The solution was then left to cool, and before it had solidified, we added 8 g blended/finely chopped prawn to the solution to make it tasteful to fish. A 3.5 cm plastic Petri dish was filled half-way with the shrimp-flavoured solution, the laminated coloured stimuli was placed on top and a clear layer of agar agar solution without prawn was poured over the top. This last step was performed as the prawn slightly discoloured the solution, and therefore the stimuli. It also ensured small pieces of prawn were hidden from the fish, which may have influenced their decision making. However, the presence of prawn under the coloured disc was sufficient to make the stimuli tasteful and attractive to the fish. The Petri dishes containing the patterned stimuli were then attached vertically to 40 cm wide feeding boards and placed at the end of each tank. Stimuli were positioned 20 cm apart, 10 cm from the bottom and 10 cm from the sides. The position in which each coloured stimulus was placed was randomized. For all experiments, triggerfish were confined to a separate chamber of the tank with an opaque partition while the models were placed in position. The trial commenced once the partition screen was lifted (see supplementary material Movie 1).

Based on modelled spectral sensitivities, the colour vision results from experiment 1 and previous colour choice experiments with Picasso triggerfish (Pignatelli et al., 2010), the 'design' of each patterned colour stimulus was chosen to be within the perceptual capabilities of the fish. Spectral reflectance measurements of all coloured stimuli (including those embedded in agar) were obtained using an Ocean Optics USB2000 spectrometer and stored using a laptop computer running OOIBASE32 software. The light reflected from each colour area of the stimuli were then measured relative to a 'Spectralon' white standard using a PX-2 pulsed xenon light source (Ocean Optics).

In any colour vision test, the potential for the animal to use differences in luminance, rather than chromatic cues, when choosing coloured stimuli must be controlled for (Kelber et al., 2003). Therefore, in experiment 1 colours were carefully equated in luminance to the known fish spectral sensitivities. As most animals, including fish (Neumeyer et al., 1991), are thought to use primarily the longwave cone sensitivity for luminance tasks (Kelber et al., 2003), we measured the difference in log quantum catch ( $Q$ ) of the long wavelength receptor ( $L$ ) for each spectral reflectance signal and adjusted the colour of the target accordingly (see Marshall and Vorobyev, 2003). In experiment 2, stimuli were designed to add noise in luminance between each quarter within a stimulus (and included a grey and white quarter to achieve this; Fig. 3). However, each coloured quarter (e.g. top right quarter of each stimuli) was matched for luminance levels.

### Experiment 1: behavioural evidence of colour vision in Picasso triggerfish

In total, we tested 24 fish to assess whether they had the ability to detect chromatic differences between coloured stimuli (blue, green, yellow, orange, red; Fig. 2B). Ten fish were tested in April and May 2004, and a further 14 fish were tested in August 2011 at Lizard Island Research Station. Fish were held in tanks with a continuous flow-through system, and water was changed at least twice per day. Fish were randomly allocated a colour and first trained to approach and turn over vial caps covered with coloured filters to receive a food

reward (small piece of prawn) that was placed under the cap, giving the fish a task it often performs in nature; that is, turning over objects to look for food. The role of olfactory cues in learning the task appeared to be minimal; fish learnt the task on a trial-and-error basis and did not appear to detect the food reward under the cap by olfactory cues alone. Fish that failed to learn the task during food-present training, or did not achieve greater than 70% success rate in training phase were omitted from the analysis ( $N=6$ ). For testing, the food reward was no longer placed under the caps, and a new set of caps that had never contained food (and never did throughout the test phase) was used. Once fish had made a correct choice and turned over the cap, fish were rewarded with a small piece of prawn held by a small pair of tweezers, which were placed into the tank from above and at the opposite end of the tank from the coloured stimuli. In each test, fish chose between the coloured cap and two other grey caps pseudo-randomly selected from a series of six grey distractor stimuli (with neutral density ND 0.15, 0.3, 0.6, 0.9, 1.2 and 1.8 units) to ensure fish were not selecting caps based on luminance. Each fish was tested on its respective colour between 15 and 26 times.

### Experiment 2: do fish have a response bias to particular colours?

Having determined that Picasso triggerfish were capable of colour vision and the approximate extent of their colour sense, we tested 16 fish in October 2008 at Lizard Island Research Station to determine whether they had a response bias to particular coloured stimuli, namely 'blue', 'green', 'yellow' or 'red' (Fig. 3). We chose these four colours as they are equally distributed across the range of the perceptual capabilities of Picasso triggerfish. We were also interested in whether blue and yellow were avoided more than red

and green, as many aposematic marine species, including nudibranch molluscs (particularly chromodorids), exhibit a blue, yellow and black colour pattern (Edmunds, 1991). In each trial, two models of semi-randomly selected colours were presented to the fish, so that each colour combination was offered to the fish. Trials were continued for 6 days, testing 1–2 times per day. We did not continue the experiment further as we wanted to record an initial response bias, rather than any learned preferences that could occur over a longer period. The trial continued for 3 min after the initial peck, but was terminated after 10 min if the fish had not consumed anything. If a fish did not peck either model in a particular trial, this trial was omitted from the analysis and the same colour combination was offered again on a subsequent day. The observer recorded the colour of the first model pecked and the total number of pecks on each model within the first 3 min.

### Experiment 3: does a complex pattern affect response biases to particular colours?

We also examined whether fish had a response bias to complex patterns consisting of multiple colours. Here, two patterns of red/green/black and blue/yellow/black with three different luminance values were used. For each colour combination, we had four differently randomly designed patterns (as per Fig. 4), each with an equal number squares for each colour, to ensure it was the colour combination and not the pattern itself that caused any decision making in the fish. For each test, we presented two stimuli (always one red/green/black and one blue/yellow/black, but randomized for luminance and pattern), to eight fish a total of 18–20 times; therefore, a total of 148 trials were conducted. Fish were tested in May 2011 at Lizard Island Research Station and were tested 2–3 times per day.

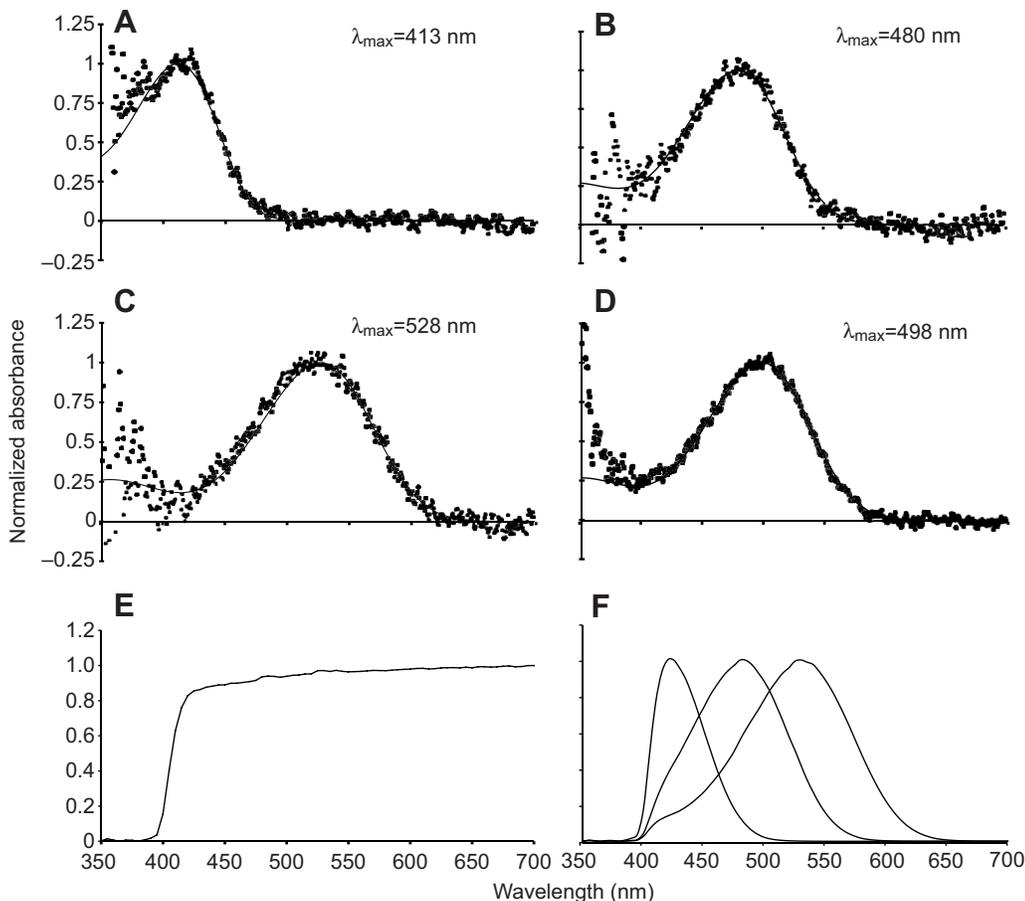


Fig. 1. Normalized pre-bleach absorbance spectra for (A) the short-wavelength-sensitive (SWS) single cones ( $N=7$ ); (B) the medium-wavelength-sensitive (MWS) visual pigment found in one member of the double cone pair ( $N=10$ ); (C) the long-wavelength-sensitive (LWS) visual pigment found in the other member of the double cone pair ( $N=12$ ); (D) the MWS visual pigment found in the rod photoreceptors ( $N=7$ ); (E) transmission measurements through the cornea, vitreous and lens; and (F) calculated spectral sensitivities of three cone photoreceptors of the Picasso triggerfish. The maximum absorbance ( $\lambda_{\max}$ ) is given in A–D.

The observer recorded the first model pecked and the number of pecks on each model over a 3 min time period.

#### Experiment 4: do fish have a response bias to patterned stimuli over monochromatic stimuli?

Here, we assessed whether conspicuous patterns, namely spots and stripes that are frequently found on aposematic nudibranch molluscs (Debelius and Kuitert, 2007), caused a response bias. We predicted that patterned stimuli may be more aversive to potential predators (Roper and Cook, 1989). For each trial, we presented a patterned stimulus and a monochromatic non-patterned stimulus of the same colour (yellow or blue; Fig. 5) to the fish in a semi-randomized design. Sixteen fish were tested in December 2008 at One Tree Island Research Station and each fish was tested once per day. Fish were tested twice with each colour and pattern combination; therefore, a total of 128 trials were conducted. The observer recorded the first model pecked and the number of pecks on each model over a 3 min period.

#### Statistical analyses

All statistical analyses were conducted in R 2.1.1 (R Development Team, 2011). For experiments 1, 3 and 4, we used a general linear mixed model with a binomial distribution with log link function, using the lmer function in lme4 package (Bates et al., 2011). Outcome (1, success; 2 non-success) was used as the dependent variable, pattern and colour were fixed factors, and fish identity was a random factor. We also included the size of the fish (all models:  $P > 0.54$ ), the position of stimuli (all models: left/right,  $P > 0.63$ ), and the colour or colour combination that was initially presented to the fish as fixed factors (all models:  $P > 0.32$ ), but all were insignificant. In experiment 1, we tested whether the success rate of coloured stimuli chosen was significantly different to 33% (as three stimuli were presented to the fish at each time) by using an offset of  $\text{logit}(0.33) = -\log(2)$ . In experiment 2, we determined whether fish showed a preference for a particular colour by analysing the data with the Bradley–Terry model (Bradley and Terry, 1952), which is specifically designed for experiments consisting of paired data (David, 1988). The Bradley–Terry model allows for a parametric test of the hypothesis that the treatments can be ordered according to preference. It supposes that  $P_{ij}$  is the probability of preferring treatment  $i$  to treatment  $j$  such that  $P_{ij} = 1 - P_{ji}$ . A preference ranking for all treatments can then be constructed based on the relative preference for each treatment compared with a baseline treatment [as  $\text{logit}(P_{ij})$ , where  $j$  is the baseline treatment]. In this analysis, ‘blue’ was the baseline treatment. The measured response variable was simply whether a particular colour was pecked first (1) or not pecked first (–1), or a colour was pecked most frequently (1) or not (–1), or the percentage missing of a colour was more (1) or less (–1). Colours not involved in a particular trial were (0). Because the experiment was performed over multiple trials over which a response bias could change, we included trial number as a fixed factor; however, this was insignificant ( $t_{141} = -0.38$ ,  $P = 0.71$ ). Analysis for experiment 2 was performed using the glmmPQL function in the MASS package (Venables and Ripley, 2002).

## RESULTS

### MSP of Picasso triggerfish and ocular media absorbance measurements

MSP of Picasso triggerfish retina revealed a single rod type and three cone types, one of which was a single cone and the other two being members of a double cone (Walls, 1942) (Fig. 1). The peak absorbance ( $\lambda_{\text{max}}$ ) determined by fitting the averages of raw spectra with a vitamin A1-based template (Govardovskii et al., 2000) of the appropriate  $\lambda_{\text{max}}$  was: rod 498 nm; single cone, short-wavelength

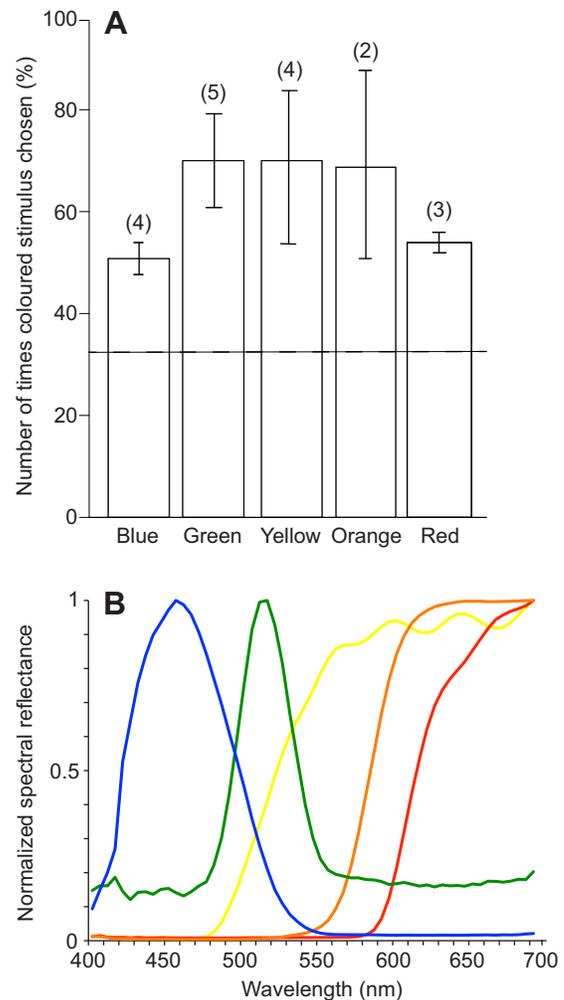


Fig. 2. (A) The number of times (%) that fish chose the coloured stimulus over two grey distractors of varying luminance (experiment 1). Bars represent means  $\pm$  1 s.e.m. Dashed lines indicate a threshold value of 33% if fish were choosing stimuli at random. Sample size (number of fish tested) is shown in parentheses. (B) Spectral reflectance of the coloured stimuli.

(S) sensitivity 413 nm; double cone, medium-wavelength (M) sensitivity 480 nm; double cone, long-wavelength (L) sensitivity 528 nm (Fig. 1A–D) (Pignatelli et al., 2010). The ocular media were found to have a 50% transmission cut off at 405 nm.

### Experiment 1: behavioural evidence of colour vision in Picasso triggerfish

Eighteen fish learnt the task within 4–5 days, but were tested after 10 days when their test performance was over 70% correct; the remaining six fish were omitted from the analysis. For all colours, fish were significantly more likely to choose a coloured stimulus over the two grey distractors, irrespective of the brightness of each distractor (blue:  $z = 2.15$ ,  $N = 4$ ,  $P = 0.03$ ; green:  $z = 5.07$ ,  $N = 5$ ,  $P < 0.001$ ; yellow:  $z = 3.63$ ,  $N = 4$ ,  $P < 0.001$ ; orange:  $z = 2.61$ ,  $N = 2$ ,  $P = 0.01$ ; red:  $z = 2.12$ ,  $N = 3$ ,  $P = 0.03$ ; Fig. 2).

### Experiment 2: do fish have a response bias to particular colours?

Fish showed a significant preference for the red stimuli in terms of the first model pecked (first model pecked:  $z = 3.55$ , d.f. = 93,  $P < 0.001$ ;

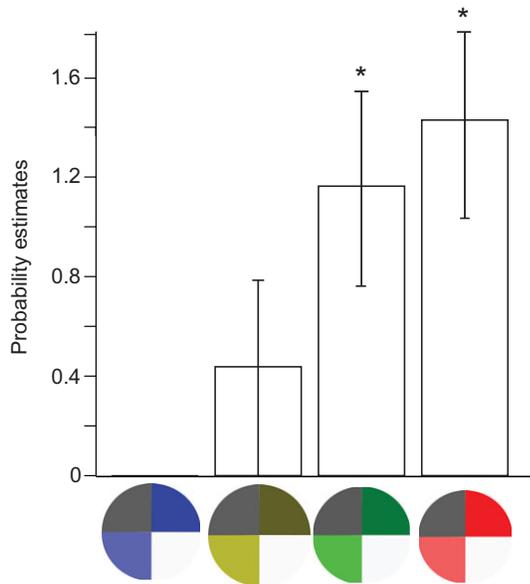


Fig. 3. Probability of choosing each coloured stimulus compared with the baseline treatment (blue) (experiment 2). Bars indicate estimate  $\pm$  1 s.e.m. \*Significant difference from blue ( $P < 0.05$ ).

number of times model pecked in first 3 min:  $z = 2.52$ , d.f. = 93,  $P = 0.01$ ; Fig. 3) followed by green, yellow and then blue. Preference for red and green was significantly greater than for blue ( $z > 2.76$ , d.f. = 93,  $P < 0.01$ ); however, there was no difference between blue and yellow ( $z = 1.13$ , d.f. = 93,  $P = 0.26$ ).

### Experiment 3: does a complex pattern affect response biases to particular colours?

The red/green/black pattern was chosen significantly more times than the blue/yellow/black pattern (first model pecked:  $z = 2.90$ ,  $N = 97$ ,  $P = 0.003$ ; total number of pecks:  $z = 2.84$ ,  $N = 97$ ,  $P = 0.003$ ; Fig. 4). Luminance was non-significant ( $z = -1.57$ ,  $N = 97$ ,  $P = 0.12$ ).

### Experiment 4: do fish have a response bias to patterned stimuli over monochromatic stimuli?

There was no difference in whether pattern or solid colours were pecked first ( $z = -0.23$ ,  $N = 135$ ,  $P = 0.82$ ), irrespective of pattern (dots, stripes;  $z = -0.55$ ,  $N = 135$ ,  $P = 0.58$ ) or colour (blue, yellow;  $z = -0.85$ ,  $N = 135$ ,  $P = 0.40$ ). There was also no significant difference in the number of pecks on each pattern ( $z = -0.32$ ,  $N = 135$ ,  $P = 0.71$ ) or colour ( $z = -0.76$ ,  $N = 135$ ,  $P = 0.48$ ) (Fig. 5).

## DISCUSSION

Here, we provide anatomical and behavioural evidence that the Picasso triggerfish has colour vision, with three distinct cone photoreceptors with spectral sensitivities of 420, 480 and 528 nm (note the shortest wavelength sensitivity S is shifted from the  $\lambda_{\max}$  of the single cone visual pigment 413 nm to 420 nm by the filtering of the ocular media). The likely trichromatic nature of the colour vision system of Picasso triggerfish has been suggested previously (Pignatelli et al., 2010), but here, using an experiment based on the classic colour vision tests of von Frisch (von Frisch, 1914), we demonstrate colour vision over an extensive colour range in this species.

Reef fish vary considerably in their spectral capabilities (Losey et al., 2003; Marshall et al., 2006). The spectral position of

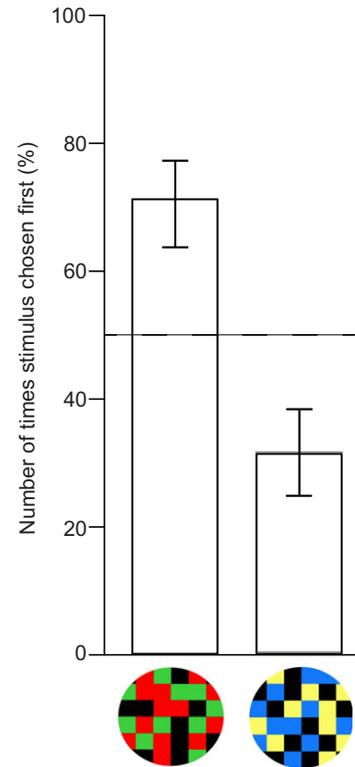


Fig. 4. The number of times (%) each 'scrambled pattern' stimulus was chosen first (experiment 3). Bars indicate means  $\pm$  1 s.e.m. Dashed line indicates a threshold of 50%, which would indicate stimuli were chosen at random.

photoreceptors between species can be partly explained by classical visual ecology trends of light transmission through water (Lythgoe, 1979; Lythgoe et al., 1994); however, more recent studies (e.g. Losey et al., 2003) indicate a complexity of different types that is as yet unexplained. In general, however, many close-to-surface dwelling reef fish possess at least three spectral sensitivities, often with one S-type housed in a single cone and the other two (M and L) in different members of a double cone (Lythgoe, 1979; Losey et al., 2003; Bowmaker and Loew, 2007). The spectral habitat of this shallow living species is rich in UV wavelengths (McFarland and Munz, 1975; Marshall et al., 2003) and some reef fish, often the smaller species, possess a spectral sensitivity peaking in the ultraviolet (UV,  $< 400$  nm) (McFarland and Loew, 1994; Losey et al., 1999; Siebeck and Marshall, 2001). However, Picasso triggerfish have both a lens and cornea that block these wavelengths, restricting the colour vision range of this species to 400–700 nm, similar to that of humans (Jacobs, 1981).

We also found that triggerfish have a preferential foraging response bias to red, followed by green, coloured stimuli. A red foraging bias has also been shown in other fish, such as sticklebacks (Smith et al., 2004); red was suggested to be the principal colour of their natural food items, which includes chironomid larvae, *Tubifex*, *Asellus* and *Daphnia* (Ibrahim and Huntingford, 1989), and also copepods, cladocerans and ostracods (Wootton, 1984). Triggerfish forage on a wide range of food items (such as forams, invertebrates, detritus and corals), which exhibit all four colours tested here. However, the bias for red food items may be adaptive and derived from a requirement for carotenoids, which range in colour from orange to red and have to be obtained

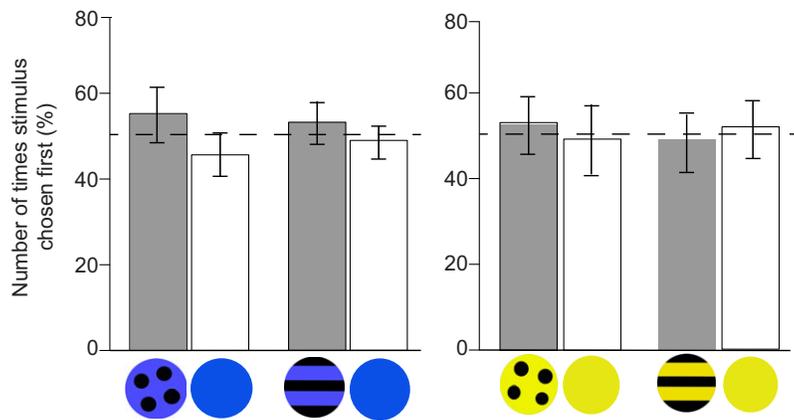


Fig. 5. The number of times (%) each plain or patterned stimulus was chosen first (experiment 4). Bars and error bars indicate means  $\pm$  1 s.e.m. Dashed line indicates a threshold of 50%, which indicates stimuli were chosen at random.

from the diet as they cannot be synthesized by the animals themselves. Carotenoids are required for a large number of physiological processes, including cancer prevention, protection from UV, protection of digestive enzymes and reproductive tissues, growth and cell differentiation (see Olson and Owens, 1998). Astaxanthin is the most commonly occurring red carotenoid in the marine environment, and is essential for the growth and survival of fish (Torrissen and Christiansen, 1995). Carotenoid pigments also play an important role in regulating fish colour and produce the yellow, orange and red colour displays of fish that may be used in intraspecific signalling as a measure of quality, territoriality and dominance. If the need for dietary carotenoids drives the bias, then we would expect to find a universal bias between coral reef fish. In an additional, slightly modified experiment 2, we also found that lunar wrasse (*Thalassoma lunare*;  $N=13$ ) exhibited a red bias for food items ( $t_{141}=3.23$ ,  $P<0.001$ ; supplementary material Fig.S2). Picasso triggerfish and lunar wrasse inhabit different habitats on the reef and exhibit different lifestyles; however, the two species overlap to some degree in their food sources as they are both omnivores and feed on a wide range of food items. Therefore, this response bias should be tested in fish with different diets, such as corallivores and piscivores.

The observed response bias could also be explained by learnt or unlearnt biases against blue and yellow, colours that often signal toxic or dangerous animals on the reef, including chromodorid nudibranchs (Rudman, 1991) and the higher-end molluscs such as the blue-ringed octopus. Unlearnt biases can potentially evolve readily when learnt avoidance to these species is too costly (Stevens and Ruxton, 2012); for example, encounters with blue-ringed octopus could result in death. Unfamiliarity with blue may also increase avoidance rates by predators (neophobia). If an unlearned response (avoidance) bias did exist in fish towards blue and yellow, it might direct the learning process and make it easier to associate particular colours and patterns with unpalatable food items. Here, we could not examine whether previous experience influenced our observations, as all fish were collected from the reef; however, we found no difference between smaller and larger individuals.

Interestingly, attraction with a yellow and blue signal is also a known strategy in marine environments, so clearly the action after seeing such a coloured pattern is context specific. Blue and yellow is the 'uniform' of cleaner fish (Cheney et al., 2009a), who are not signalling their toxicity, but are trying to attract the attention of client reef fish to visit cleaning stations and have their ectoparasites removed. Several reef fish most likely use yellow and blue in sexual selection decisions or in other intraspecific signals, and these are the

predominant colours of Picasso triggerfish, suggesting that this species is one of them (supplementary material Fig.S1). Blue and yellow is an effective colour combination in marine environments where it transmits well (Lythgoe, 1979; Marshall, 2000a; Marshall, 2000b). Most colour vision systems have a short wavelength photoreceptor and at least one other sensitive to longer wavelengths meaning the yellow–blue axis in perceptual space is both evolutionarily ancient, or at least well used, and conspicuous to a wide variety of animals (Hurlbert, 1997; Jacobs, 1981; Cheney et al., 2009a).

When considering pattern, we also found that fish exhibited a response bias to complex patterned stimuli containing red, green and black compared with patterns that contained blue, yellow and black, which indicates colour is the main cue that drives the response bias as opposed to pattern. Furthermore, we found that fish did not avoid patterned objects over solid coloured objects. In general, the relative importance of pattern, colour and luminance in visual signalling is unclear. Chicks also appear to use colours, rather than pattern, when learning and memorizing a signal (Aronsson and Gamberale-Stille, 2008; Osorio et al., 1999). Specific colours may transmit information, but pattern, in particular high contrast within a colour pattern or against the background, may increase the speed at which a signal is learnt (Rothschild, 1984), increase initial wariness in predators (Lindström et al., 2001) and increase the time that avoidance learning by predators is retained (Roper and Wistow, 1986; Roper and Redston, 1987).

In conclusion, understanding the visual capabilities and psychology of signal receivers will help us elucidate the evolution and function of colour and patterns. Response biases differ between taxa, and can be dependent on the visual capabilities of the signal receiver, varying physiological requirements, different behaviours, and the environment and context in which the signal is viewed.

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#### AUTHOR CONTRIBUTIONS

K.L.C. and N.J.M. conceived and designed the study, executed some of the experimental work (experiment 1 and 3), and interpreted the findings. K.L.C. drafted and revised the article. C.N. and E.C.M. executed experiment 2 and 4, respectively.

#### COMPETING INTERESTS

No competing interests declared.

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