

Colour vision in coral reef fish

U. E. Siebeck^{1,*}, G. M. Wallis² and L. Litherland¹

¹Sensory Biology Group, School of Biomedical Sciences, University of Queensland, St Lucia 4072, Australia and ²Visuo-motor control laboratory, School of Human Movements, University of Queensland, Australia

*Author for correspondence (e-mail: u.siebeck@uq.edu.au)

Accepted 30 October 2007

SUMMARY

Over many millions of years, sea creatures have developed a range of light reflectance properties. One example is the large variation in the patterns and colours of fish inhabiting the world's coral reefs. Attempts to understand the significance of the colouration have been made, but all too often from the perspective of a human observer. A more ecological approach requires us to consider the visual system of those for whom the colours were intended, namely other sea life. A first step is to understand the sensitivity of reef fish themselves to colour. Physiological data has revealed wavelength-tuned photoreceptors in reef fish, and this study provides behavioural evidence for their application in colour discrimination. Using classical conditioning, freshly caught damselfish were trained to discriminate coloured patterns for a food reward. Within 3–4 days of capture the fish selected a target colour on over 75% of trials. Brightness of the distracter and target were systematically varied to confirm that the fish could discriminate stimuli on the basis of chromaticity alone. The study demonstrates that reef fish can learn to perform two-alternative discrimination tasks, and provides the first behavioural evidence that reef fish have colour vision.

Key words: colour vision, classical conditioning, coral reef fish, behaviour.

INTRODUCTION

Coral reef fish are often colourful and live in a colourful environment. The question of why coral reef fish are so colourful has received much attention over the years and yet still remains largely unresolved (Longley, 1917; Lorenz, 1962; Crook, 1997; Marshall, 2000a). Colour signals have been found to be important for a large range of behaviours across a large range of animals (Bradbury and Vehrencamp, 1998). One shortcoming of many studies has been the tendency to describe the animal colours from the perspective of the human visual system rather than that of a conspecific or other relevant observer (Bennett et al., 1994; Barber et al., 2001). The recent discovery that many reef fish colours contain ultraviolet components (Marshall, 2000b), that many of these fish appear to be sensitive to ultraviolet light due to ultraviolet transparent ocular media (Siebeck and Marshall, 2001) or ultraviolet sensitive photoreceptors (for a review, see Marshall et al., 2006) and that at least one, *Pomacentrus amboinensis*, has been shown to use ultraviolet signals for communication (Siebeck, 2004) serves to heighten the need for a new approach based on the visual system of the fish themselves.

How fish colours are perceived by organisms observing them depends on three variables: (i) the spectrum of the light present in their habitat (downwelling light and transmission properties of the water), (ii) the properties of the fish colours, and (iii) the visual system of the observer (Lythgoe, 1979; Vorobyev et al., 2001). A large body of work exists on the transmission properties of different types of water in different parts of the world (Frank and Widder, 1996; Kirk, 2003) and there is evidence to show that the visual system of organisms is often adapted to the specific spectral properties of the water they live in (Lythgoe, 1972; Shand, 1993; Novales Flamarique, 2000) and may even depend on the specific visual signals they are interested in within their spectral environment (Cummings, 2007).

Reef fish colours can be simple or complex (with single or multiple reflectance peaks) and often include peaks in the ultraviolet range (Marshall, 2000b). Importantly, the choice of reflectances may say little or nothing about the visual system of a fish since the patterns may be intended for the eyes of fish other than conspecifics. For example, not all fish with UV patterning possess UV transparent ocular media (Siebeck and Marshall, 2001). Ultimately, if we wish to understand the colour systems of fish we need to conduct physiological and behavioural experimentation.

One major prerequisite for colour vision in any organism is the presence of at least two photoreceptor (cone) types with different spectral sensitivities. A first step towards investigating colour vision is, therefore, to analyse the number of photoreceptor types present in the retina. With the help of microspectrophotometry (MSP), the spectral absorbance of individual photoreceptors can be measured directly (Hart, 2004). Around 70 species of marine fish have been measured to date, and have been found to have at least two different spectral types of photoreceptors (for a review, see Marshall et al., 2006).

Another prerequisite for colour vision is that photoreceptors with different spectral sensitivities form separate channels that are compared during signal processing, allowing for the discrimination of colours on the basis of their wavelength composition. If the signal from all photoreceptors is combined into a single channel the discrimination of two colours is only possible if they differ in brightness. Thus an animal can be shown to have colour vision if it can distinguish colours on the basis of their wavelength composition independently of their brightness (Kelber et al., 2003). Investigations into colour vision, therefore, must somehow eliminate brightness as a possible cue. One approach is to produce isoluminant stimuli. This has been a common approach in human behavioural work (Medina and Mullen, 2007). However, such an approach requires extremely careful control of colour production

and, in the case of animal studies, knowledge of the subject animal's photoreceptor characteristics. A simpler alternative is to render luminance irrelevant by adding luminance noise to both target and distracter stimuli.

The first behavioural experiments on colour vision in fish were conducted nearly a century ago (von Frisch, 1912). For a good review on different approaches used to investigate colour vision in animals see Kelber et al. (Kelber et al., 2003). Using a variety of behavioural methods, colour vision has been demonstrated for a number of freshwater fish (Schiemenz, 1924; Neumeyer, 1984; Neumeyer, 1992). However, no behavioural studies exist that test the ability of marine fish to see colour, and, to our knowledge, no studies exist that test the ability of reef fish to perform visual discrimination tasks. The reef fish species selected for this study, the damselfish *Pomacentrus amboinensis* Bleeker 1868 (Pomacentridae), is a territorial omnivore. It was chosen because previous studies have shown that the fish accept aquaria as new territories within less than a day, easily adjust to commercially available fish flakes, and readily interact with objects placed into the aquaria (Siebeck, 2004).

Here, we test whether freshly captured coral reef fish, *Pomacentrus amboinensis* are able to learn to perform a two-alternative, forced-choice task designed to test whether they have the ability to distinguish between two colour stimuli, independently of their brightness.

MATERIALS AND METHODS

Fish

The fish were collected while SCUBA diving using hand nets on reefs around Lizard Island, Australia. They were held individually in glass aquaria (30×20×30 cm) at the Lizard Island Research Station. All aquaria received fresh saltwater continuously (flow through system). A plastic tube (10 cm long, 4 cm diameter) was placed into each tank and served as shelter for the fish. All fish were released back to their reef following completion of the experiments.

Food preparation and feeding apparatus

The composition of food as well as the feeding apparatus was simplified from that of Neumeyer (Neumeyer, 1984). Ten grams of commercially available fish flakes for tropical marine fish (Flake Frenzy, Marine Flakes, HBH, Springville, UT, USA) were mixed with 7.5 ml of water. The mixture was stirred and kneaded with a wooden spoon until both components had fully combined and had a shiny texture.

The feeding apparatus consisted of a tube (3 mm diameter, 150 mm long) that was attached to a 5 ml syringe filled with the food mixture. The amount of food available to the fish was controlled manually and could be adjusted by varying the pressure applied to the syringe. In this way, different amounts of food could be delivered to the fish in a controlled manner.

In Neumeyer's set-up the food preparation is a lengthy process that involves various processes to ensure that the mixture is homogenous and does not contain any air bubbles (Neumeyer, 1984). This is important as the food is delivered via two long tubes (>40 cm) that extend from the syringe in the experimenters hands sitting in front of the aquarium all the way to the back of the aquarium along the back of a feeding plate that is inserted into the aquarium (Neumeyer, 1984). An air bubble somewhere inside the tube impairs the precisely controlled delivery of food, as pushing on the syringe will compress the air rather than pushing the food along. This leads to delayed delivery of the food, and, if the

experimenter keeps pushing, to the delivery of too much food too late. As the feeding tubes are fixed in position this situation cannot easily be rectified. In our case, the food was delivered to the front of the aquarium and as a consequence much shorter tubes were required. The effect of air bubbles in a short tube is much smaller than in a very long tube as less food is pushed around. Also, in the case that too much food is expelled, the feeding apparatus could be simply removed from the aquarium.

Training and testing procedures

The goal was to train the fish to push the rewarded stimulus with their mouth (from here on referred to as a 'tap') at least ten times before they received a reward. The high number of taps was chosen to ensure that the fish selected the target deliberately. The first step was to introduce the food to the fish by dropping small amounts of food near their shelter. Then, they were presented with food that was still hanging on a tube attached to the feeding syringe. Once they were used to eating off the end of the tube anywhere in their aquarium, a coloured stimulus was attached to the tube so that the end of the tube remained visible to the fish. Finally, the stimulus was moved down so that the end of the tube was obscured and the fish had to tap the stimulus before the experimenter applied pressure to the syringe and food appeared below the stimulus. Once the fish reliably tapped the stimulus ten times in at least three consecutive trials, a second (distracter) stimulus (also attached to an identical feeding apparatus) was presented together with the rewarded stimulus and testing commenced. Both syringes and tubes contained food to avoid any olfactory cues giving away the position of the rewarded stimulus. Between trials, any food the fish had not eaten was removed from the end of the rewarding tube. Also, a small amount of food was removed from the distracter tube so that the food in both tubes was equally fresh.

During testing, two stimuli were held inside the aquarium against the wall closest to the observer. A second observer tallied all taps, including those made on the distracter stimulus. The trial ended when the fish had achieved a correct response, or if the trial lasted more than 2 min, in which case the fish was not rewarded. Each testing block included four trials, and two blocks were completed each day. The positions of the reward and distracter stimuli were randomised under the constraint that each stimulus was presented for the same number of times on each side.

Experimental sequence

Ten specimens of the damselfish species, *Pomacentrus amboinensis*, were trained to a blue stimulus while another ten specimens of the same species were trained to a yellow stimulus. The colours yellow and blue were chosen for a number of reasons. They are highly contrasting colours that are used by many reef fish and therefore appear important for colour signalling (Marshall, 2000a). Also, *P. amboinensis* is largely yellow and as their habitat is found right at the edge of the reef they often view conspecifics against the blue underwater background illumination.

During initial testing, the blue-trained fish were tested against a yellow distracter and *vice versa*. This was done in order to verify that performance was independent of the training colour. A total of ten trials were conducted.

In part two of the experiment, the fish were tested against three different luminance levels of the distracter colour to test whether their behaviour could be explained by luminance differences. Six trials were conducted in which each luminance level was tested against the training colour twice in reversed positions.

Next, the fish were presented with three different luminance levels of the rewarded colour and tested against the original distracter stimulus. Six trials were conducted in which each brightness level was presented once in each position.

Finally, the fish were tested on all combinations of three brightness levels of distracter against three brightness levels. This was done to test whether the fish were able to categorise all brightness levels of the rewarded colour into one group of rewarded stimuli and distinguish them from all brightness levels of the distracter stimulus. Eighteen trials were conducted such that all combinations of distracter and learning luminance levels were tested against each other twice, and on both sides (left and right).

Stimuli

Stimuli were made of latex and painted with either blue or yellow acrylic paint (OPTIMAcryl®, Schmincke, Erkrath, Germany). The targets were finger-shaped and controlled by casting them in a plaster mould. In order to create darker and lighter shades of each colour, black or white was added to the plain colour. Three shades of yellow and three shades of blue were created in this way (Fig. 1). Two lighter shades of blue were created and a darker and a lighter yellow, with the aim of approximately matching the range of luminance levels of both sets of colours.

As the spectral sensitivities of the *P. amboinensis* are unknown, three brightness levels of distracter and reward stimuli were used. The ocular media were found to have a 50% transmission cut off at 330 nm (U.E.S., unpublished). The reflectance spectra of the stimuli were quantified using a fibre optic spectrometer (USB2000, Ocean Optics, Dunedin, USA). The reflectance of the targets was measured relative to a 99% reflectance standard. A PX-2 (Ocean Optics, Dunedin, USA) was used as light source.

Analysis

Before inclusion in the analysis, all responses (right/left) of each fish within each experiment were tested for possible bias towards one side with paired *t*-tests. None of the fish were excluded from analysis as no bias was detected (all *t*-tests, $P > 0.05$).

Fisher's exact test was used to test whether the fish could distinguish the trained from the distracter stimulus. The number of correct and incorrect taps (summed over all replicate conditions) was compared to the distribution of taps if no discrimination was achieved (50% correct). This analysis was done for each fish and each condition within each experiment. Confidence intervals were calculated assuming a binomial distribution.

The total number of first correct taps was also calculated for each condition over all fish.

RESULTS

Reflectance

Fig. 1 shows the reflectance of the colours used. All experiments were conducted under a transparent plastic roof that did not transmit ultraviolet light. The wavelength range available for vision under the experimental conditions was therefore limited to wavelength longer than 400 nm.

Training

All fish acclimatised to their new environment within 24 h. A fish was thought to have acclimatised when it was seen to explore the aquarium when no observer was present, it retreated to the shelter tube whenever it was approached and it was seen to observe the experimenter from the entrance of the tube. This behaviour was distinctly different from the first 24 h during which the fish never

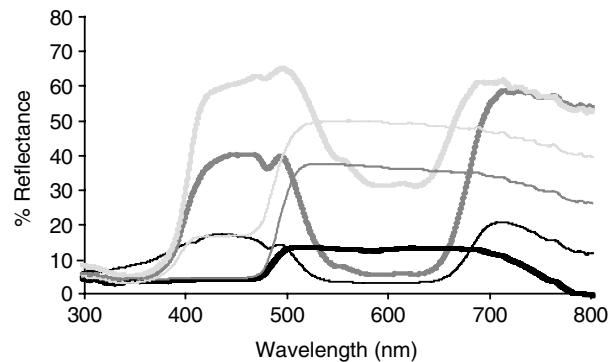


Fig. 1. Reflectance measurements of the coloured targets used. Three brightness levels of yellow (thin lines) and blue (thick lines) are shown. The range of wavelengths available to the fish was limited to wavelengths above 400 nm because of the absorption properties of the clear plastic roof material.

left their shelter and positioned their body so that the head was facing away from the observer. The individual behaviour of the fish towards the stimuli and the experimenter varied, but all fish (blue and yellow trained group) learned to associate food with coloured stimuli within 4 days post capture (Fig. 2). The critical and most time intensive step (2–3 days) in the training process was to convince the fish to 'trust' the experimenter and approach the feeding tube to receive their reward. Once feeding from the tube all fish rapidly completed the remaining training steps. Blue- and yellow-trained fish reached the training target (10 taps on correct stimulus) within seven sessions.

From the beginning of training, different levels of 'confidence' could be observed as judged by the fish's readiness to leave their shelter in the presence of the experimenter and the target. A common behaviour observed early on during training was the 'tail slap', where the fish approached the target tail first, slapped it several times and darted back into their shelter. As the behaviour was no longer observed during later stages of experimentation it appears that *P. amboinensis* use this behaviour to assess the level of danger of novel objects.

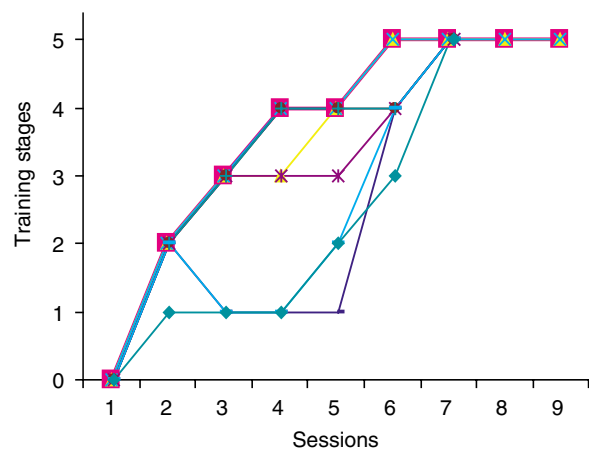


Fig. 2. Learning process of the ten fish trained on blue (each indicated by a different colour and symbol). Stage 0, day of capture; 1, only feeds after observer has left; 2, feeds from tube at shelter; 3, feeds from tube with target attached; 4, taps target at least once; 5, taps target ten times.

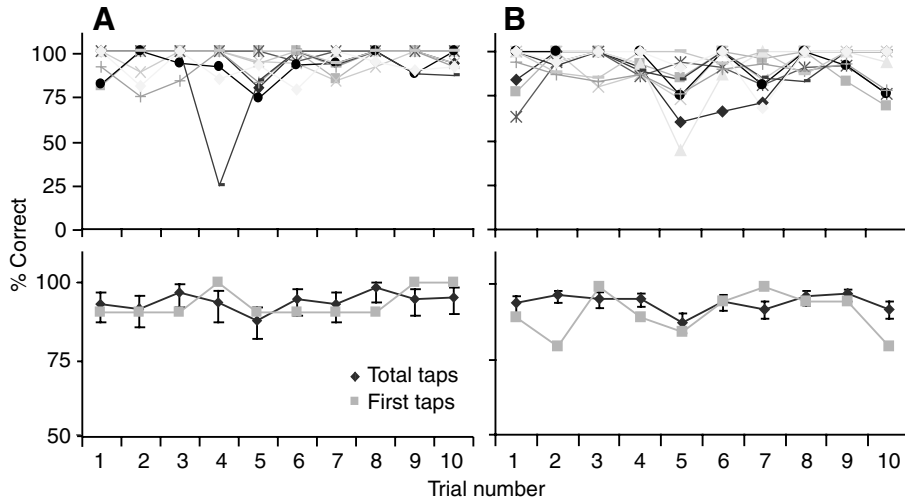


Fig. 3. Test 1: results for ten individual fish trained on yellow (A) and blue (B). The top graphs show the choice frequency of the fish for the correct target over ten consecutive trials. The bottom graphs show the average (\pm CI) of the total number of choices (diamonds) and the total number of correct first choices only (squares) for each group.

First test: blue versus yellow

All ten fish trained on the yellow stimulus were able to distinguish the yellow from the blue target from the first trial onwards (Fig. 3). On average they tapped the correct stimulus in 94.7% (98.3, 88.6; upper, lower 95% CI) of cases when all taps were counted. The fish tapped the correct target first 93 times out of 100.

All ten fish trained on the blue stimulus were also able to distinguish the two stimuli on average in 91.3% (95.8, 84.8) of cases when all taps are taken into account and they tapped the correct colour first in 82 of 100 cases.

In both groups of fish, all fish were able to distinguish the two colours as their tapping distribution was significantly different from chance (Fisher's exact test $P < 0.0001$, in all cases).

Second test: trained colour versus three distracter luminance levels

The fish trained on medium yellow were all able to distinguish their trained colour from all three levels of blue [light blue: 93.2% (97.1, 86.1); medium blue: 95.1% (98.4, 88.7); dark blue: 87.9% (93.6, 79.9)] (Fig. 4). In total, the fish tapped the correct target first 18 out of 20 times for light blue and medium blue trials and 16 times for dark blue trials. All fish showed significant results for the conditions yellow versus medium and light blue and all but two showed significant results for the yellow versus dark blue condition. The two fish that failed the task both showed a clear preference for the correct colour in their first trial (14 out of 17 correct taps), but their performance was reduced in their second trial (16 out of 28 and 22 out of 37 taps correct).

In general, the fish trained on dark blue were able to distinguish their trained colour from all three levels of yellow [total taps: light yellow: 90.1% (95.1, 82.4); medium yellow: 91.3% (95.8, 83.6); dark yellow: 92.0% (96.5, 84.8)]. All fish tapped the correct colour first for the light yellow condition, 15 out of 20 first taps were correct for the medium yellow condition, and 16 for the dark yellow condition (Fig. 4). In all cases the results were significantly different from chance (Fisher's exact test $P < 0.0001$).

Third test: three levels of trained colour brightness levels versus distracter colour

In general, fish trained on yellow were able to perform this task (Fig. 5). They correctly tapped their training colour independently of its brightness on average in 89.5% (94.4, 81.2) (light yellow), 91.3% (95.8, 83.6) (medium yellow, original rewarded stimulus) and 87.7% (93.6, 80.0) (dark yellow) of cases. The results of all but one fish are significant in all conditions (Fisher's exact test $P < 0.0001$). In the condition light yellow versus the distracter colour, fish number 1 tapped the correct stimulus 23 out of 31 times in the first trial and 30 out of 78 times in the second trial.

On average, the ten fish trained on blue correctly identified their training colour independently of its brightness in 76.9% (84.8, 67.5) (light blue), 90.8% (95.7, 83.7) (medium blue) and 94.6% (98.4, 88.7) (dark blue, original training colour) of cases (Fig. 5). Eight of the ten fish trained on blue were able to perform this task to at least 75% correct for all three brightness levels (Fisher's exact tests in those cases $P < 0.05$), whereas two fish had problems with generalising from the original training colour (dark blue) to light

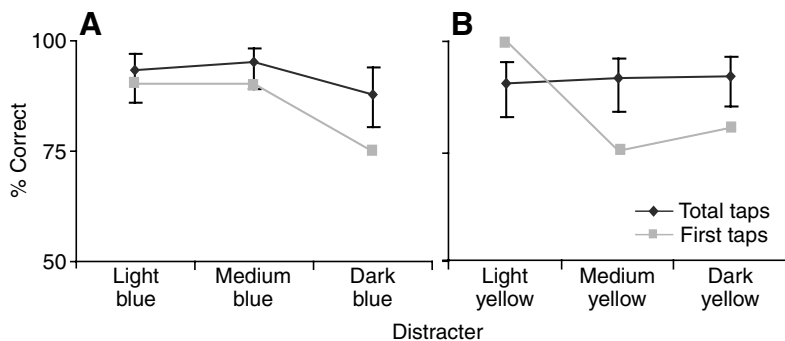


Fig. 4. Test 2: rewarded colour versus three brightness levels of distracter colour. Choice frequencies (\pm 95% CI) are shown for the correct stimulus for yellow trained fish (A) and blue trained fish (B) when a light, medium and dark distracter stimulus was presented.

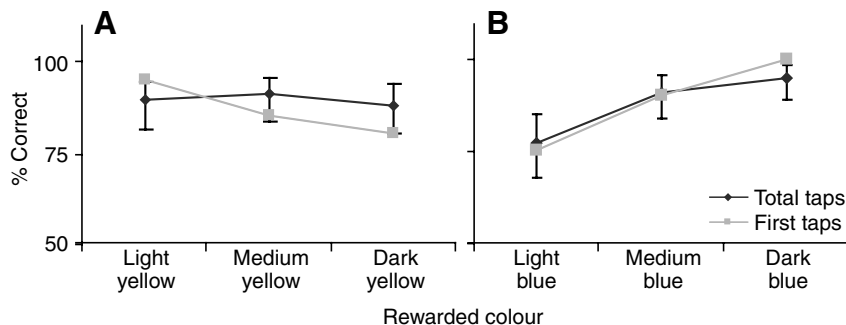


Fig. 5. Test 3: constant distracter (dark blue for yellow trained fish and medium yellow for blue trained fish) versus three brightness levels of the rewarded colour. Choice frequencies ($\pm 95\%$ CI) are shown for the correct stimulus for yellow-trained fish (A) and blue-trained fish (B) when a light, medium and dark rewarded stimulus was presented.

blue (14 correct out of 41 taps and 24 correct out of 35 taps, respectively).

Final test: all combinations of all brightness levels

On average, the group of fish trained on yellow, achieved a frequency of 89% (94.4, 81.2) correct choices (Fig. 6A). Correct choices varied between treatments and ranged between 76% (83.6, 66.1) (light yellow versus dark blue) and 94% (97.8, 87.4) (dark blue versus medium yellow) correct for total taps (Fig. 6). Three fish achieved significant results in all nine conditions, six fish in eight conditions and one fish in seven conditions (Table 1A). Overall, in 82 of 90 conditions (9 conditions \times 10 fish) significant results were achieved (Table 1A). The combination where they failed was not consistent (Table 1A).

On average, the group of fish trained on blue, reached a frequency of 87% (92.8, 78.8) correct choices (Fig. 6B). Correct choices varied between treatments and ranged between 77.8% (85.5, 68.3) (light blue versus light yellow) and 94.6% correct (98.3, 88.6) (light blue versus dark or medium yellow; Fig. 6). The number of correct first taps was best for the condition medium or light blue versus light yellow (17/20) and worst for light blue versus medium yellow (14/20). Three fish achieved significant results in all nine conditions, six fish in eight conditions and one fish in seven conditions (Table 1B). Overall, in 82 of 90 conditions (9 conditions \times 10 fish) significant results were achieved

(Table 1B). The combination where they failed varied between the fish (Table 1B).

DISCUSSION

This study shows for the first time that, within just a few days of capture, it is possible to train coral reef fish to learn and perform a visual discrimination task based on colour cues alone. Classical conditioning using a two-alternative forced choice procedure adapted from Neumeyer (Neumeyer, 1984) was used to show that the damselfish, *Pomacentrus amboinensis*, has colour vision. A series of experiments demonstrated that two groups of fish trained on blue or yellow are able to identify their training colour irrespective of its brightness or that of the distracter.

Various methods have been used to demonstrate colour vision using behavioural experiments in a range of animals (for a review, see Kelber et al., 2003). The method used in this study successively reduced the number of available cues for the fish until a correct response could only be made on the basis of chromatic cues. The approach employed here, in which the level of difficulty was incremental in each new experiment, might not be necessary for accurate performance in the final, decisive experiment, but was employed here because at the time of testing it was unknown how reef fish would perform in visual learning and discrimination experiments.

The first experimental task required the fish to distinguish a blue from a yellow stimulus. The main conclusion from this experiment

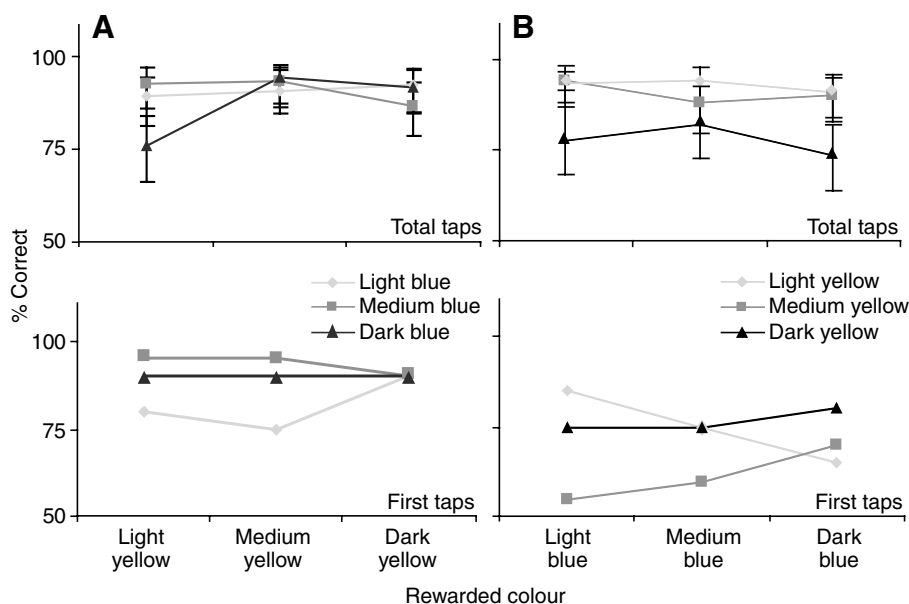


Fig. 6. Test 4: variation of the brightness of the rewarded and distracter stimuli. Frequencies ($\pm 95\%$ CI) of correct taps for yellow trained (A) and blue trained (B) fish. The top graphs show the results for the total number of taps and the bottom graphs show the results for first taps only.

Table 1. Results of the Fisher's exact test for test 4

	db/dy	db/my	db/ly	mb/dy	mb/my	mb/ly	lb/dy	lb/my	lb/ly
(A) Yellow-trained fish									
1	0.0068	0.0003	0.0551*	0.0002	<0.0001	0.0004	0.0079	0.0004	0.0012
2	0.0004	0.0004	0.0014	0.9999*	0.0004	0.0004	0.0004	0.0004	0.0004
3	0.0002	0.0023	0.0008	0.0004	0.0004	0.0023	0.0004	0.0004	0.0004
4	0.0734*	<0.0001	0.1948*	0.0007	0.0008	0.0054	0.0456	0.0082	0.0013
5	0.0004	0.0004	0.6586*	0.0004	0.0003	0.0004	0.0015	0.0004	0.0004
6	0.0201	0.0002	0.2314*	0.0203	0.0006	0.0068	0.0004	0.0082	0.0004
7	0.0004	<0.0001	0.0025	0.0014	0.0036	0.0004	0.0004	<0.0001	0.0007
8	0.0004	0.0004	0.0376	0.0007	0.0004	0.0015	<0.0001	0.0504*	0.0025
9	0.0004	0.0004	0.0235	0.0007	0.0004	0.0015	<0.0001	0.0504*	0.0025
10	0.0008	0.0004	0.0004	0.0004	0.0004	0.0125	0.0004	0.0004	0.3637*
(B) Blue-trained fish									
1	0.0167	0.0068	0.0004	0.0013	0.0006	0.0003	0.0004	0.0167	0.0004
2	0.0004	0.0012	0.0002	0.0305	<0.0001	0.0014	0.0408	0.0571*	0.4216*
3	0.0003	0.0004	0.0004	0.0002	0.0456	0.0028	0.0305	0.0184	0.0305
4	0.0068	0.0007	0.0002	<0.0001	0.0007	0.0004	0.0159	0.0114	0.0008
5	0.0088	0.0114	0.0051	0.0006	<0.0001	0.0014	0.0066	<0.0001	0.2047*
6	0.0033	0.0014	0.0004	0.0012	0.0003	0.0517*	0.0014	0.0184	0.0014
7	0.0004	0.0004	0.0546*	0.0002	0.0015	0.0002	0.0043	0.0406	0.0054
8	0.0003	0.0040	0.0004	<0.0001	0.0002	0.0023	0.0025	0.0059	0.3122*
9	0.0028	0.0016	0.0001	0.0010	0.0004	0.0009	0.2819*	0.0031	0.0387
10	0.0001	<0.0001	<0.0001	0.0004	0.0017	0.0167	0.6324*	0.0014	0.0013

*Non-significant conditions.

dy, dark yellow; my, medium yellow; ly, light yellow; db, dark blue; mb, medium blue; lb, light blue.

is that freshly caught reef fish are able to learn to associate food with a specific target and are able to perform two alternative discrimination tasks. This opens up a large range of possibilities for future experiments testing the visual abilities of reef fish, similar to what has been done with goldfish (Neumeyer, 1984; Neumeyer et al., 1991; Neumeyer, 1992). Such associative learning had not been described in marine fish before but has been used to test colour vision in a range of other animals, including insects (Shafir, 1996; Nakamura and Yamashita, 2000; Hempel de Ibarra et al., 2001; Lehrer and Campan, 2004), freshwater fish (Schiemenz, 1924; Neumeyer, 1984; Ohnishi, 1991; Neumeyer, 1992), crustaceans (Marshall et al., 1996), birds (Peiponen, 1992; Swaddle and Johnson, 2007), marsupials (Hemmi, 1999) and primates (Pessoa et al., 2003; Pessoa et al., 2005a; Pessoa et al., 2005b). So it is perhaps not surprising that reef fish also showed the ability for associative learning. What is surprising, however, is the speed with which the freshly caught fish adapted to their new habitat, their new food and the tasks they had to perform (identify and tap a stimulus ten times) in order to get food.

Experiments two to four tested the ability of the fish to identify their trained colour when (i) the distracter brightness (ii) the trained colour brightness and (iii) the brightness of both stimuli was varied. Colour vision experiments are only conclusive if it can be demonstrated that an animal can distinguish colours irrespective of their brightness. It becomes impossible to conclude that an animal has colour vision if, as is the case with a study on jumping spiders, brightness and chromatic cues are available for discrimination (Nakamura and Yamashita, 2000). In this study, *P. amboinensis* did not need brightness cues to distinguish the rewarded from the distracter stimuli, and thus it can be concluded that *P. amboinensis* has colour vision.

In order to distinguish blue from yellow, *P. amboinensis* must have at least two photoreceptors with different spectral sensitivities. This is supported by the finding that a close relative, *Pomacentrus coelestis*, has three spectral sensitivities (one single, two members of the double cone) in the 400–600 nm wavelength

range of interest here (McFarland and Loew, 1994a; McFarland and Loew, 1994b). Together with previous results for *P. amboinensis* (Siebeck, 2004) it is likely that they have at least three photoreceptors, one in the UV and two in the visible part of the spectrum. The ocular media of *P. amboinensis* absorb well below the peak absorbance found for the ultraviolet-sensitive cones (peak sensitivity around 365 nm) of other damselfish (Marshall et al., 2006) and thus fit well with this hypothesis. Whether or not all three photoreceptors are combined in a trichromatic system, or whether the UV signal is used for wavelength-specific behaviour, such as found for the butterfly, *Pieris brassicae*, remains to be tested (Scherer and Kolb, 1987). *Pomacentrus amboinensis* have complicated UV-reflective patterns all over their otherwise yellow body that vary between different individuals and that differ in brightness depending on the behaviour of the fish (U.E.S., unpublished). It appears that it would thus be advantageous for the fish to be able to compare the yellow and UV signals to assess the identity, condition or possibly also mood of a conspecific. Future studies are designed to investigate species and individual recognition based on colour patterns.

In each experiment two measures were analysed, the total number of taps and the first tap per trial. In most cases, the two measures reached similar values suggesting that the first tap is a good predictor of the performance of the fish. However, there are exceptions to this rule. In the last two experiments, the blue-trained fish had a much lower number of correct first taps than would be expected looking at their overall correct taps. It therefore appears that counting all taps is a more reliable method for evaluating the behaviour of the fish. This is further supported by the fact that *P. amboinensis* are territorial fish that will attack new objects placed in their territory. Especially at the beginning of a new experiment, when they see a stimulus for the first time, they tend to bite and push the novel stimulus with maximum force, before settling down and tapping their rewarded stimulus. Hence counting multiple taps allows the experimenter to distinguish attacks from true target selection.

The method developed here has the advantage that it is very simple and adaptable and therefore perfectly suited to field conditions. The fish only have to be held in captivity for a relatively short period of time and can be released back into their habitats following the experiments. Also, rather than using filter wheels and light sources to project stimuli at the back of the aquaria (Neumeier, 1992), simple painted stimuli were attached to short feeding tubes and held into the aquaria. With the help of spectrometers, the reflectance properties of the targets can be monitored, which can then be adapted by mixing different colours, or by using different printer inks to create coloured targets that then have to be laminated before insertion into the water. The disadvantage, however, is that it is not possible to perform tests where colour stimuli with narrow wavebands are required (e.g. wavelengths discrimination or colour mixture experiments).

In summary, damselfish are able to learn to associate coloured targets with a food reward and perform colour discrimination tasks. The experimental protocol described here can easily be used in the laboratory as well as during field trips to test various aspects of the visual abilities of reef fish. The described approach will be even more powerful once the spectral sensitivities are known and specific hypotheses about the visual system of the experimental animal can be tested, such as has been shown for bees (Hempel de Ibarra et al., 2002; Hempel de Ibarra and Giurfa, 2003) and goldfish (Neumeier, 1992).

We would like to thank the wonderful Lizard Island Research Station staff for their continuing support and Misha Vorobyev for many interesting discussions about colour vision and help with statistics. This research was supported by the Australian Research Council (grant # DP0557285).

REFERENCES

- Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J. and Huntingford, F. A. (2001). Indirect fitness consequences of mate choice in sticklebacks: offspring of brighter males grow slowly but resist parasitic infections. *Proc. R. Soc. Lond. B Biol. Sci.* **268**, 71-76.
- Bennett, A. T. D., Cuthill, I. C. and Norris, K. J. (1994). Sexual selection and the mismeasure of color. *Am. Nat.* **144**, 848-860.
- Bradbury, J. W. and Vehrencamp, S. L. (1998). *Principles of Animal Communication*. Sunderland, MA: Sinauer.
- Crook, A. C. (1997). Colour patterns in a coral reef fish. Is background complexity important? *J. Exp. Mar. Biol. Ecol.* **217**, 237-252.
- Cummings, M. E. (2007). Sensory trade-offs predict signal divergence in surfperch. *Evolution* **61**, 530-545.
- Frank, T. M. and Widder, E. A. (1996). UV light in the deep-sea: *in situ* measurements of downwelling irradiance in relation to the visual threshold sensitivity of UV-sensitive crustaceans. *Mar. Freshw. Behav. Physiol.* **27**, 189-197.
- Hart, N. S. (2004). Microspectrophotometry of visual pigments and oil droplets in a marine bird, the wedge-tailed shearwater *Puffinus pacificus*: topographic variations in photoreceptor spectral characteristics. *J. Exp. Biol.* **207**, 1229-1240.
- Hemmi, J. M. (1999). Dichromatic colour vision in an Australian marsupial, the tammar wallaby. *J. Comp. Physiol. A* **185**, 509-515.
- Hempel de Ibarra, N. and Giurfa, M. (2003). Discrimination of closed coloured shapes by honeybees requires only contrast to the long wavelength receptor type. *Anim. Behav.* **66**, 903-910.
- Hempel de Ibarra, N., Giurfa, M. and Vorobyev, M. (2001). Detection of coloured patterns by honeybees through chromatic and achromatic cues. *J. Comp. Physiol. A* **187**, 215-224.
- Hempel de Ibarra, N., Giurfa, M. and Vorobyev, M. (2002). Discrimination of coloured patterns by honeybees through chromatic and achromatic cues. *J. Comp. Physiol. A* **188**, 503-512.
- Kelber, A., Vorobyev, M. and Osorio, D. (2003). Animal colour vision – behavioural tests and physiological concepts. *Biol. Rev.* **78**, 81-118.
- Kirk, J. T. O. (2003). The vertical attenuation of irradiance as a function of the optical properties of water. *Limnol. Oceanogr.* **48**, 9-17.
- Lehrer, M. and Campan, R. (2004). Shape discrimination by wasps (*Paravespula germanica*) at the food source: generalization among various types of contrast. *J. Comp. Physiol. A* **190**, 651-663.
- Longley, W. H. (1917). Studies upon the biological significance of animal coloration. I. The colors and color changes of west indian reef-fishes. *J. Exp. Zool.* **23**, 533-601.
- Lorenz, K. (1962). The function of colour in coral reef fishes. *Proc. R. Inst. G. B.* **39**, 282-296.
- Lythgoe, J. N. (1972). The adaptations of visual pigments to the photic environment. In *Handbook of Sensory Physiology*. Vol. VII/1 (ed. H. Autrum, R. Jung, W. R. Loewenstein, D. M. MacKay and H. L. Teuber), pp. 567-600. Berlin: Springer.
- Lythgoe, J. N. (1979). *The Ecology of Vision*. Oxford: Clarendon Press.
- Marshall, N. J. (2000a). Communication and camouflage with the same 'bright' colours in reef fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1243-1248.
- Marshall, N. J. (2000b). The visual ecology of reef fish colours. In *Animal Signals. Adaptive Significance of Signalling and Signal Design in Animal Communication* (ed. Y. Espmark, T. Amundsen and G. Rosenqvist), pp. 83-120. Trondheim: Tapir Publishers.
- Marshall, N. J., Jones, J. P. and Cronin, T. W. (1996). Behavioural evidence for colour vision in stomatopod crustaceans. *J. Comp. Physiol. A* **179**, 473-481.
- Marshall, N. J., Vorobyev, M. and Siebeck, U. E. (2006). What does a reef fish see when it sees a reef fish? Eating 'Nemo'. In *Fish Communication*. Vol. 2 (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 393-422. Enfield, NH: Science Publisher.
- McFarland, W. N. and Loew, E. R. (1994a). Ultraviolet visual pigments in marine fishes of the family pomacentridae. *Vision Res.* **34**, 1393-1396.
- McFarland, W. N. and Loew, E. R. (1994b). Ultraviolet visual pigments in marine fishes of the family pomacentridae [published erratum appears in *Vision Res.* 1994 Nov; 34(21):2939]. *Vision Res.* **34**, 1393-1396.
- Medina, J. M. and Mullen, K. T. (2007). Colour-luminance interactions in binocular summation. *Vision Res.* **47**, 1120-1128.
- Nakamura, T. and Yamashita, S. (2000). Learning and discrimination of colored papers in jumping spiders (Araneae, Salticidae). *J. Comp. Physiol. A* **186**, 897-901.
- Neumeier, C. (1984). On spectral sensitivity in the goldfish. Evidence for neural interactions between different "cone mechanisms". *Vision Res.* **24**, 1231-1231.
- Neumeier, C. (1992). Tetrachromatic colour vision in goldfish: evidence from colour mixture experiments. *J. Comp. Physiol. A* **171**, 639-649.
- Neumeier, C., Wietsma, J. J. and Spekrijse, H. (1991). Separate processing of 'color' and 'brightness' in goldfish. *Vision Res.* **31**, 537-549.
- Novalés Flamarique, I. (2000). The ontogeny of ultraviolet sensitivity, cone disappearance and regeneration in the sockeye salmon *Oncorhynchus nerka*. *J. Exp. Biol.* **203**, 1161-1172.
- Ohnishi, K. (1991). Goldfish visual information-processing patterns in food-reinforced discrimination-learning between compound visual-stimuli. *J. Comp. Physiol. A* **168**, 581-589.
- Peiponen, V. A. (1992). Color discrimination of 2 passerine bird species in the Munsell system. *Ornis Scand.* **23**, 143-151.
- Pessoa, D. M. A., Araujo, M. F. P., Tomaz, C. and Pessoa, V. F. (2003). Colour discrimination learning in black-handed tamarin (*Saguinus midas niger*). *Primates* **44**, 413-418.
- Pessoa, D. M. A., Perini, E. S., Carvalho, L. S., Tomaz, C. and Pessoa, V. F. (2005a). Color vision in *Leontopithecus chrysomelas*: a behavioral study. *Int. J. Primatol.* **26**, 147-158.
- Pessoa, D. M. A., Tomaz, C. and Pessoa, V. F. (2005b). Color vision in marmosets and tamarins: behavioral evidence. *Am. J. Primatol.* **67**, 487-495.
- Scherer, C. and Kolb, G. (1987). Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera). *J. Comp. Physiol. A* **160**, 645-656.
- Schiemenz, F. (1924). Über den Farbensinn der Fische. *Z. Wiss. Biol. C Vergl. Physiol.* **1**, 175-220.
- Shafir, S. (1996). Color discrimination conditioning of a wasp, *Polybia occidentalis* (Hymenoptera: Vespidae). *Biotropica* **28**, 243-251.
- Shand, J. (1993). Changes in the spectral absorption of cone visual pigments during the settlement of the goatfish *Upeneus tragula*: the loss of red sensitivity as a benthic existence begins. *J. Comp. Physiol. A* **173**, 115-121.
- Siebeck, U. E. (2004). Communication in coral reef fishes – the role of ultraviolet colour patterns for the territorial behaviour of *Pomacentrus amboinensis*. *Anim. Behav.* **68**, 273-282.
- Siebeck, U. E. and Marshall, N. J. (2001). Ocular media transmission of coral reef fish – can coral reef fish see ultraviolet light? *Vision Res.* **41**, 133-149.
- Swaddle, J. P. and Johnson, C. W. (2007). European starlings are capable of discriminating subtle size asymmetries in paired stimuli. *J. Exp. Anal. Behav.* **87**, 39-49.
- von Frisch, K. (1912). Über farbige Anpassung bei Fischen. *Zool. Jb. Physiol.* **32**, 209-214.
- Vorobyev, M., Marshall, N. J., Hempel de Ibarra, N. and Menzel, R. (2001). Colourful objects through animal eyes. *Color Res. Appl.* **26**, S214-S217.