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Do coral reef fish learn to avoid unpalatable prey using visual cues?

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Keywords: aposematism bluehead wrasse chemical defence colour learning pattern predation Thalassoma bifasciatum Colour and pattern provide important visual cues that affect animal behaviour. Learned avoidance of unpalatable prey by coral reef fish in response to visual cues has been studied before, but visual cues have not been separated from distastefulness to make certain that fish were learning solely in response to visual signals. Here, we used artificial foods to test the ability of wild-caught bluehead wrasse, Thalassoma bifasciatum, a common Caribbean reef fish, to learn to avoid unpalatable prey in laboratory experiments. First, we tested whether the relative position of foods of the same or different colours affected avoidance of unpalatable prey. Next, we tested whether blueheads could learn to avoid different colours of unpalatable prey. These experiments also tested whether blueheads could learn to avoid unpalatable prey against a pre-experimental colour bias. Finally, we determined whether blueheads learn to avoid unpalatable prey more effectively using colour or pattern signals. Fish learned to avoid unpalatable prey using colour independent of prey position, and they learned to avoid some colours (red, blue and orange), but not others (yellow, purple and green), based on visual cues alone. Fish avoided yellow, purple and green coloured prey, regardless of whether they were palatable or unpalatable. Fish only avoided white prey when unpalatable. Fish rapidly learned to overcome a strong pre-experimental bias against red prey. There was no difference in the ability of fish to learn to avoid prey using colour or pattern. We conclude that blueheads learn to avoid unpalatable prey using visual signals alone, that they have different responses to different colours and that they adapt quickly to novel prey appearance. Our results do not support the concept that specific prey colours, such as red or orange, strictly signal a chemical defence, but that blueheads continually assess and adapt to prey palatability using visual cues. © 2012 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Bright body coloration and pattern are widespread among animals and serve many purposes (Cott 1940; Edmunds 1974; Osorio & Vorobyev 2008). Colour can be important for thermoregulation, camouflage, mimicry or communication (Cott 1940; Endler 1990), with colour and pattern frequently used by animals to communicate with conspecifics as well as predators (Cott 1940; Edmunds 1974; Osorio & Vorobyev 2008). Nevertheless, animal body colour can also be the indirect result of physiological processes unrelated to the previously stated examples (Edmunds 1991; Pawlik et al. 1995), and in aquatic habitats, turbidity, dissolved pigments and the differential attenuation of light frequencies with water depth can greatly affect colour perception (Edmunds 1974; Pawlik 2012).

Animal body colour and pattern are controlled by three distinct mechanisms: pigments, thin-layer interference and scattering. Pigments are chemical compounds that absorb specific wavelengths of light and reflect the remaining wavelengths. The eye

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perceives the colour of the light that is not absorbed. Interference is a mechanism by which iridescent coloration is produced. Often, a thin layer of wax or keratin causes light to be refracted as it enters and exits, causing two reflections of light that are in phase for specific wavelengths at specific angles. Scattering produces a blue colour in birds, fish and lizards. This blue coloration is created by a coating of transparent material imbedded with tiny particles or air spaces covering the body of the animal. As visible light enters the surface coating, violet, blue and green wavelengths are scattered and longer wavelengths are absorbed by lower layers, resulting in a blue or greenish-blue coloration. Colour can also be controlled behaviourally, such as colour flashing in lizards (Bradbury & Vehrencamp 1998). Amphibians, reptiles, fish, cephalopods and insects can change their body colour using dermal chromatophores, with muscle fibres controlling the dispersion or occlusion of pigment granules in the skin (Parker 1948; Cloney & Florey 1968; Bagnara & Hadley 1973).

In many terrestrial ecosystems, brilliant colours and disruptive body patterns are important warning signals that increase the ability of predators to learn to avoid undesirable or unpalatable prey (Cott 1940; Edmunds 1974; Ruxton et al. 2004). Aposematism, or the use of warning signals to denote the unpalatable or harmful

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aspects of an organism, is potentially a very important aspect of predator—prey relationships, because warning signals may be more evolutionarily advantageous than camouflage in many terrestrial systems (Cott 1940; Edmunds 1974; Ruxton et al. 2004). In addition to colour and pattern, warning signals can also be auditory, olfactory or behavioural (Hauglund et al. 2006).

According to Edmunds (1987), four criteria need to be met for an organism to be considered an aposeme, or having an aposematic appearance or behaviour: the organism must (1) be sufficiently unpalatable to deter predators, (2) conspicuously display itself, (3) be avoided by some predators because of its conspicuousness, and (4) be better protected from predation by the foregoing than by using an alternative strategy, such as crypsis. While aposematism and mimicry are well-studied phenomena among terrestrial animals such as insects, snakes and frogs (Mappes et al. 2005) and freshwater fish (Kruse & Stone 1984), much less is known about the ecological importance of the warning signals of marine animals. There are many brightly coloured or highly contrasting marine animals that are also chemically defended (Edmunds 1991), particularly on tropical coral reefs, where light availability and water clarity permit the use of visual cues. These animals include sponges, corals, polyclad flatworms, molluscs (particularly nudibranchs), annelids and the larvae of some marine organisms (Edmunds 1991; Pawlik et al. 1995; Ang & Newman 1998; Meredith et al. 2007). Many of these animals are assumed to be aposematic, however, very few examples of aposematism have been demonstrated rigorously (Edmunds 1991; Pawlik 2012). Fishes are the most important consumers on tropical coral reefs (Sale 1991), so a first step in testing for aposematism would be determining whether fishes are able to avoid unpalatable prey based on visual signals. Tropical marine fishes use a variety of signals to learn to avoid unpalatable prey (Gerhart 1991; Long & Hay 2006; Ritson-Williams & Paul 2007), but it has not been demonstrated whether this avoidance is due to visual signals alone (i.e. with specific experiments in which visual cues are tested in the absence of any olfactory or gustatory cues).

Marine fishes, like other vertebrates, have a complex and highly specialized eye (Marshall 2000). The eyes of over 70 species of marine fishes have been analysed and have at least two different types of cone cells (Marshall et al. 2006). Multiple types of cone cells are a requirement for colour vision (Siebeck et al. 2008). Five distinct types of photopigments have been isolated from the eyes of coral reef fishes: ultra-violet-sensitive, violet-sensitive, bluesensitive, green-sensitive, and red-sensitive (Munz & McFarland 1975; Loew & Lythgoe 1978; Levine & MacNichol 1979; Lythgoe et al. 1994; McFarland & Loew 1994). Based on this evidence, many coral reef fishes are physiologically capable of colour vision, although some may only be able to distinguish between two colours, while others may distinguish three or more (Marshall 2000).

Physiological studies have been performed on the visual systems of coral reef fishes of the family Labridae (Barry & Hawryshyn 1999; Siebeck & Marshall 2000). Electrophysiological recordings were made from the optic nerve of the Hawaiian saddle wrasse, *Thalassoma duperrey*, to determine whether this species has the ability to see the colours of conspecifics, and peak spectral sensitivities correlated with the spectral reflectance of conspecific body colours (Barry & Hawryshyn 1999). A study on the transmission capabilities of ocular media of the eye of the bluehead wrasse, *Thalassoma bifasciatum*, the subject of the present study, revealed that the eye is capable of transmitting light throughout the majority of the visible light spectrum, indicating that there is no physical boundary to colour vision in this species (Siebeck & Marshall 2000). Moreover, the visual sensitivity of wrasses does not extend to the UV range (Losey et al. 2003).

Bluehead wrasses, like all coral reef fishes, live in one of the world's most colourful ecosystems. Colourful fishes and invertebrates act as habitat, food and predators, with the full spectrum of visible colours represented among animals as diverse as sponges, cnidarians, molluscs and fishes (Feddern 1965; Pawlik et al. 1995). Conspecific fishes are well understood to signal social dominance and mate attraction by changing body colour (Warner & Swearer 1991).

Until recently, no behavioural studies had been performed to determine whether marine fishes could discriminate colours. Siebeck et al. (2008) sought to test whether a coral reef fish, the damselfish, *Pomacentrus amboinensis*, had the ability to distinguish between two colours. The results of their study suggested that damselfish could distinguish between at least two colours, blue and yellow, independent of brightness.

Many studies have advanced our understanding of the ability of terrestrial predators to learn to avoid unpalatable prey (Sillen-Tullberg 1985; Ham et al. 2006; Aronsson & Gamberale-Stille 2008), but only a few have addressed marine fishes (Gerhart 1991; Long & Hay 2006; Ritson-Williams & Paul 2007). None of the latter, however, tested the ability of fishes to learn to avoid unpalatable prey based on visual cues alone.

In the present study, we investigated whether the bluehead, a common Caribbean reef fish species, has the ability to learn and remember to avoid unpalatable artificial prey items based on the visual signals of colour and pattern. Wild-caught blueheads were subjected to feeding assays using artificial prey of different types to assess the impact of various prev colours and patterns on predation. We used blueheads in this study because they are one of the most abundant fish on Caribbean coral reefs, as well as being generalist predators (Feddern 1965; Randall 1967). Blueheads have been used in many other behavioural and feeding assays (Warner & Hoffman 1980; Pawlik et al. 1995) and respond well to laboratory conditions. We addressed six questions below, which could only be answered in the laboratory because the same replicate group of fish had to be exposed to each phase of an experiment. (1) Does relative prey position affect the ability of blueheads to learn and remember to avoid unpalatable prey? (2) Do wild-caught blueheads have a preexperimental bias against specific colours of prey? (3) Are blueheads able to learn and remember to avoid unpalatable prey using colour alone? (4) Do specific colours affect the ability of blueheads to learn to avoid defended prey? (5) Are blueheads able to overcome a pre-experimental bias against a particular prey colour? (6) Do blueheads learn to avoid unpalatable prey more effectively using the visual signals of colour or pattern?

METHODS

Fish Maintenance

Yellow-phase bluehead wrasse (*T. bifasciatum*), were collected off the coast of Key Largo, FL, U.S.A., and shipped to the University of North Carolina Wilmington, Center for Marine Science, U.S.A. Approximately 100 fish were kept among five $90 \times 40 \times 35$ cm aquaria filled with filtered, natural sea water from Masonboro Sound, NC, U.S.A. Fish used in experiments were ~ 6–10 cm standard length, making them ~1 year old based on length. The five tanks were part of a recirculating sea water system, from which 25% of the volume was changed weekly. Two of the aquaria were used as experimental tanks and the other three were used to house the remaining fish. All five aquaria were subjected to a 12:12 h light:dark cycle using 175 W metal halide lights suspended 15 cm above each tank. Sea water temperature was maintained at 21-23 °C and salinity was maintained at 30-32 practical salinity units (psu). Fish were fed to satiety two to three times each day with dry fish flakes (TetraMin Tropical Flake Food) and were never food deprived. Flake food was allowed to soak in water prior to feeding, causing the food to form a brown slurry of small particles. The two aquaria used for experiments were each divided into five cells using opaque perforated plastic sheets, resulting in 10 cells measuring $20 \times 40 \times 35$ cm each. Aquaria bottoms were covered in limestone gravel and provided with several 5–10 cm pieces of PVC pipe in which fish could hide, at least one piece of pipe per cell for divided aquaria.

Artificial Prey

To create artificial prey, the gel-based food used by Chanas & Pawlik (1995) was made from 1 g of powdered squid mantle, 0.5 g of carrageenan and 20 ml of deionized water, which was blended together until homogeneous. Powdered food colouring was added (Creative Cutters[®] Blossom Tints, Richmond Hill, ON, Canada), and then the mixture was microwaved for 20 s or until it began to boil. The molten mixture was then poured into a 1.5×1.7 cm plastic mould approximately 1 mm thick, which was placed over fibreglass window-screen mesh that was completely wrapped around a 10×10 cm stone tile. When the mixture had cooled, the mould was removed, leaving an artificial prey item that covered exactly 10×10 rectangles of window-screen, with each rectangle measuring about $1.5 \times 1.7 \times 1$ mm.

Prey colour was carefully controlled in the formulation of all artificial prey. The concentration of food colourant used to create each colour prey, as well as the resulting colour on the Munsell scale and grey scale, is listed in Table 1. Before experiments began, all colourants were tested to make certain they were palatable. First, colourants were tested in pellet feeding assays (Pawlik et al. 1995) and determined to be palatable at a four-fold higher concentration than that used in the present study. Additionally, artificial prey made in the manner described above with colourant at two-fold higher concentration used in the present study, but masked with black food colouring (vegetable charcoal), were determined to be palatable.

Artificial prey were made unpalatable by adding denatonium benzoate (Sigma-Aldrich D5765) to the artificial food mixture at 2 mg/ml prior to microwaving. Denatonium benzoate is an extremely bitter, nontoxic compound, marketed under the names Bitrex (Macfarlan Smith, Edinburgh, U.K.) and Aversion (Aversion Technologies, Bowie, MD, U.S.A.), and used to prevent nail biting in humans, chewing in dogs, and to make denatured alcohol unpalatable.

Pre-experimental Training

Prior to the beginning of experiments, groups of fish were presented with a brown prey item (Table 1) for 3 h each day to

Table 1

List of experimental colours, food colouring names and product numbers, concentration of food colouring used in experiments, and approximate Munsell colour achieved

Colour	Product name, number	Concentration (g/ml)	Munsell colour/grey scale
Red	Red, C443	0.002	7.5 R 5/18
Orange	Tangerine, C446	0.002	10 R 6/16
Yellow	Egg Yellow, C429	0.0015	10 Y 9/12
Blue	Ocean Blue, C418	0.0015	10 B 3/6
Green	Foliage Green, C419	0.0015	7.5 GY 4/10
Purple	Aubergine, C449	0.0015	5 RP 2/10
Brown	Brown, C424	0.002	2.5 YR 3/8
White	None	Х	10
Light Grey	Black, C422	0.0001	8
Grey	Black, C422	0.00025	5
Black	Black, C422	0.001	3

habituate them to the use of the artificial foods on screen-wrapped tiles. Brown prey were not otherwise used in any experiments. When fish consumed 50% of the brown prey within 3 h, experiments could begin with that set of fish the following day.

Experiment 1

In experiment 1, we examined whether relative prev position affects the ability of blueheads to learn to avoid unpalatable prev. The experiment consisted of three parts: a 3 h learning phase, a 1 h delay, and a 3 h memory test (Fig. 1). The 10 cells in the two aquaria were haphazardly divided into five control cells and five experimental cells, with three fish in each cell. At the beginning of the learning phase, identical tiles were added to both the experimental and control cells, with each tile bearing two prey items, a yellow unpalatable prey and a grey palatable prey (yellow was chosen because it had been determined that there was no preexperimental bias against this colour). We removed the tiles after 3 h and determined prey consumption by counting the number of mesh rectangles exposed when prey was eaten. After a 1 h delay, we added tiles as before, but reversed the position of the yellow and grey prey in the experimental cells. Again, tiles were removed and consumption determined at the end of the memory test. If fewer than 10 squares of prey were eaten from any tile after any part of the experiment, that tile was scored as having no response and not used in statistical analyses. If more than five of the 10 tiles in any experimental run resulted in no response, the experimental run was discarded. No set of fish was used more than once in each experiment.

Experiment 1 was repeated with both prey items coloured grey. This second run was performed to determine whether fish could learn to avoid an unpalatable prey item based on relative position when prey colour was the same.

Experiment 2

In experiment 2, we examined whether wild-caught blueheads (1) have a prelearned bias towards different colours of prey, (2) learn to avoid unpalatable prey using visual signals and/or (3) respond differently to different colours in learning to avoid defended prey.

This experiment consisted of seven parts over 2 days: on day 1, we conducted a 3 h colour bias test, followed by a 1 h delay, followed by a 3 h bias durability test; on day 2, after a 16–18 h delay,



Figure 1. Diagram of experiment 1 examining whether relative prey position affects the ability of bluehead wrasse to learn to avoid unpalatable prey.

we conducted a 3 h learning phase, followed by a 1 h delay, followed by a 3 h memory test (Fig. 2). The experiment was repeated with seven prey treatment colours (red, orange, yellow, blue, green, purple, white) and a grey control colour, all of which were prepared fresh for each component of the experiment (Table 1). Following pre-experimental training, groups of three fish were placed into each of the 10 cells within the experimental aquaria. For the colour bias test, one treatment colour and one control prey, both palatable, were presented to each group of fish. For the bias durability test, fish were presented with identical prey to test whether any colour bias persisted. On the second day, for the learning phase, fish were presented with prey of the same colours as the previous day, but the treatment colour was unpalatable. For the memory test, prey of the same colours were again presented, but both were palatable (Fig. 2). No set of fish was used more than once in each experiment.

Experiment 3

In experiment 3, we examined whether blueheads could be trained to overcome a pre-experimental bias and feed on prey of a previously avoided colour. This experiment followed the results of experiment 2, which showed that blueheads have a prelearned or innate negative bias against the colour red. All methodological aspects of experiment 3 were identical to those in experiment 2, but experiment 3 consisted of only five parts, with the bias durability test of experiment 2 removed (Fig. 3). During the colour bias test and the memory test, both red (treatment) and grey (control) prey were palatable. During the learning phase, the grey prey was made unpalatable while the red control prey remained palatable.

Experiment 4

In experiment 4, we examined whether blueheads learn to avoid unpalatable prey more effectively using colour or pattern signals. The protocol was identical to that in experiment 2 except (1) each treatment prey was yellow, and four dots of black prey mixture were imbedded into each prey (in a pattern similar to the four-face of a throwing die) throughout the first five parts of the experiment, and (2) during the memory test, the experimental fish were further divided into three subgroups, with subgroup 1 receiving treatment prey identical to the previous parts of the experiment, subgroup 2 receiving treatment prey that was yellow with no dots, and subgroup 3 receiving treatment prey having dots, but with the same colour as grey control prey (Fig. 4).



Figure 2. Diagram of experiment 2 examining whether bluehead wrasse (1) have a pre-experimental bias towards different colours of prey, (2) learn to avoid unpalatable prey using visual signals and/or (3) respond differently to different colours in learning to avoid defended prey.



Figure 3. Diagram of experiment 3 examining whether bluehead wrasse could be trained to overcome a pre-experimental bias and feed on prey of a previously avoided colour.

Statistical Analyses

Data were arcsine transformed prior to statistical analyses. Paired *t* tests were used to determine whether there was a difference in the amount of treatment and control prey consumed for each part of each experiment. Unpaired *t* tests were used to determine whether there was a difference in the proportion of control prey consumed between the colour bias test and memory test in each experiment, which was done to determine whether the fish learned to avoid the treatment prey based on visual signals alone. For experiment 1, unpaired *t* tests were used to determine whether there was a difference in the proportion of control prey consumed between experimental and control cells during the memory test. For experiment 4, an ANOVA was used to determine whether there were differences in the proportions of control prey consumed between subgroups during the memory test.

RESULTS

Experiment 1

Blueheads learned to avoid unpalatable prey using a visual cue, colour, regardless of the relative position of the unpalatable prey item (Fig. 5). When yellow was tested as the treatment colour, both experimental fish (positions switched) and control fish (positions constant) consumed a significantly greater amount of grey control prey in the learning phase (paired *t* test: control fish: $t_8 = -4.8$, P < 0.05; experimental fish: $t_{12} = -2.9$, P < 0.05) and in the memory text (control fish: $t_{10} = -4.6$; P < 0.05; experimental fish: $t_{18} = -3.4$, P < 0.05; Fig. 5) of the experiment. There was no difference in the proportion of grey prey consumed between the control and experimental groups during the memory test ($t_{14} = 1.2$, P = 0.12; Fig. 5).

When the experiment was performed with grey as both the control and treatment colour, fish consumed significantly more palatable control prey in both the control and experimental groups during the learning phase (control fish: $t_{18} = -2.7$, P < 0.05; experimental fish: $t_{16} = -2.5$, P < 0.05; Fig. 6). There was no difference in the mean percentage of prey consumed during the memory test for both the control fish and experimental fish: $t_{18} = -0.38$, P = 0.36; experimental fish: $t_{16} = -1.6$, P = 0.07; Fig. 6). There was also no difference in the proportion of control prey consumed between the control and experimental groups during the memory test ($t_{17} = -0.43$, P = 0.34; Fig. 6).



Figure 4. Diagram of experiment 4 examining whether bluehead wrasse learn to avoid unpalatable prey more effectively using colour or pattern signals.

Experiment 2

Blueheads were biased against red prey, consuming a significantly greater amount of grey prey than red prey during both the colour bias test ($t_{24} = -2.8$, P < 0.05) and the bias durability test ($t_{26} = -3.8$, P < 0.05; Fig. 7). Fish also learned to avoid unpalatable red prey, consuming a significantly greater proportion of grey prey in the memory test than in the colour bias test ($t_{27} = -2.6$, P < 0.05;



Fig. 7). Similarly, blueheads were biased against blue prey, consuming a significantly greater amount of grey prey than blue prey during both the colour bias test ($t_{28} = -2.6$, P < 0.05) and the bias durability test ($t_{28} = -1.8$, P < 0.05; Table 2). Fish also learned to avoid unpalatable blue prey, consuming a significantly greater proportion of grey prey in the memory test than in the colour bias test ($t_{28} = -1.9$, P < 0.05; Table 2).

Blueheads were weakly biased against yellow prey, consuming a significantly greater amount of grey prey than yellow prey during



Figure 5. Mean \pm SE percentage of yellow and grey prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse during experiment 1. Unpalatable prey are indicated with a \blacklozenge . The arrow indicates the time interval between sessions. The position of yellow and grey prey was switched between the learning phase and the memory test in the experimental treatment. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the amount of prey consumed during each part of the experiment.

Figure 6. Mean \pm SE percentage of grey prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse during experiment 1. Unpalatable prey are indicated with a \blacklozenge . The arrow indicates the time interval between sessions. The position of grey prey was switched between the learning phase and the memory test in the experimental treatment. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the amount of prey consumed during each part of the experiment.



Figure 7. Mean \pm SE percentage of red and grey prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse during experiment 2. Unpalatable prey are indicated with a \blacklozenge . Arrows indicate time intervals between sessions. Sample sizes were as follows: N = 13 for session 1, N = 14 for session 2, N = 14 for session 3 and N = 16 for session 4. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the mean percentage of red and grey prey consumed during each session of the experiment.

the colour bias test ($t_{28} = -2.2$, P < 0.05; Table 2), but not the bias durability test ($t_{30} = -1.5$, P = 0.07; Table 2). Fish were not able to learn to avoid unpalatable yellow prey, with no statistical difference in the amount of yellow and grey prey consumed in the memory test and colour bias test ($t_{28} = -1.3$, P = 0.11; Table 2).

Blueheads were biased against purple prey, consuming a significantly greater amount of grey prey than purple prey during both the colour bias test ($t_{30} = -4.3$, P < 0.05) and the bias durability test ($t_{28} = -2.6$, P < 0.05; Fig. 8). But, fish were unable to learn to avoid unpalatable purple prey, with no statistical difference in the amount of purple and grey prey consumed in the memory test and colour bias test ($t_{25} = 0.11$, P = 0.46; Fig. 8). Similarly, blueheads were biased against green prey, consuming a significantly greater amount of grey prey than green prey during both the colour bias test ($t_{34} = -3.0$, P < 0.05) and the bias durability test ($t_{30} = -3.0$, P < 0.05; Table 2). Again, fish were unable to learn to avoid unpalatable green prey, with no statistical difference in the amount of grey prey consumed in the memory test and colour bias test ($t_{34} = -3.0$, P < 0.05; Table 2). Again, fish were unable to learn to avoid unpalatable green prey, with no statistical difference in the amount of green and grey prey consumed in the memory test and colour bias test ($t_{34} = -0.68$, P = 0.25; Table 2).

Blueheads were also biased against orange prey, consuming a significantly greater amount of grey prey than orange prey during

Table 2	
Summary of results	from experiment 2

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Colour	Pre-existing bias	Bias against colour	Learned to avoid
Red	Strong	Yes	Yes
Blue	Strong	Yes	Yes
Yellow	Weak	Yes	No
Purple	Strong	Yes	No
Green	Strong	Yes	No
Orange	Strong	Yes	Yes
White	None	None	No

Pre-existing bias was (1) strong if fish consumed significantly less treatment prey in both the colour bias test and the bias durability test, (2) weak if fish only consumed significantly less treatment prey in the colour bias test, and (3) none if there was no difference in treatment prey consumed for either session. Fish learned to avoid treatment prey based on colour alone if they consumed a significantly greater proportion of control prey in the memory test than in the colour bias test.



Figure 8. Mean \pm SE percentage of prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse while testing purple during experiment 2. Unpalatable prey are indicated with a \blacklozenge . Each session of the experiment lasted 3 h. Arrows indicate the time intervals between sessions. Sample sizes were as follows: N = 16 for session 1, N = 15 for session 2, N = 13 for session 3 and N = 11 for session 4. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the mean percentage of purple and grey prey consumed during each session of the experiment.

both the colour bias test ($t_{28} = -1.9$, P < 0.05) and the bias durability test ($t_{32} = -2.7$, P < 0.05; Fig. 9). Fish were able to learn to avoid unpalatable orange prey, consuming a significantly greater proportion of grey prey in the memory test than in the colour bias test ($t_{29} = -2.1$, P < 0.05; Fig. 9).

Finally, blueheads showed no bias against white prey, with no statistical difference in the amount of white and grey prey consumed during the colour bias test ($t_{32} = 1.1$, P = 0.14) or the bias durability test ($t_{32} = 0.38$, P = 0.36; Fig. 10). Fish were also unable to learn to avoid unpalatable white prey, with no statistical difference in the amount of white and grey prey in the memory test or the colour bias test ($t_{33} = -1.5$, P = 0.08; Fig. 10).



Figure 9. Mean \pm SE percentage of prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse while testing orange during experiment 2. Unpalatable prey are indicated with a \blacklozenge . Each session of the experiment lasted 3 h. Arrows indicate time intervals between sessions. Sample sizes were as follows: N = 15 for session 1, N = 17 for session 2, N = 15 for session 3 and N = 16 for session 4. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the mean percentage of orange and grey prey consumed during each session of the experiment.



Figure 10. Mean \pm SE percentage of prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse while testing white during experiment 2. Unpalatable prey are indicated with a \blacklozenge . Each session lasted 3 h. Arrows indicate the time interval between sessions. Sample sizes were as follows: N = 17 for session 1, N = 17 for session 2, N = 15 for session 3 and N = 16 for session 4. *Denotes a significant difference (paired t test: $P \le 0.05$) in the mean percentage of white and grey prey consumed during each session of the experiment.

Experiment 3

As in experiment 2, blueheads were biased against red prey, consuming a significantly greater amount of grey prey than red prey during the colour bias test ($t_{34} = -2.3$, P < 0.05; Fig. 11). But, fish learned to overcome a bias against red prey, with no statistical difference in the amount of red and grey prey consumed during the memory test ($t_{24} = -0.90$, P = 0.20; Fig. 11).

Experiment 4

Blueheads showed no bias against yellow patterned prey (with four black dots), with no statistical difference in the amount of



Figure 11. Mean \pm SE percentage of prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse while testing red during experiment 3. Unpalatable prey are indicated with a \blacklozenge . Each session lasted 3 h. Arrows indicate the time intervals between sessions. Sample sizes were as follows: N = 18 for session 1, N = 11 for session 2 and N = 13 for session 3. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the mean percentage of red and grey prey consumed for each session of the experiment.

yellow patterned prey and grey prey consumed during both the colour bias test ($t_{56} = -0.044$, P = 0.48) and the bias durability test ($t_{54} = -1.4$, P = 0.08; Fig. 12). Fish also were unable to learn to avoid unpalatable yellow patterned prey, with no statistical difference in the amount of yellow patterned and grey prey consumed in the memory test and colour bias test in the yellow patterned subgroup ($t_{35} = -1.7$, P = 0.12; Fig. 12). Fish showed no difference in their ability to learn to avoid unpalatable prey using colour or pattern signals, as there were no differences in the proportion of grey prey consumed between subgroups (ANOVA: $F_{2,25} = 2.0$, P = 0.16; Fig. 12).

DISCUSSION

Blueheads learned to avoid unpalatable prey using colour as a visual signal, and this ability was dependent on the specific colour of prey offered to them. While blueheads learned to avoid red, blue and orange prey, they were unable to learn to avoid purple, green and yellow prey, because they continued to avoid prey of these three colours through each step of experiment 2 (Table 2). This result contrasts with their response to white prey, which they never learned to avoid through each step of experiment 2 (Table 2). This variation in response could be due to a number of factors, including innate or prelearned biases, but the fact that blueheads can be so easily trained against a red colour bias (Fig. 11) may suggest that the inability of these fish to learn to avoid certain colours is evidence of limitations of the sensitivity of their visual system. Interestingly, our results for coral reef fish contrast those for wild-caught great tits. Parus major, which showed no difference in response to different colours when they were trained to avoid unpalatable prey (Ham et al. 2006). To our knowledge, no comparable study of fish behaviour exists in which visual cues have been decoupled from gustatory or olfactory cues, and the ability of fish to learn against a colour bias has been tested.

Not surprisingly, relative prey position had no effect on the ability of fish to learn to avoid unpalatable prey (Figs 5, 6). Instead, colour was the visual cue used by blueheads to learn to avoid unpalatable prey within the context of our experimental protocol, with pattern having no discernable effect on learning, with or without colour (Fig. 12). It may be that other visual cues, such as shape and texture, are also important learning cues, but these cues were beyond the scope of this study.

Because blueheads have a microscopic, pelagic larval phase, obtaining fish that were fully naïve to differences in prey colour was not practical, and it was assumed that wild-caught fish would show pre-experimental biases. In fact, they showed these biases against red, orange, blue, purple and green (Table 2). For some birds, a prelearned bias against colour appears to be innate, as demonstrated in a study of naïve and wild-caught great tits, which both avoided similar colours of prey (Lindström et al. 1999). Despite their strong, prelearned bias against red, however, blueheads quickly learned to eat palatable red prey (Figs 7, 11), suggesting that whether their prelearned bias is genetically predisposed or learned by early experience, it can rapidly be overcome by learning. Interestingly, the cleaner wrasse, Labroides dimidiatus, shows a high level of learning flexibility in performing a reverse reward contingency task, an ability previously demonstrated only in mammals (Danisman et al. 2010). Zebrafish, Danio rerio, also show a high capacity for reversal learning, demonstrating an ability to rapidly learn and relearn to attend to a reinforced stimuli after switching the reinforced stimuli with a previously unreinforced stimuli (Parker et al. 2012).

The presence of a pattern of four black dots did not enhance the ability of blueheads to learn to avoid unpalatable prey (Figs 4, 12). Fish had no bias against the yellow prey patterned with black dots



Figure 12. Mean \pm SE percentage of prey consumed (bars) and proportion of grey prey consumed (circles) by bluehead wrasse while testing yellow with pattern during experiment 4. Unpalatable prey are indicated with a \blacklozenge . Each session lasted 3 h. Arrows indicate the time intervals between sessions. Sample sizes were as follows: N = 29 for session 1, N = 28 for session 2, N = 28 for session 3, N = 10 for the yellow-only group in session 4, N = 8 for the yellow with pattern group in session 4 and N = 10 for the pattern-only group in session 4. *Denotes a significant difference (paired *t* test: $P \le 0.05$) in the mean percentage of yellow with pattern prey and grey prey consumed during each session of the experiment.

and they were unable to learn to avoid this prey type based on visual signals. During the memory test there was no difference in the proportion of grey prey consumed by any of the groups, indicating that blueheads respond to colour and pattern comparably. This result is contrary to a response observed in domestic chicks, *Gallus gallus domesticus*, in which the chicks attended to colour and not to pattern during avoidance learning (Aronsson & Gamberale-Stille 2008).

In all our experiments for which a learning phase was followed by a memory test, blueheads sampled some portion of the previously unpalatable prey, indicating that these fish continually test potential prey for palatability, which may explain the rapidity with which they learn against a colour bias. This flexibility is contrary to what has been observed for the avoidance of chemically defended monarch butterflies, *Danaus plexippus*, by blue jays, *Cyanocitta cristata*, which consume a whole insect and then reject or regurgitate it and subsequently avoid that butterfly species and all similarly coloured and patterned mimics (Brower 1958a, b, c).

Perception of light and colour in aquatic environments is a complex issue, primarily because of the differential attenuation of light frequencies with water depth and clarity. As the physiological capabilities of the visual systems of marine predators are better characterized (Levine & MacNichol 1979; Barry & Hawryshyn 1999; Siebeck & Marshall 2000; Marshall et al. 2006), a better understanding can be gained of the cognitive capacities and behavioural responses of these animals. The responses of blueheads in the present study do not reveal an obvious pattern related to the colour spectrum, with similar responses to red and blue, and a mixture of responses to colours in between (Table 2).

There has been considerable debate over the existence and importance of aposematic coloration among marine invertebrates, particularly brightly coloured nudibranchs and flatworms found on coral reefs (e.g. Faulkner & Ghiselin 1983; Edmunds 1987, 1991; Pawlik 1993, 2012; Ritson-Williams & Paul 2007). The foregoing study provides evidence that, while coral reef fish have the ability to learn to avoid unpalatable prey using visual cues, they adapt to changes in the appearance of defended prey much more rapidly than terrestrial predators, such as birds. This may help to explain why the often brilliant colours of tropical sponges are unrelated to tissue palatability (Pawlik et al. 1995). More importantly, it may have a bearing on the absence of complicated mimicry schemes seen among terrestrial animals bearing bright colours and contrasting patterns, particularly butterflies. It seems likely that predation is a much more important selective force for terrestrial insects than for marine invertebrates, resulting in the greater sophistication of visual cues among the former than the latter (Pawlik 2012).

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