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Disruptive colouration in reef fish: does matching the background reduce predation risk?

Running title (40 characters):
Disruptive colouration in reef fish

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Summary Statement (15-30 words)
Vertically barred patterns provide protection for prey from visual predators even when they mismatch the spatial frequency of background habitats.

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Animals use disruptive colouration to prevent detection or recognition by potential predators or prey. Highly contrasting elements of colour patterns, including vertical or horizontal bars, are thought to be effective at distracting attention away from body form and reducing the risk of being detected. However, it is unclear whether such patterns need to be a good match to the spatial characteristics of the background to gain cryptic benefits. We tested this hypothesis using the iconic vertically-barred humbug damselfish, *Dascyllus aruanus*, a small reef fish that lives amongst the finger-like projections of branching coral colonies. Using behavioural experiments, we demonstrate that the spatial frequency of humbug pattern does not need to exactly match the spatial frequency of the coral background to reduce the likelihood of being attacked by two typical reef fish predators: slingjaw wrasse, *Epibulus insidiator* (Pallas, 1770) and coral trout, *Plectropomus leopardus* (Lacépède, 1802). Indeed, backgrounds with a slightly higher spatial frequency than the humbug body pattern provided more protection from predation than well-matched backgrounds. These results were consistent for both predator species, despite differences in their mode of foraging and visual acuity, which was measured using anatomical techniques. We also show that a slight mismatch in the orientation of the vertical bars did not increase the chances of detection. However, the likelihood of attack did increase significantly when bars were perpendicular to the background. Our results provide evidence that fish with highly contrasting patterns do not need to exactly match the spatial characteristics of the background.
Introduction

Animals use visual camouflage to avoid detection and/or recognition by predators and prey (Thayer, 1909, Cott, 1940) by using different strategies such as background matching, masquerade, countershading, and disruptive colouration (defined in (Stevens and Merilaita, 2009a). Background matching requires that the body colour (hue), brightness (luminosity) and/or pattern elements closely resemble that of a specific background (in specialist camouflage), or a number of backgrounds (in generalist camouflage) (Stevens and Merilaita, 2009a, 2011). For example, the body colouration of the green tree frog, *Agalychnis callidryas*, has a similar spectral reflectance to the leaves of the tree on which they rest (Emerson et al., 1990); while the giant cuttlefish, *Sepia apama*, is able to change body patterns to match a wide range of backgrounds (Zylinski et al., 2011). Background matching is most effective when animals are relatively stationary, as movement will often break camouflage and cause an animal to become more detectable to predatory visual systems (Julesz, 1971, Ioannou and Krause, 2009, Hall et al., 2013).

Disruptive colouration uses highly contrasting pattern elements that occur near the edge of the animal or across the body to break up the body outline, interrupting normal object recognition pathways so the animal form is no longer recognisable (Thayer, 1909, Cott, 1940, Stevens and Cuthill, 2006, Stevens and Merilaita, 2009b, Stevens et al., 2009, Cuthill and Székely, 2009). In Cott’s (1940) pioneering work on animal colouration, he suggested that the sub-principle of maximum disruptive contrast (in terms of colour or luminance) between adjacent pattern elements was one of the most effective mechanisms for distracting attention away from a focal animal. Cott used the black and white, vertically-barred humbug damselfish *Dascyllus aranus* as one of the main examples to illustrate this tenet. However, disruptive colouration should also have some resemblance to the background against which it is viewed, in terms of colour, pattern and luminance (Fraser et al., 2007). For example, (Kelman et al. 2007) demonstrate that the degree of luminance contrast in disruptive markings displayed by cuttlefish did not exceed the luminance contrast in the experimental backgrounds. Additionally, others found there that when the luminance contrast
between the pattern and background were similar, moths with disruptive edge markings had higher survival rates compared to those with non-disruptive elements, suggesting that disruptive colouration rarely is acting in isolation (Stevens et al., 2006). While matching the background luminance is important in disruptive colouration, whether disruptive markings additionally have to match the background in terms of spatial scale to prevent detection has not been specifically tested, to our knowledge. This is despite knowledge that there are significant differences in the spatial frequency of conspicuous and cryptic animal body patterns (Godfrey et al., 1987, Cheney et al., 2014). Interestingly, Cott (1940) did not make any predictions about the spatial characteristics of optimal disruptive colouration in animal body patterns.

In this study, we used humbug damselfish to examine whether the spatial frequency of disruptive pattern elements needs to closely match the spatial frequency of coral backgrounds to provide the fish benefit from a reduction in the likelihood of attack. As outlined in Fig. 1i, fish with a similar pattern to the background, whether that background is plain (a) or patterned (c), is more likely to be cryptic from the perspective of a predator, compared to if a pattern is highly contrasting with the background (b). To understand the design and success of various camouflage strategies, we must consider how colour patterns are viewed by relevant signal receivers (Endler, 1983). Therefore, we first measured the visual acuity of two reef fish predators using information on the anatomy of their eyes and the density of photoreceptors in the area of the eye most likely used for focussing a clear image, similar to the fovea in humans (Collin and Pettigrew, 1989, Ullmann et al., 2012). This information was combined to apply relevant blurring to images used in behavioural assays and natural scenes, so that they represent a predators-eye-view of a scene. Next, we used behavioural experiments with the same two predatory fish species to investigate whether there is a reduction in the likelihood of attack for humbugs when viewed against backgrounds of similar and mismatched spatial frequencies (number of within-pattern-elements), measured using Fast Fourier Transform (FFT) analysis (similar to previous methods (Cortesi et al., 2015b). Finally, we assessed field images from the Great Barrier Reef to quantify the spatial frequency of humbug damselfish
against natural coral backgrounds. We discuss the implications of our findings in relation to disruptive contrast strategies in both marine and terrestrial predator-prey relationships.

Materials and Methods

Study species

The three-barred humbug, *D. aruanus*, (hereafter referred to as a humbug) forms close associations with branching scleractinian coral heads with vertical finger-like protrusions (Randall and Allen, 1977, Randall et al., 1997, Allen et al., 2003). Humbugs are diurnally active, and rarely move more than 1 m from their home coral head, preferring to hide within coral branches when predators approach (Sale, 1970, McCormick and Weaver, 2012, Sale, 1971). The two predators in this study were chosen for their different hunting strategies: slingjaw wrasse (*Epibulus insidiator*) are slow-moving, short-range predators (over a distance of a few cm), while coral trout (*Plectropomus leopardus*) are ambush predators that approach their prey rapidly, attacking from a few metres away (Schott et al., 2014). Predators were caught from the waters surrounding Lizard Island, Great Barrier Reef, Australia [14°41’06” S, 145°26’32” E] using barrier and hand nets (slingjaw wrasse, n = 6), or hook and line (coral trout, n = 9). These two species are also relatively easy to house and train in aquaria (Vail et al., 2013, Vail et al., 2014, Cortesi et al., 2015a), and have been shown to feed on humbugs in the wild (St John, 1999, John et al., 2001).

Predator spatial acuity

We calculated the spatial acuity of both predators to estimate how they would potentially perceive humbug patterns against experimental and natural backgrounds. We used retinal wholemounts from five fish of each species to measure the highest density of photoreceptors in their retinas. This region is likely to be the area of highest acuity within the retina, similar to the *fovea* in humans (Land and Nilsson, 2012). Retinal wholemounts are generated by removing the retina from the eye, fixing the tissue and then mounting the entire retina on a slide with the
photoreceptors pointing towards the viewer. Photoreceptors are then counted using specialised software on a modified microscope, using methods previously published elsewhere (Ullmann et al., 2012, de Busserolles et al., Coimbra et al.) and described in detail in the Supplementary Information. Spatial resolution was calculated both as the minimum resolvable angle (\(\theta\), in degrees) by the eye, and in the number of cells subtended by 1° of visual arc (spatial resolving power, SRP in cycles per degree). The minimum resolvable angle (\(\theta\)) can then be used to calculate the smallest detectable size of an object at a given distance. In comparison, the SRP provides us with the reciprocal information, in terms of how many cycles (i.e. black and white lines) could be discriminated in 1° of visual arc (Land and Nilsson, 2012).

Calculations of the minimum resolvable angle (\(\theta\)) followed previously published methods (Land and Nilsson, 2012), where the finest grating an eye can resolve has an angular period of \(2\Delta\phi\), where \(\Delta\phi\) is the inter-receptor angle and is calculated as follows:

\[
\Delta\phi = \frac{s}{f}
\]

where \(s\) = distance between photoreceptor centres and \(f\) = focal length or posterior nodal distance (PND) = 2.55 x lens radius.

Only cone cells were used for calculating minimum separation, and we assumed that all cone cells contribute to the visual task. It has been suggested that only double/twin (D/T) cones within fish visual systems convey spatial information, similar to chickens (Osorio et al., 1999). However, as D/T cones make up the majority of cone cells in both species, and as the contribution of single cones remains unknown, only using D/T cones for spatial acuity calculations is potentially incorrect. Minimum separation was measured using the average number of cells in 1 mm in the densest region of cone cells in the retina (Table 1). Our results represent the highest possible visual acuity, before any summation can occur in the ganglion cell layer, or beyond.

Image analyses
We then quantified how well humbug body patterns matched the spatial frequency of experimental and natural backgrounds. To do this, we used Fourier analysis techniques to quantify the frequency characteristics of specific objects within images that had been blurred to reflect predator visual acuity. First, bitmap images of the experimental backgrounds with humbugs were created in Adobe Photoshop (Adobe Photoshop CS5.1) and saved as 2048 x 2048 pixel images (.bmp). These images were then manipulated using previously published methods (Caves et al., 2016) to approximate predator visual acuity. In brief, the Fast Fourier Transform of a single channel of the image was multiplied by a modulation transfer function (MTF) with a contrast of < 2% at the minimum resolvable spatial frequency (i.e. smallest detail). This value was chosen as it represents the minimum contrast threshold for fish under bright light conditions (Douglas and Hawryshyn, 1990). Full images were then recovered using a reverse Fourier transform, resulting in an image where spatial information spanning angles < θ were not present. The following parameters were used to blur the image: distance from the viewer to the image (initially set at 100 cm as this approximates the viewing distance in behavioural trials), the width of the image (estimated using the length of the humbug, set at 5.5 cm total length), and the minimum resolvable angle of the predator in degrees.

To analyse the spatial frequency contrast between the vertically barred humbug and the background, we applied a one-dimensional Fast Fourier Transformation (1D FFT) to the blurred images in a custom-designed MATLAB script (R2014a, Mathworks, Nantick, USA). The frequency of ten same-length horizontal transects (0°) through the body of the humbug were averaged to calculate the peak spatial frequency of the vertical bars, which was then compared to an average of ten randomly positioned background transects of the same length and orientation (see Supplementary Information Fig. 1). If the peak frequency of the humbug bars is similar to that of the background, it suggests that the two objects contain similar frequencies along the horizontal plane. The results are plotted showing the peak frequency of the humbug and the background, and also showing the difference between the peak frequency of the fish and the background.
Behavioural experiments

A series of paired-choice experiments were used to test the hypothesis that humbugs benefit from a reduction in the likelihood of attack when their body patterns exactly match the spatial frequency of their coral background. Predators were housed in individual aquaria (slingjaw: 1.2 x 1.2 x 0.4 m; coral trout: 2.2 x 1.2 x 0.4 m) in the flow-through seawater system at Lizard Island Research Station and were fed either pilchards or prawn twice per day during the acclimation period. Each aquarium was divided into two arenas using black corrugated plastic board (thickness = 6 mm) as a partition. A door was cut into the middle of partition to allow fish access into the experimental arena (Fig. 2). Tanks were screened off with shade cloth to minimise external disturbances. A submersible video camera (Hero2, GoPro, San Mateo, USA) was placed on a stainless steel tripod in the middle of the aquarium to film the experiment. The tripod did not interfere with the fish entering the test arena and after a period of acclimation they were not distracted by the camera and tripod.

Behavioural experiments took place over five separate periods of 8-12 weeks from January 2012 - March 2014. In each trial, individual predators were required to search for humbugs that were placed against experimental backgrounds that varied in spatial frequency. With slingjaw wrasse, we were able to use a laminated cut-out photograph of a humbug placed against each background, which the wrasse approached and attacked to receive a food reward from above. However, we were unable to train coral trout to perform the experiment without real prey items, therefore we used euthanized humbug individuals.

Experimental backgrounds were designed in Adobe Illustrator (CS5.1, Adobe Systems Incorporated, Mountain View, USA). To reduce any response bias that could be caused by hue or saturation contrast, all backgrounds were designed and constructed using black, white or monochromatic grey (50%) with the total number of pixels in the image altered to a 50:50 ratio of black:white. All backgrounds were printed using a Deskjet Printer (HP470, Hewlett-Packard, Palo
Alto, USA) and laminated prior to testing. We did not test whether lamination affected the reflectance of the objects or backgrounds as most experimental objects were made using the same materials, thus standardising the glare amongst the backgrounds and objects.

We tested the hypothesis that experimental backgrounds with the same spatial frequency as the vertical bars of humbugs would provide the most protection from predation (Fig. 1ii). The spatial frequency of backgrounds varied slightly for each of the two predator species, as slingjaw wrasse are likely to target juveniles and sub-adults, while coral trout tend to predate on larger adult humbug damselfish (St John, 1999). The laminated photograph of a humbug used in slingjaw experiments was 3 cm (total length). The width of the middle bar in the pattern of this humbug was 4 mm. We used multiple copies of the same humbug photograph, which was presented to slingjaw in a randomised manner. Humbugs used in coral trout experiments were all adults (determined by a lack of blue pigmentation on the ventral fins; size range: standard length (SL) = 26 – 63 mm), and the width of their middle bar was approximately 8 mm.

Predators were tested in five separate behavioural experiments in which the prey and the background varied in terms of spatial frequency (Fig. 1ii). Backgrounds were either regular black and white vertical bars (Experiments 1 & 2) or stylised natural coral head backgrounds generated from photographs (Experiment 3). We also tested the hypothesis that humbugs would more likely to be attacked when the orientation contrast between humbug body pattern (based on the middle bar) and background was high (Experiments 4 & 5).

**Training:** Predators were first trained to associate an A4 laminated paper target mounted on a Perspex board with food. This training was carried out differently for each predator species due to differences in their behaviour and physiology (Randall et al., 1997). Both species of predator were trained to enter an experimental arena and eat a piece of prawn (slingjaw wrasse) or pilchard (coral trout) attached via clear fishing line to a plain white laminated paper background. Once slingjaw wrasse were able to approach and eat food from a background, they were trained using positively
reinforced operant conditioning to attack a laminated image of a plain black humbug and given a
food reward in the middle of the arena from the experimenter. Images of humbugs and the humbug
shape used in training were attached to the backgrounds using double-sided Velcro®. Once coral
tROUT entered the arena to eat the pilchard within 60 seconds consistently, they moved on to the
testing phase, where the pilchard was replaced with a euthanized humbug. Humbugs were
euthanised in a seawater solution of 0.2 ml clove oil per litre of seawater (according to ethics
approval QBI/192/13/ARC). Prior to each trial, humbugs were rinsed thoroughly to remove any
traces of clove oil and were attached using colourless fishing line to the background.

Testing: A distractor background (with no humbug fish) was present for each trial to ensure that
predators searched for humbugs on a background, rather than striking backgrounds at random. We
pseudo-randomised the end of tank in which the backgrounds were placed (left or right), the
location of each background (left, right, centre), and the spatial frequency of the distractor
background (no humbug attached) to prevent the predator associating a particular location or
background with food. Fish blood (2-5 ml) from defrosted, commercially available pilchards was
added to the experimental arena in front of all three backgrounds to reduce olfactory cues from
individual humbugs and to motivate predators to attack humbugs. The water was agitated to
distribute the blood and to ensure that it did not interfere with the predator’s ability to see the
background or humbugs.

Trials started when the door was opened and the predator could enter the experimental
arena, and ended when the predator attacked a humbug. If the predator took longer than four
minutes to attack the humbug, this indicated a lack of motivation to feed and the trial was
terminated. In all trials, the humbug and background combinations, time of day of the trial (am or
pm), standard length of the humbugs (coral trout only), and the location of the chosen background
were recorded. A total of six slingjaw wrasse were used (experiment 1: n = 6; experiment 2: n = 6;
experiment 3: n = 5), one of which failed to complete experiment 3 (Supplementary Information
A total of 9 coral trout were used throughout the study (experiment 1: n = 5; experiment 2: n = 8; experiment 3: n = 6), all of which completed at least one experiment, with three fish completing all three experiments (Supplementary Information Table 1). All predators were presented with a minimum of six repeats of each background combination in each experiment (total trials completed by each predator in individual experiments: minimum n = 14; maximum n = 30).

Three predators did not complete all background combinations presented to them: two refused to complete one trial each (BMJ12 & BJF12), while one refused to complete 4 trials (DJF13; Supplementary Information Table 1). Experiments were conducted in early morning and early evening for the coral trout and during daylight hours of 10 am and 3 pm for the slingjaw to simulate their respective crepuscular and diurnal predation behaviours. Additionally, experiments that were trialled during winter months (May – June) were less successful than those in the summer months (January – March) as predators were more motivated to attack prey during these months, presumably due to an increase in metabolism (and therefore hunger) with the increased summer water temperatures.

**Statistical Analyses**

The likelihood of attack for humbugs against particular backgrounds was analysed using a modified Bradley-Terry generalised mixed effects model (Bradley and Terry, 1952) with a binomial response (background attacked or not attacked). To take into account individual variability between predators and the repetition of tests on each individual, fish identity was added as a random factor within the model. Time of day (am, pm), end of tank in which the backgrounds were placed (left or right), the distractor background used, the location of each of the test backgrounds (left, right, and centre), and the trial number were also included as fixed factors; however, these were insignificant (all Z > 0.26 and all p ≥ 0.07) and removed from the final model. In coral trout experiments, predators did not choose prey based on body size alone: the size of prey that were attacked was not significantly different to the size of prey that was not attacked (Experiment 1, size range = 34 – 55
mm, paired t_{84} = -0.98, p = 0.33; Experiment 2, size range = 26 – 57 mm, t_{167} = 0.46, p = 0.65; Experiment 3, size range = 32 – 63 mm, t_{107} = -0.70, p = 0.48). Therefore, we did not consider prey size further in our analyses. The results of the Bradley-Terry model were then fitted to a logistical function (plogis) to determine the probability of the humbug being chosen (based on background). Analyses were performed in R 3.1.3 (2015-03-09, ‘Smooth Sidewalk’ (2015) using the glm, t.test and lmer functions (MASS package, Venables and Ripley, 2002), kruskal.test (stats package), and the posthoc.kruskal.nemenyi.test functions (PMCMR package, Pohlert, 2015)).

**Results**

**Predator spatial acuity**

Both predators had distinct differences in photoreceptor density across the retina, with the highest concentrations of cone cells generally falling within the central region. The highest density of cone cells in the slingjaw wrasse was 2569 photoreceptors cm\(^{-1}\) compared to 1222 photoreceptors cm\(^{-1}\) in the coral trout (Table 1). The highest visual acuity calculated with photoreceptor counts agrees with previously published calculations using ganglion cell densities (*Choerodon albigena*, blue-tusk fish, Labridae): 2880 ganglion cells cm\(^{-1}\); *P. leopardus*: 1225 ganglion cells cm\(^{-1}\) (Collin, 1989, Collin, 2008). Therefore we assume no convergence from photoreceptor to ganglion cell in these retinal regions at least and that photoreceptor densities provide a good estimate of acuity for tasks involving small objects. The minimum resolvable angle (θ) was calculated as 0.092° and 0.103° for the slingjaw and coral trout respectively based on the number of cone cells in the highest density regions (Table 1). These spatial acuities correspond to minimum resolvable gratings of 1.74 mm and 1.45 mm at a viewing distance of 100 cm respectively. To simplify the subsequent analysis, a minimum resolvable angle of 0.10° to approximate both predators’ visual resolution.

**Image analysis**
All eight backgrounds used in behavioural experiments were blurred to a minimum resolvable angle of 0.10° to determine how predators would perceive the difference between background and humbug pattern. Fig. 3 shows that the humbug peak frequency was most similar to the ‘similar width’ barred background (orange lines), while the other barred backgrounds have distinctly different peak frequencies. Unlike the humbug pattern, there is no one distinctive peak frequency in the ‘natural’ coral backgrounds (Fig. 4), which has peaks both lower and higher in spatial frequency than that of the humbug. When the orientation of the humbug is analysed, it is clear that fish angle influences the peak frequency contrast between the humbug and the background in the horizontal plane, with the greatest differences seen when the fish is at 45° or 90° to the background (Fig. 5).

We also blurred an image of humbugs against a coral head from the field (humbugs in the wild) and analysed it using the FFT analysis. Only the green channel of the image was analysed to prevent additional colour information influencing the spatial frequency analysis. At close range, the peak frequencies of the humbug body pattern were very different from the coral background (Fig. 6), perhaps due to the wider range of natural frequencies in the background. Additionally, compared to the boldly barred humbug peak in frequency is seen within the fish that is not observed in the coral, possibly corresponding to the regular, bold patterning of the humbug, compared to the irregular, and less-bold patterning of the branching coral head (branching coral heads lose the structure of their branching when flattened in a 2D image). This image was then blurred to represent increasing viewing distances beyond 1m from both a human and predator’s perspective, and to identify if at a particular distance, the humbug pattern more closely matched that of the background (Fig. 6). Indeed, by a distance of 5 m, the humbug body statistics do more closely match that of the coral background from a fish predator’s perspective, but not a human’s perspective.

Behavioural experiments
The likelihood of humbugs being attacked varied depending on the spatial frequency of the background. In Experiment 1 (grey, similar width and ¼ width bars), humbugs were least likely to be attacked when viewed against a background with a similar spatial frequency to its own body pattern (Fig. 7a: *slingjaw*: grey vs. similar width: \( z = 2.16, n = 6, \text{d.f. residuals} = 15, p = 0.031 \); ¼ width vs. similar width: \( z = 2.75, n = 6, \text{d.f. residuals} = 15, p = 0.006 \); *coral trout*: grey vs. similar width: \( z = 5.66, n = 5, \text{d.f. residuals} = 12, p < 0.001 \); ¼ width vs. similar width: \( z = 3.48, n = 5, \text{d.f. residuals} = 12, p = 0.000501 \)), with the grey background providing the least protection from both predators. When viewed by the *slingjaw* wrasse, there was no statistical difference in the likelihood of attack for the humbug when viewed against the 1 mm background, compared to the monochromatic grey (Fig. 7a: *slingjaw*: grey vs. ¼ width: \( z = 0.405, n = 6, \text{d.f. residuals} = 15, p = 0.686 \)).

In Experiment 2 (½ width, similar width, 2 x width bars), humbugs were again least likely to be attacked when viewed against a background with a similar spatial frequency and, interestingly, when also viewed against a slightly higher spatial frequency to their body pattern (Fig. 7b: *slingjaw*: ½ width vs. similar width: \( z = 1.10, n = 6, \text{d.f. residuals} = 15, p = 0.28 \); 2 x width vs. similar width: \( z = 2.16, n = 6, \text{d.f. residuals} = 15, p = 0.031 \); *coral trout*: ½ width vs. similar width: \( z = -0.93, n = 9, \text{d.f. residuals} = 23, p = 0.35 \); 2 x width vs. similar width: \( z = 2.38, n = 9, \text{d.f. residuals} = 23, p = 0.017 \)). The lowest frequency background provided the least protection, consistent with results from Experiment 1.

In Experiment 3, when stylised coral backgrounds were used, there was a decreased likelihood of being attacked when viewed against the similar width or ½ width branching corals. There was no significant difference in the protection afforded by the coral that was most similar in terms of spatial frequency to the humbug body pattern, and that of the smallest branching coral (Fig. 7c: *slingjaw*: ½ width vs. similar width branches: \( z = -1.50, n = 5, \text{d.f. residuals} = 12, p = 0.134 \); *coral trout*: ½ width vs. similar width branches: \( z = -1.63, n = 6, \text{d.f. residuals} = 15, p = 0.103 \)). When viewed by the *slingjaw*, there was no difference in the risk of attack between the
similar width and 2 x width branching corals (Fig. 7c: **slingjaw**: 2 x width vs. similar width branches: \( z = 1.06, n = 5, \) d.f. residuals = 12, \( p = 0.29 \)). In fact, in slingjaw behavioural trials, the only time humbug stimuli showed reduced likelihood of attack against natural backgrounds was when the ½ width branching coral was paired with the 2 x width branching coral. In this case, there was a significant reduction in attack likelihood if viewed against the ½ width branching coral (Fig. 7c: **slingjaw**: 2 x width vs. ½ width branches: \( z = -2.49, n = 5, \) d.f. residuals = 12, \( p = 0.0128 \)). For the coral trout predator, humbugs gained significantly more protection when viewed against the coral with similar spatial frequency to their own body pattern, compared to the widest branching corals (Fig. 7c: **coral trout**: 2 x width vs. similar width branches: \( z = 1.34, n = 6, \) \( p < 0.001 \)).

In Experiments 4 and 5, there was a significant increase in the likelihood of being attacked when the angle of the humbug was at 90° to the background (Fig. 8a: **slingjaw**: 90° vs. 180°: \( z = 2.081, \) d.f. residuals = 2, \( n = 4, \) \( p = 0.038 \); **coral trout**: 90° vs. 180°: \( z = 2.94, n = 4, \) d.f. residuals = 14, \( p = 0.003 \)). In all other angle contrast scenarios, the likelihood of attack did not significantly change with angle contrast although for both species there was a non-significant trend for decreased attack likelihood (Fig. 8b: **slingjaw**: stripe matched vs. 45°: \( z = -1.81, n = 5, \) d.f. residuals = 16, \( p = 0.071 \); stripe matched vs. 180°: \( z = -0.51, n = 5, \) d.f. residuals = 16, \( p = 0.608 \); 45° vs. 180°: \( z = 0.383, n = 5, \) d.f. residuals = 16, \( p = 0.702 \); **coral trout**: stripe matched vs. 45°: \( z = -1.317, n = 4, \) d.f. residuals = 13, \( p = 0.188 \); stripe matched vs. 180°: \( z = -1.615, n = 4, \) d.f. residuals = 13, \( p = 0.106 \); 45° vs. 180°: \( z = -0.932, n = 4, \) d.f. residuals = 13, \( p = 0.351 \)).
Discussion

Summary

Our results demonstrate that highly contrasting pattern elements used in disruptive colouration do not have to exactly match the spatial characteristics of the background to reduce the likelihood of attack by potential predators. Interestingly, backgrounds with a slightly higher spatial frequency provided humbugs with a reduction in the likelihood of attack, presumably due to predators being unable to detect the prey items against these backgrounds compared to other backgrounds. Our results are consistent when considering the orientation of the humbug against the background, as the likelihood of attack only increased when the orientation contrast was maximised (humbug at 90° to the background). The results for both behavioural testing and image analysis were similar between the two predator species, despite differences between their visual systems and hunting strategies, suggesting that the humbug body pattern has evolved to be effective against a wide range of vertebrate visual systems.

Background matching & disruptive colouration

Predator behavioural trials suggest that perfect background-matching is not necessary for increased survival. Our results with fish predators are similar to those found with avian predators, where the disruptive elements of moth body patterns (in particular, the spatial positioning of highly contrasting elements across the body) provided increased survival (or reduction in likelihood of attack) without perfect background-matching (Schaefer and Stobbe, 2006, Stevens et al., 2006). Our results suggest an additional benefit to having a body pattern that is both background-matching and disruptive (so-called differential blending; (Cott, 1940)), as the disruptive colouration provides crypsis on a range of backgrounds, possibly due to the varying angle and width of the highly contrasting black and white bars within the body pattern. Humbugs are therefore likely to be protected from detection by fish predators when they are viewed against a range of spatial frequency backgrounds, supporting the idea that disruptive elements are particularly important in
concealing animals that reside in heterogenous environments (Thayer, 1909). Indeed, the humbug lives around and within several species of branching corals that are likely to have a range of spatial frequencies (Sale, 1972).

Higher spatial frequency background decreases likelihood of attack

It was particularly interesting to us that a higher spatial frequency background provided as much (or even greater) reduction in the likelihood of predatory attack than backgrounds with a similar spatial frequency to the humbug body pattern. There are a number of possible explanations why this may occur. Backgrounds that have a greater degree of complexity could be distracting to the visual system of the predator (Dimitrova and Merilaita, 2009) and indeed, avian predators take longer to find prey on more complex backgrounds, regardless of the spatial frequency contrast (Dimitrova and Merilaita, 2011). This suggests that a greater number of elements within the background transfers visual attention away from the target or prey. A slight mismatch with the background may also enhance the disruptive effect of the humbug body pattern by decreasing predatory edge detection. Edge detection is a key phase in early visual processing in vertebrates, occurring in the retinal ganglion cells (Land and Nilsson, 2012). Body form can be detected by luminance contrast between the animal and the background (Canny, 1986, Hubel and Wiesel, 1962, Marr and Hildreth, 1980). However, the disruptive vertical bars of the humbug body pattern would create ‘false edges’ that breakup the body shape of the fish, termed coincident disruptive colouration (Cott, 1940). In frogs, highly contrasting body patterns and enhanced borders unlike those seen in natural scenes have also shown to act as a camouflage strategy due to the lack of suitable detection methods in predator visual systems (Osorio and Srinivasan, 1991). The disruptive effect may be greater on a background that contains more ‘natural edges’ than the body pattern (as found on a higher spatial frequency background), as the contrast between the body outline and background would be further blurred, causing a greater visual illusion to the predators.
Our experimental trials, and subsequent Fourier Analyses were performed at a set predator viewing distance of 100 cm. With increased viewing distances (similar to those used by coral trout when attacking prey in the wild) it is likely that the black-and-white bars on the humbug merge into one grey object, as is predicted with many of the bright and intricate reef fish colour patterns (Marshall, 2000, Vorobyev et al., 1999, Wilkins et al., 2016). Indeed, as the reef contains many dappled shadows and grey-areas, merged grey objects are likely to be well camouflaged through a direct match to background luminous intensity. We took an underwater field image of humbugs against a coral head and blurred it to examine how well humbugs would match the natural background at a range of viewing distances. To human visual systems, humbugs will remain conspicuous even at a distance of 10 m in clear ocean water. However, when modelled using the predators’ visual acuity, the effectiveness of the humbug’s camouflage increased with increasing viewing distance, until the spatial frequency of the humbug and coral appear to be similar at a distance of 5m.

Coral trout ambush prey from a distance of a few metres and therefore it is likely that the combination of their limited spatial acuity, the visual complexity of the reef background, and the unique characteristics of the humbug body pattern, allow this species to avoid being eaten as often as other damselfish species, as suggested by gut contents analyses (John, 1995, 1999, 2001). The slingjaw wrasse approaches prey at close distances, suggesting other aspects of the humbug ecology play a role in avoiding predatory attacks, such as sheltering within the coral head, or the effect of movement combined with the disruptive body pattern.

**Visual acuity of predators**

Our backgrounds were designed to be at the limit of spatial discrimination for both predators, and therefore the predators may have been unable to distinguish the individual bars within the background, due to behavioural visual resolution being lower than theoretical resolution,
as demonstrated in other fish (Champ et al., 2014). We calculated minimum resolvable angles using the distance between two cone photoreceptors (both double and single cones), and did not take into account potential summation within the retinal ganglion cell (RGC) layer, optic nerve, or optic tectum. Further processing may reduce the spatial acuity of the predator (Collin and Pettigrew, 1989) thereby enhancing the cryptic potential of the prey body pattern against the background. Additionally, lower light levels would encourage regional summation of the signal within the retina, further reducing visual acuity. As the light levels in this experiment were high (all the experiments were carried out during daylight hours), it is unlikely that summation had a significant effect on the visual acuity of the predators.

Movement and orientation of prey

When swimming up and down in the water column, and moving to different areas of the coral head, humbugs do not always swim at the same orientation to the coral. We show that humbugs still retain a significant reduction in predation risk even when they are not completely aligned with the background habitat. Indeed, the orientation of an animal against a background can enhance or reduce the individual’s crypsis depending on the alignment of features within the background and body pattern (Webster et al., 2009). It has been shown that in some species of moth, individuals rest in non-random orientations that maximise crypsis with the background (Endler, 1984). Moths will change their orientation to a more cryptic position (Kang et al., 2013) using both visual and tactile information (Kang et al., 2015), thereby reducing predator detection (Webster et al., 2009) and suggesting an adaptive advantage of being in-phase with the background. Our results agree to some extent with the alignment hypothesis: the humbug only suffered increased predation when its patterns were perpendicular (90°) to the background, which would be rare in the natural environment.

In this study, we did not incorporate movement, and although high contrast markings are likely to increase predator search times in comparison to low contrast markings independent of
motion (e.g. Dimitrova et al., 2009), high contrast patterns are hypothesised to increase protection when moving (Allen et al., 2013, Thayer, 1909). This ‘motion dazzle’ (sensu Cott, 1942) has been shown to be particularly effective when the pattern is striped or barred as opposed to spotty or plain (Stevens et al., 2008) and can affect the distance travelled, speed and directional information relayed to the viewer’s visual system (Ashida and Kitaoka, 2003, Conway and Livingstone, 2005, Jackson et al., 1976). It has been suggested that, in terrestrial animals, bars and stripes in body patterns may distract predator’s visual systems and provide misinformation about the direction of movement of a group of animals (How and Zanker, 2014). However, some studies have shown that the details of the pattern (i.e. whether background matching or disruptive) are not influential in decreasing predation likelihood, as long as the pattern is at least similar to the background. It is the presence of other similarly patterned objects that increase protection (Hall et al., 2013, Stevens et al., 2011). For interest, we have included a blurred movie of the humbug in its natural environment to simulate how they may appear to predators in the wild (Supplementary Information Movie 1).

The humbug has a body pattern similar to that of a zebra and congregates in small groups suggesting an increased confusion effect due to the presence of similarly patterned objects (both humbug and branching coral background). When combined with the attenuating properties of water, it is likely that movement will only serve to increase the camouflage potential of the humbug body pattern. Clearly this is an area that warrants further research.

**Limitations of study**

In this study we have looked exclusively at the humbug and background in greyscale and not incorporated colour into any visual models. As the humbug is black and white, and coral is likely to vary in spectral reflectance between individual colonies, there will be colour contrast between the coral background and humbug body pattern, particularly as many coral reef fish predators are likely to have functional colour vision (Losey et al., 2003, Marshall et al., 2003a, Marshall et al., 2003b, Phillips et al., 2016). It would be interesting to identify whether colour adds
another element of disruption to the humbug body pattern, and recent methods have been developed to answer exactly these types of question (Endler, 2012).

Conclusions

In summary, the results of this study are the first to show quantitatively that although coral head backgrounds serve to increase the crypsis of disruptive colouration, it is not achieved through matching the spatial frequency of the background as previously assumed (Cott, 1940). Instead, a slight mismatch to a higher spatial frequency background enhances the crypsis of disruptive colouration. Therefore, humbugs should be found in environments with a variety of spatial frequency backgrounds, including many higher spatial frequency backgrounds. Further research should identify whether humbugs actively choose higher spatial frequency backgrounds to reduce the likelihood of detection when approached by predators, as has been suggested in killifish (Kjernsmo and Merilaita, 2012).

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