# Dazzling Damselfish: Investigating background complexity and motion dazzle in humbug damselfish (*Dascyllus aruanus*)

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High-contrast body patterning is prevalent in nature. When combined with movement this patterning can disrupt visual cues of observers and reduce the likelihood of identification, a phenomenon called motion dazzle. These dazzle patterns generate different forms of optical illusion and can mislead observers about the shape, location and direction of the target. However, it is also possible that when these highly contrasted animals move against similarly contrasted backgrounds that there is further disruption to visual signals. In this study, we use the humbug damselfish (*Dascyllus aruanus)* to model motion cues of its high contrast body stripes against high contrast background gratings of different widths and orientations, from the perspective of a predator. We found that when the background is more uniform (or indiscriminable to a viewer) that the humbugs may rely on the confusing motion cues created by internal stripes, but where the background is high contrast that they can rely more on disruption of edge detection, and confusing motion cues induced by both the background and body patterning. We also assessed whether humbugs altered their behaviour in response to different backgrounds. When detecting backgrounds with a spatial structure similar to their own striped body pattern, humbugs move closer and move less overall to remain cryptically camouflaged against the background and thus avoid revealing themselves to potential predators. At backgrounds with higher frequency gratings, humbugs moved more which may represent a greater reliance on the internal contrast of the fish's striped body pattern to generate disruptive motion cues. This study demonstrates that striped animals alter their behaviour depending on the background to maximise the protection offered via motion dazzle.

# Dazzling Damselfish: Investigating background complexity and motion dazzle in humbug damselfish (*Dascyllus aruanus*)

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## Abstract

High-contrast body patterning is prevalent in nature. When combined with movement this patterning can disrupt visual cues of observers and reduce the likelihood of identification, a phenomenon called motion dazzle. These dazzle patterns generate different forms of optical illusion and can mislead observers about the shape, location and direction of the target. However, it is also possible that when these highly contrasted animals move against similarly contrasted backgrounds that there is further disruption to visual signals. In this study, we use the humbug damselfish (*Dascyllus aruanus)* to model motion cues of its high contrast body stripes against high contrast background gratings of different widths and orientations, from the perspective of a predator. We found that when the background is more uniform (or indiscriminable to a viewer) that the humbugs may rely on the confusing motion cues created by internal stripes, but where the background is high contrast that they can rely more on disruption of edge detection, and confusing motion cues induced by both the background and body patterning. We also assessed whether humbugs altered their behaviour in response to different backgrounds. When detecting backgrounds with a spatial structure similar to their own striped body pattern, humbugs move closer and move less overall to remain cryptically camouflaged against the background and thus avoid revealing themselves to potential predators. At backgrounds with higher frequency gratings, humbugs moved more which may represent a greater reliance on the internal contrast of the fish's striped body pattern to generate disruptive motion cues. This study demonstrates that striped animals alter their behaviour depending on the background to maximise the protection offered via motion dazzle.

## Introduction

Avoiding detection is key to survival for many animals and there are several tactics that animals may use to decrease detection. Visual camouflage, chemical concealment and modification of vocalisations (see Ruxton 2009 and examples within) are all strategies that animals possibly use to maintain crypsis. However, visual camouflage is the most understood approach with strategies such as the alteration of colouration or patterning, body positioning or self-shadowing all assisting an animal to blend into its environment. However, many of these camouflage strategies are only thought to be effective when prey is still, with movement likely to increase conspicuousness and provide locational information to predators (Tan and Elgar 2021). Despite movement seeming contradictory to camouflage, there are likely mechanisms that are used to confuse and distract predators. Several strategies incorporating movement have been reported over recent years, such as flicker-fusion, motion dazzle and motion camouflage (Stevens and Ruxton 2019). However, given the lack of evidence in biological systems of flicker-fusion and motion camouflage, to date the most well substantiated movement camouflage strategy in compromising predator detection cues is motion dazzle.

Motion dazzle is a phenomenon where high-contrast body patterning of moving targets can disrupt the visual cues of predators, thus reducing the likelihood of capture. Motion dazzle is not a form of crypsis *per se,* but rather a mechanism that inhibits an observer's perception of a target by making the estimation of speed and / or trajectory harder (Stevens 2007; Stevens *et al.* 2008; Thayer 1909). The most effective dazzle patterns are repetitive high contrast coloration such as stripes, bands and zig-zags (Hall *et al.* 2016). When combined with movement, these dazzle patterns generate different forms of optical illusion and can mislead observers about the shape, location and direction of the target (How and Zanker 2014). Historically, warships were painted with high contrast geometric patterns, or 'dazzle camouflage' in attempt to disrupt the perception of range, size, heading and speed (Scott-Samuel *et al.* 2011). More recently, several studies have explored the mechanisms underlying the effects of motion dazzle. Using computer generated scenarios with human observers it has been shown that motion dazzle hinders the ability of human 'predators' to accurately capture targets (Stevens *et al.* 2008), distorts the perceived speed of targets (Hall *et al.* 2016; Kodandaramaiah *et al.* 2020) and affects direction perception of targets (Hughes *et al.* 2017).

Recent research has provided further validation to the concept of motion dazzle. In snakes, a comparative phylogenetic approach found relationships between longitudinal stripes and small, fast, exposed snakes, suggesting contrasting body patterns function efficiently during movement (Allen *et al.* 2013). Similarly, a phylogenetic approach to lizard patterning found that conspicuously striped lizards were substantially more mobile than cryptic lizards, indicating that the striping my enhance escape strategies via motion dazzle (Halperin *et al.* 2017). Furthermore, using comparative methods and eco-physiological factors it was found that lizards with longitudinal striped tails are likely ground dwelling, have higher body temperatures, diurnally active and can lose their tail, supporting the notion that striped tails in lizards may have protective functions based on motion dazzle effects (Murali *et al.* 2018). Motion dazzle patterns have also been associated with the evolution of smaller body size in animals as they are less detectable when stationary, have greater manoeuvrability and have higher sprint speed (Kodandaramaiah *et al.* 2020; Murali and Kodandaramaiah 2020). However, to date, the most biologically relevant experiment done on motion dazzle applied a simulated biological visual system based on two dimensional motion detection (2DMD) algorithm to high contrast patterning of zebras. How and Zanker (2014) found that zebra movement created confusing motion signals including motion opposing the direction that the animal was moving. These varied motion signals can potentially confuse an observer's motion perception system, providing a predator avoidance strategy. This study provided some excellent insights into motion dazzle and how contrasted patterning may offer protection when combined with movement. But it is likely that when these highly contrasted animals move against similarly contrasted backgrounds that there is even further disruption to visual signals due to interruption at the edge of the animal and greater confusion of motion cues.

An excellent model species to explore further components of motion dazzle is a small species of coral reef fish, the humbug damselfish (*Dascyllus aruanus*) (hereafter 'humbugs'). Humbugs have high contrasted black and white striped patterning and inhabit branching *Acropora* sp. and *Pocillopora* sp. coral colonies. The humbugs rarely stray more than one metre from their home colonies (Kent *et al.* 2019) and have been shown to seek refuge in the complex architecture of branching corals (Holbrook and Schmitt 2002). A recent study used static humbug images to demonstrate they may receive protection through disruptive camouflage, particularly when the backgrounds are of similar or slightly higher spatial frequency to the humbug stripes (Phillips *et al.* 2017). The study also found that the greatest camouflage was achieved when the humbug stripes were orientated like the background (Phillips *et al.* 2017). However, humbugs are rarely static and regularly move within and between coral patches in groups of up to 80 individuals (Holbrook *et al.* 2000; Mann *et al.* 2014). As demonstrated with the zebra pattern, it is likely that, when combined with movement, the humbug pattern offers protection via motion dazzle (How and Zanker 2014). But it is possible that there is even more motion disruption attained when humbugs are found against highly contrasted backgrounds, such as coral habitat, or other humbug fish. Further, there are reports that some animals can assess their degree of camouflage and predation risk and adjust behaviour to maximise camouflage (Kang *et al.* 2015; Wilson-Aggarwal *et al.* 2016). Given evidence of disruptive camouflage against backgrounds of similar spatial frequencies, and that motion dazzle may also be enhanced with similarly contrasted backgrounds, we wanted to explore whether humbugs can perceive which backgrounds provide better protection and modify their behaviour accordingly.

In this study we first built on the work conducted by How and Zanker (2014) by using the same motion model that was applied to zebra patterning. We extended this to assessing if high contrast backgrounds interact with the humbug pattern to enhance motion dazzle and if motion cues may also be disrupted at the edges of animals, particularly against highly contrasted backgrounds. By using uniform square-wave gratings at different spatial frequencies (i.e. grating width) and orientations we explored baseline differences obtained by different spatial frequencies without complexities found in the natural environment. We predicted that those backgrounds with similar frequency and orientation to that of the humbugs would provide the greatest disruption to motion cues and be most effective in reducing detectability from the perspective of a moving predator. We then explored whether humbugs could perceive different backgrounds and modify behaviour to maximise camouflage. We examined the humbug eye to establish whether humbugs have the visual acuity to perceive differences in backgrounds of varying spatial frequencies. We then assessed whether humbugs spent more time closer to particular backgrounds and examined if humbugs moved more in response to different backgrounds, or if they moved more in certain tank regions (close, middle or far) in response to the different backgrounds. Backgrounds with spatial frequencies similar or slightly higher to humbug stripes, and that are orientated in similar direction (Phillips et al., 2017) provide humbugs with the greatest disruptive camouflage. Thus, we hypothesised that humbugs would spend more time closer, and move more in response to those backgrounds that are more effective in reducing detectability. Given the width of the humbug stripes were around 0.5 cm, we predicted the grating best at reducing detectability would be 0.5 cm with a vertical orientation.

## Materials & Methods

### Animal Acquisition and Housing

All procedures were approved by the Macquarie University Animal Ethics Committee (ARA 2017/039).

Six humbugs were obtained from a local aquarium supplier in Sydney, Australia and transported in aerated seawater to the Macquarie University Seawater Facility. This facility comprises 45,000 L of recirculated seawater, which is collected from depth in Sydney Harbour. Humbugs were housed individually in opaque white polythene tubs (600 × 350 x 400 mm) and maintained at a water flow rate of 3 L min$^{-1}$, salinity of 35 ppt and temperature of 26°C. Aquaria were illuminated with aquarium LED lights (Aqua One Strip Glo Marine 90cm Aquarium LED Light) on a 12:12 h light:dark regime. A white PVC pipe (100 mm long, 100 mm diameter) in each tank served as a shelter for the fish. Once a day, aquaria were cleaned and fish were fed to satiation with Nutridiet marine flakes (Seachem, Madison US). Fish were acclimated to the aquaria for two weeks before trials commenced.

### Test Backgrounds / Gratings

We created several different square-wave test gratings to test as backgrounds for motion dazzle and subsequent behavioural trials. The square-wave test gratings were created in Adobe Illustrator (version 22.1, 2018) and consisted of repeating black (RGB: 0,0,0) and white (RGB: 255,255,255) bars of equal width. Five different spatial frequencies were generated where the widths of the individual grating 'bars' (black or white) were 1 cm, 0.5 cm, 0.25 cm, 0.1 cm and 0.05cm (see Figure S1 in supplemental information for examples of the gratings). These gratings cover the range of spatial frequencies of Acropora branching corals in which the humbugs are usually found (Phillips *et al.* 2017). Cards were printed on 250GSM A3 matte photographic paper (Krisp, Hoppers Crossing VIC, Australia). Background cards were cut to size (25 x 25 cm) and laminated using transparent plastic pouches (gloss, A3, 125 µm thick; GBC® Signature laminating pouches, USA). Although we use unnatural striped backgrounds, these results can provide insight into the potential camouflage high contrast body patterns may offer particularly in a highly complex and structured visual environment and/or for animals that school or live in packs.

### Estimating the motion dazzle

We recorded video footage of three humbugs from the perspective of a moving predator. A GoPro video camera was attached to a camera dolly and pulled along a track fixed parallel to the grating so that the movement of the camera from one side of the arena to the other was smooth, level, and at a consistent speed. We moved the GoPro from side-to-side at ~15-20 cm $s^{-1}$. Fish were recorded against background gratings of three different spatial frequencies (1 cm, 0.25 cm and 0.05 cm) which were presented in both vertical and horizontal orientation. These frequencies were used as they represent a grating width that could not be discriminated (0.05 cm), is close to the limit (0.25 cm) and easily discriminated by the virtual predator (1 cm) and from these sizes we can deduce the results of a broader range of grating widths. In total, fish were recorded against six different backgrounds.

The motion cues created by the humbug were analysed with respect to the visual abilities of a potential predator, the slingjaw wrasse (*Epibulus insidiator*) or coral trout (*Plectropomus leopardus*). The analysis was performed on a 600 × 600-pixel grid extracted from each video frame, starting from when the humbug entered and finishing when it exited this grid. To keep the speed of motion consistent we analysed video clips in which the humbug did not move, thus all motion was created due to the movement of the camera (i.e., the potential predator). All videos were analysed so that the humbug moved from the left to the right of the screen, for some videos this was achieved by flipping the video horizontally. Between 8 and 23 frames were analysed.

Visual motion at the level of the retina was estimated from the videos using a 2-dimensional motion detection (2DMD) model (How and Zanker 2014; Pallus *et al.* 2010) written in Matlab. The 2DMD model is based on elementary motion detectors, with the spatial and temporal correlation of pixel intensities between consecutive video frames used to determine the strength and direction of object motion. The 2DMD model uses two orthogonal arrays of elementary motion detectors to compare each pixel at a set pixel spacing and between frames based on a set temporal and spatial filter. The frame rate of the videos (30 frames per second, i.e., 30 Hz) is similar to the temporal resolution thresholds in fishes (Fritsches *et al.* 2005; Matsumoto *et al.* 2009; Pallus *et al.* 2010; Pusch *et al.* 2013; Ryan *et al.* 2017), thus, the temporal filter was set to one (i.e., a temporal resolution of 30Hz). A Difference-of-Gaussians (DOG) spatial filter was applied to each video frame to mimic the effect of the opponent centre-surround receptive field organisation of the retinal ganglion cells. The spatial extent of the DOG filter and the spacing parameter were chosen to reflect the peak spatial resolving power of a potential predator i.e., a sligjaw wrasse or coral trout, which has been estimated at ~10-12 cycles $deg^{-1}$ based on the packing density of photoreceptors. As videos were made at distances of 30 cm, we further reduced the spacing parameters to reflect spatial resolving power of 2.5 cycles $deg^{-1}$ to assess the view from a predator at 1 m away, which reflects the distance in nature in which the potential predators may see a humbug (Phillips *et al.* 2017).

Each video clip was analysed to determine the total mean strength of motion, and the mean strength of motion in 72 direction vectors, i.e., each vector was the mean across five-degree wide directional bins. We were interested in the motion cues at the edge of the humbug where the internal pattern and background pattern interact. Thus, we account for motion created by the background pattern by performing the motion analysis on a 600 × 600-pixel region of the same video frames with just the background grating. The mean motion strength at each direction vector for the background motion analysis was then subtracted from the motion strength of the corresponding humbug motion analysis. If background motion was greater than the motion of the humbug, the motion cues were treated as zero.

The total magnitude of motion cues and the magnitude of motion over direction vectors of the humbug were fit a linear mixed-model using the lme4 package in R. Strength of motion was square root transformed and residuals from the models met linearity and normality assumptions. The individual fish nested in trial number was set as the random effect. Significance of the interactions were confirmed using log likelihood ratios. Motion strength was included as the response variable with direction vector, and the size and orientation of the background grating included as fixed effects with an interaction term between them. Pairwise comparisons were obtained using the pairwise method and a p-value adjustment equivalent to the Tukey test in the emmeans package (Lenth 2022). Significance and pairwise comparisons were undertaken as above.

**Anatomical measurements of visual acuity**

Animals were euthanised with an overdose (1:2000) of methane tricaine sulfonate salt MS222 (Sigma) buffered with an equal amount of sodium bicarbonate. Retinal wholemount procedures were adapted from Ullmann *et al.* (2012). Retinal Ganglion Cell (RGC) counts were performed on an Olympus BX53 compound microscope fitted with a motorised stage and an Olympus DP80 camera. Stage movement and the camera was controlled by cellSens Dimension software (Olympus, version: 4.1). The total number of neurons in the RGC layer were counted using a ×100/1.40NA oil immersion objective. Counts were made at 0.25 mm intervals with a 50 x 50 µm counting frame, providing approximately 400 sample locations across the retina. Given the difficulty in differentiating between ganglion and amacrine cells in the areas of high density, all neural cells were counted together. The theoretical (anatomical) peak spatial resolving power was estimated for *D. aruanus* from the maximum density of RGCs in the retina and the focal length as outlined by Lisney and Collin (2008). We assume that RGCs are the limiting factor for spatial resolving power and they are packed in a hexagonal array.

**The effect of visual background on humbug behaviour**

Humbug behaviour in response to different visual backgrounds was tested in open field trials. The backgrounds were large square-wave gratings of five different spatial frequencies (i.e., grating period) that were presented at two different orientations (vertical or horizontal) (see Figure S1 in supplementary material for examples of the gratings). We assessed two behaviours in response to the different backgrounds: 1. the mean distance that fish positioned themselves from each different grating, and 2. the mean distanced moved throughout the tank as well as the distance moved within different regions of the test arena (close, middle and far) in response to the different gratings.

*Behavioural Assay*

Fish behaviour was tested using an open-field trial (Champagne *et al.* 2010) in a 40 × 22 cm aquarium. The test board with the background test grating was placed 5 cm from the rear of the tank prior to commencement of the trial. The aquarium was filled with 5 cm of aged water at the same temperature as the holding tanks and an air stone was placed behind the test board. Fish were transferred from their home tanks to the middle of the experimental arena and given a one-minute acclimation period before the trial commenced, with each trial lasting five minutes. Fish were tested against a total of 10 different test gratings, i.e., the five different spatial frequencies in both vertical and horizontal orientation. The order of presentation of the gratings for each fish was randomised using a pseudorandom number generator before commencement of the trials. At the completion of the trial, fish were returned to their home tank and a 70% water change in the experimental arena was performed. Fish behaviour was recorded using a GoPro$^{TM}$ Hero 9 digital video camera positioned centrally above the tank. The GoPro camera was set to linear mode and video recorded at 30 frames per second (fps). Observers remained out of the view of the fish for the entirety of the trial (Figure 1). After a five-day break, the trials were repeated with the grating and fish tested in a different randomised order compared to the first set of trials.

*Video Tracking*

The location of the fish in each video frame was obtained using the automatic tracking software DeepLabCut (version 2.2) (Mathis *et al.* 2018; Nath *et al.* 2019). Because the water level in the test arena was maintained at 5 cm and, therefore, the humbugs remained at similar depth throughout the trials, only X and Y coordinates were tracked. DLC was used to track two points of interest as reference landmarks, the black stripes on the head and tail of the humbugs, but subsequently only the X,Y coordinates of the head were used for analysis. Videos were modified from 30 fps to 15 fps to reduce processing time. To train the networks to automatically track the fish, we labelled 200 frames taken from 10 videos and used a MobileNetV2.1 based neural network with default parameters for 30,000 training iterations. We validated the training algorithm with one shuffle and found the test error was: 3.2 pixels, train: 3.4 pixels. We then used a p-cutoff of 0.9 to condition the X,Y coordinates for future analysis. This network was then used to analyse the videos of all the trials which were all recorded under the same experimental settings. We obtained 4,500 positional X,Y coordinates (one per video frame) for each individual fish for a single trial. Pixels were converted to centimetres (cmperpixel =

0.02763) before calculating the distance that each fish positioned itself from each grating and the total distance travelled by each fish during a trial.

Because assessing whether behavioural responses in relation to different backgrounds changed over time is an important first step, we obtained behavioural measurements every 15 seconds (or 225 frames). To obtain the distance that the humbugs positioned themselves from the grating, the perpendicular distance from the top of the grating to the head of the fish (i.e. grating Y – fish Y) was taken every 225 frames. For the distance moved by each fish we aggregated and summed the distance moved between coordinates every 225 frames. To assess whether fish travelled more in different regions of the tank, we divided the tank into three regions using the *cut* function in Rstudio (R Core Team 2022). The regions were defined as close (0–11 cm from grating), middle (12–22 cm from grating) and far (13–35 cm from grating).

*Statistical Analyses*

Before investigating differences in the distance in fish position from the grating we first assessed the distribution of the data. Due to the bimodal distribution of the data, a linear model could not be fit. Because the perpendicular distance of the fish to the grating was limited to values between 0 and 35 cm due to the dimensions of the test arena, distance values were normalised between 0 and 1. Consequently, the distance data were beta distributed and a beta regression model was fitted to the data using *glmmTMB* in the glmmTMB package (Brooks *et al.* 2017). We included grating orientation (horizontal or vertical), grating size and time as fixed factors with a three-way interaction term between them. We included a random effect of fish nested in trial. To check for overdispersion we used the *overdisp* function (Gelman and Hill 2006). Residuals were checked with the *residuals* function in the DHARMa package (Hartig 2022). Significance of each interaction term was confirmed using log likelihood ratios. Where interactions were not significant, they were dropped from the model. Pairwise comparisons were obtained using the pairwise method and a p-value adjustment equivalent to the Tukey test in the emmeans package (Lenth 2022).

To examine differences in the total distance travelled during the trial we fitted a linear mixed-model using the lme4 package (Bates *et al.* 2015). The mean distance travelled was included as the response variable with grating size and grating orientation included as fixed effects and with an interaction term between them. The individual fish nested in trial number was set as the random effect. The data were log-transformed and residuals from the models met linearity and normality assumptions. Significance of the interactions were confirmed using log likelihood ratios. Pairwise comparisons among main effects were obtained using the pairwise method and a p-value adjustment equivalent to the Tukey test in the emmeans package (Lenth 2022). To examine distances travelled in the different tank regions we fitted a model with mean distance travelled as the response variable with grating size, grating orientation and tank region included as fixed effects with a three-way interaction specified. Significance and pairwise comparisons were undertaken as above.

## Results

*Disruption to visual motion cues caused by background patterns.*

To understand the how the size and orientation of background stripes effects motion dazzle, we compared motion cues of humbugs from the perspective of a potential predator. The motion cues created at the edge of the humbug varied significantly between motion direction vectors depending on the size and orientation of the grating ($X^2$= 656.8, p<0.001). As expected, there was no significant difference between vertical and horizontal gratings at the smallest grating of 0.05 cm, as the grating was not discriminable by the virtual predator. Visual inspection of motion over pixel position (top inset, Figures 2A and 2B) shows most motion arises from the internal stripes of the fish. The colour wheel (bottom inset, Figure 2A) indicates the direction of motion and the blue and red colours within the fish show that the motion cues are generated both forwards and backwards in relation to the direction of the fish.

The 0.25 cm gratings produced greater total motion. Visual inspection of motion over pixel position (top inset, Figures 2C and 2D) shows a large amount of background motion caused by the gratings being close to the visual threshold of the virtual predator. However, when the background motion colour was removed (bottom inset, Figures 2C and 2D), the outline and shape of the humbug is easily identified. At 0.25 cm size gratings, motion strength was significantly different at 17 out of 72 motion directions between the horizontal and vertical gratings. Greater motion was produced in the vertical motion directions in the vertical gratings, and vice versa in the horizontal gratings. The motion is dominated by the internal stripes of the humbug when viewed against horizontal gratings, whereas the motion at the edges of the humbug dominates when viewed against vertical gratings.

At a grating size of 1 cm, 5 out of the 72 motion directions were significantly different between the horizontal and vertical gratings (shown as red markers in Figure 2). Similar to grating sizes of 0.25 cm greater motion was produced in the vertical motion directions in the vertical gratings, and vice versa in the horizontal gratings. Visual inspection of motion over pixel position (top inset, Figures 2E and 2F) show the individual background stripes are easily resolved by the virtual predator. However, the vertical gratings cause a large amount of conflicting motion cues producing both motion in the direction travelled (red) and opposing it (blue), similar to the internal stripes of the fish. When the background motion colour was removed (bottom inset, Figures 2E and 2F) the outline of the humbug is broken up and is more conspicuous when viewed against the vertical gratings. Thus, vertical gratings above 0.25 cm, in which the individual gratings become discriminated would be expected to make it more difficult for a predator to detect the edges of the humbug and the apparent direction of movement.

### Anatomical measurements of visual acuity

Three retinas from the left eyes of fish were examined to establish visual acuity and areas of highest cell density. The mean peak RGC density across the three fish was 28,983 (± 2203) cells $mm^{-2}$ and the acuity estimate from this is 2.65 cpd.

### The effect of visual background spatial frequency on humbug behaviour

*Distance in relation to different gratings*

The three-way interaction of grating, orientation and time was not significant ($\chi^2 = 5.007$, $P = 0.286$) (Figure S2). The interaction with the co-variate of time was dropped and the final model specified an interaction between grating and orientation with time included as a covariate. There was a significant interaction between grating orientation and grating size ($\chi^2 = 31.299$, $P < 0.001$) but there was no effect of time ($\chi^2 = 0.507$, $P = 0.476$).

At the grating stripes widths of 0.05, 0.10 and 0.25 cm there was no difference in fish distance between the vertical and horizontal orientations. At the grating stripe width of 0.50 cm, fish remained significantly closer to the vertical grating (mean distance 13.9 cm) when compared with the horizontal grating (mean distance 19.5 cm; $t = 7.160$, $P < 0.001$). Similarly, when tested with a grating stripe width of 1.00 cm fish remained significantly closer to the vertical grating (mean 16.65 cm) than to the horizontal grating (mean distance 19.22 cm; $t = 2.148$, $P = 0.0312$). When comparing within different orientations, there was not a large amount of variation in the distance from the different horizontal gratings. However, fish moved significantly closer to the 0.50 cm vertical grating when compared to other vertically orientated gratings (Figure 3, see Table S1 & S2 for all pairwise comparisons and test statistics).

*Distance moved in relation to different gratings.*

The three-way interaction of grating, orientation and time was not significant ($\chi^2 = 9.4089$, $P = 0.06$) (Figure S3). The interaction with the co-variate of time was dropped and the final model specified an interaction between grating and orientation with time included as a covariate. There was a significant interaction between grating orientation and grating size ($\chi^2 = 90.216$, $P < 0.001$). There was a significant effect of time ($\chi^2 = 58.545$, $P < 0.001$) with fish significantly increasing their movement over time.

With the 0.25 cm and 0.50 cm gratings, the fish moved significantly more in response to the horizontal, rather than the vertical gratings. There was no difference in the total distance moved between the vertical and horizontal orientations for the 1 cm grating. When comparing within different orientations, fish moved significantly less in response to the 0.05 horizontal grating but there were no other differences in distanced moved in response to horizontal orientations. The fish moved significantly less in response to the 0.25 and 0.5 cm gratings when they were vertically orientated. There were no significant differences between distance moved for 0.05, 0.1 and 1 cm when vertically orientated (Figure 4, see Table S3 and S4 for pairwise comparisons).

Fish moved most in response to the 0.05 cm vertical grating, a mean (±1 SE) distance of 224.7 cm (± 3.6 cm). The fish also moved significantly more in response to the 0.10 cm vertical gratings (mean 91.06 cm ± 10.72 cm). The large standard deviation seen for the horizontal grating of 0.10 cm (Figure 5) was due to one fish that moved drastically more than all other trials. During one trial, the individual fish moved 14,000 cm over the five minutes. This was compared with an average distance moved of 714 cm for all other trials. This suggests some unknown, possibly external factors may have influenced its behaviour. Fish moved least in response to the vertical 0.25 cm, averaging 33.9 ± 2.3 cm (see Table 1 for all distances moved).

*Distance moved in different tank regions*

There was a significant effect of the three-way interaction between grating size, grating orientation and tank region (close, middle and far from the grating) ($X^2 = 179.52$, $P < 0.001$). Fish travelled significantly more in all tank regions in response to the 0.05cm vertical grating. Fish also travelled significantly more in response to the vertical grating at the spatial frequency of 0.10 cm when in the region closest to the grating (Figure 5, see Table S5 for pairwise comparisons).

## Discussion

In this study, we found that the striped pattern of the humbugs can generate confusing directional visual motion cues (motion dazzle). This motion dazzle is further enhanced when viewed against high contrast backgrounds which have similar spatial frequency and orientation to humbug stripes. At these backgrounds the edges of the humbugs are also harder to detect, particularly when the body stripes and background align. Furthermore, this study found humbugs can likely perceive the different visual backgrounds and modify their behaviour to optimise camouflage. Grating frequency and orientation influenced both the fish proximity to the grating background and the total distance moved by the fish. These findings suggest that the humbugs can resolve and distinguish between the different grating patterns and that this change in behaviour may indicate a behavioural adjustment to optimise camouflage.

*Importance of the background for motion dazzle*

The motion dazzle effect, in which motion cues are generated in the opposing direction to animal movement, occurred from both the striped humbug body pattern as well as the background, but only when gratings were orientated vertically and discriminable by the virtual predator. Comparable with previous modelling that assessed zebra patterning, the humbug stripes created similar confusing motion cues at all backgrounds tested (for detailed mechanisms of this, see

How and Zanker, 2014). However, as the aim of this paper was to understand how high contrast backgrounds interact with body pattern to further disrupt motion cues, the discussion has been largely focussed on how background complexity may enhance motion dazzle effects. Background gratings close to the limits of the virtual predator's visual system (0.25 cm), did not produce dazzle camouflage but rather created a large amount of motion in a single direction. At grating sizes larger than 0.25 cm where gratings are more easily resolved, motion dazzle is generated from both the humbug pattern as well as the background (see Figure 2F (1 cm vertical) for example). Motion, induced either from the movement of the humbug or the predator, plays an important role in camouflage particularly when viewed against complex backgrounds. A previous study found that prey with high-contrast stripes viewed on complex backgrounds become less visible to praying mantis predators than prey with background-matching patterns particularly when moving with speed (Umeton *et al.* 2019). We propose that at vertical grating sizes larger than 0.25 cm motion dazzle occurs both as a result of the humbugs striped body pattern and the high contrast background, which may maximise camouflage.

The motion analysis also revealed that vertical background gratings which are resolved by the virtual predator may have the added benefit of disrupting detection of the edge of the humbug. Of the gratings examined, the greatest disruption to the humbug edge was at the 1 cm grating, when the humbug body stripes were aligned with the vertical background gratings, making the humbug more inconspicuous. There are other striped reef fish that employ similar techniques for reducing edge detection, for instance the blue-spotted cornetfish (*Fistularia commersonii*) frequently changes body colouration to present dark bands when on visually complex coral habitats, reducing detectability through disruptive camouflage (Castillo and Tavera 2022). At background gratings of 0.25 cm, large amounts of motion were produced, and the edges of the humbug were detected and intact. Whilst this may suggest the humbug would standout against this grating, it is well established that background noise and complexity can enhance camouflage (Matchette *et al.* 2018; Rowe *et al.* 2021). Further behavioural work which compared capture rates of dummy humbugs by potential predators shows no heightened capture of humbugs against background grating sizes similar to 0.25 cm (Phillips *et al.* 2017). The results from this study suggest that vertical gratings above 0.25 cm (where individual gratings become discriminated) would be expected to make it more difficult for a predator to detect the edges of the humbug and the apparent direction of movement. It is likely that edge disruption is optimised when background gratings are closer to the width of the stripes on the humbugs body. Future studies should model motion cues of humbugs in nature viewed both in schools and against branching corals, which have a wider range of spatial frequencies than that of the humbug stripes.

*Behavioural modification in response to different background gratings*

The vital first step in evaluating how humbugs respond to different backgrounds was to understand their capacity to resolve the different grating sizes. The humbugs used in this study were found to have a peak anatomical visual acuity of 2.65 cycles deg$^{-1}$, which is relatively low when considering that the average acuity for 159 teleost fish is 8.4 (± 6.5) cycles deg$^{-1}$ (Caves *et al.* 2017). However, it is likely that the functional (behavioural) acuity is even lower, as studies on other small coral reef fishes show that behavioural acuity is typically around half that estimated from anatomical measurements (Champ *et al.* 2014; Parker *et al.* 2017). Given this relationship, we have estimated here that the behavioural acuity of the humbugs is likely around 1.1 cycles·deg$^{-1}$. This estimate of behavioural acuity can be used to determine how far away the humbugs can potentially resolve objects or patterns. A behavioural acuity of 1.1 cycles·deg$^{-1}$ means that one just-resolvable cycle will subtend an angle of 0.909 degrees. A grating cycle is one black and one white band, thus for the 0.50 cm grating (1 cycle = 0.1 cm) the stripes should become unresolvable by the humbug at a distance greater than ~6.25cm. Similarly, a grating stripe width of 0.25 cm (1 cycle = 0.50 cm) would be unresolvable at a distance greater than ~30 cm.

In this study, we observed significant differences in the position of free-swimming humbugs between the horizontal and vertical gratings of 0.5 cm and 1 cm stripe width, with fish moving closer to the vertical gratings at these spatial frequencies. Of all the gratings tested, these stripe widths are the most similar to the spacing of the black and white stripes on the humbug's body. Remaining close to these backgrounds offers the greatest camouflage, as detection of the edges of the humbug and the apparent direction of movement may be more difficult, as suggested by our motion models. Interestingly, the humbugs located slightly further away from the 1 cm vertical gratings compared to the 0.5 cm vertical grating, although still remained relatively close. This may suggest that the humbugs are able to adjust their distance to a cryptic background based on its spatial characteristics to optimise camouflage. As closer objects appear larger to a viewer, when the humbug moves further from larger gratings, hence closer to the potential predator, the striped pattern of the humbug better match the background from the perspective of the predator. However, understanding if this is indeed an approach to improve camouflage needs to take predator perception and stereo (3D) vision into account (Pike 2018). This observation also suggests that the humbugs did not remain closer to the larger gratings simply because the striped pattern looked like a grille that the fish could escape through, as the 1 cm grating would likely seem easier to fit through than the 0.5 cm grating.

Contrary to expectations, the fish moved significantly less in response to the vertical gratings that were most similar, or slightly smaller than the width of their stripes (0.25 and 0.5 cm). Our motion modelling suggests that background motion dazzle and edge disruption is most effective when gratings are larger than 0.25 cm. Given that the background motion dazzle effect is driven by the movement of the predator, at background gratings above 0.25 cm remaining still may improve camouflage by reducing motion parallax as well as conserve energy. Alternatively, it could be when the fish pattern is similar to the background that the fish remains still, relying on background or disruptive camouflage for protection. This is in line with the findings by Phillips *et al.* (2017), where disruptive camouflage and subsequent predation on static humbugs was less where the background was similar, or slightly smaller than the humbug stripes. It is likely that high contrast stripes have a dual benefit to animals through both disruptive camouflage and motion dazzle (Caro and Koneru 2021; Stevens and Merilaita 2011). Indeed, there are examples of animals such as longitudinally striped snakes which use the stripes to blend into their environment when still, but likely benefit from motion dazzle when rapidly fleeing predators (Wolf and Werner 1994). Studies that explore how humbugs respond to different backgrounds in the presence of predators are certainly warranted.

Humbugs moved a significantly greater distance overall in response to the smaller gratings, with the most movement observed in response to the 0.05 cm vertically orientated grating, and that this difference held for all regions of the test arena. At this higher spatial frequency, it is likely that the fish could not resolve the striped pattern while in areas of the arena far from the test grating (> 30 cm away) and this led to the change in behaviour. However, the fact that the fish moved significantly more only when the grating was oriented vertically suggests that they could resolve the pattern at some points within the tank and that this acquired knowledge created a persistent change in behaviour even in areas of the arena where it could not be resolved. The increase in movement by the fish may be a response to a perceived lack of background against which to conceal itself, leading to greater fear or exploratory behaviour in the relatively unfamiliar surrounds of the test arena. Alternatively, as demonstrated earlier in the motion modelling, the increased motion could represent a greater reliance on the internal contrast of the fish's striped body pattern to generate disruptive motion cues (see Figure 2A&B) (motion dazzle) that could momentarily confuse a potential predator in the final moments of a predatory strike. Interestingly, humbugs also moved more in response to the 1 cm gratings. Given background motion dazzle and edge disruption was highest for gratings between 0.25 and 1 cm we would expect humbugs to move similar distances when responding to these to these gratings.  However, it is possible that humbugs camouflage strategy is be more complex when the spatial frequencies are lower than their body stripes. Here we observed greater movement around the 1 cm gratings but remained significantly closer to the vertical compared to the horizontal gratings. Future studies should explore how humbugs modify behaviour around larger gratings, particularly those significantly larger than that of body stripes.

Taken together, these observations suggest that humbugs have evolved an understanding of how different backgrounds offer different levels of visual camouflage and modify their behaviour accordingly.  When detecting backgrounds with a spatial structure similar to their own striped body pattern, they move closer and reduce movement to remain cryptically camouflaged against the background and avoid revealing themselves to predators. But where cryptic camouflage is not attainable, they may use motion camouflage to confuse predators. Indeed, it is better not to be seen at all but if, and when required, the humbugs can rely on motion dazzle to avoid capture. In their natural environment it is likely that the humbugs use the branching coral colonies and other humbug fish to attain camouflage. While 2D striped backgrounds provide a good foundation for initial exploration, nature scapes and 3D complex structures should also be assessed to get a more comprehensive picture of how humbugs attain camouflage in their environment.

Humbugs are not the only animals that modify their behaviour according to the spatial structure of the background. Studies have demonstrated that shore crabs (*Carcinus maenas*) (Twort and Stevens 2023), Aegean wall lizards (*Podarcis erhardii*) (Marshall *et al.* 2016) and the least killifish (*Heterandria formosa*) (Kjernsmo and Merilaita 2012) prefer background habitats that help facilitate camouflage. In the case of the bark-resting moth (*Jankowskia fuscaria)* individuals have been found to increase camouflage after resting on tree bark by realigning and shifting their body position (Kang *et al.* 2015). Some other species of reef damselfish, *Pomacentrus moluccenis* and *Chromis viridis*, likely use a combination of body colouration and behaviour to communicate with conspecifics and maintain obscurity to predators (Marshall 2000). Likewise, some ground-nesting birds can assess their degree of camouflage and predation risk, and adjust their behaviour accordingly (Wilson-Aggarwal *et al.* 2016). In this study, we found that humbugs altered their behaviour by moving closer to backgrounds that potentially offered greater camouflage and may move more in response to less cryptic backgrounds, thereby utilising the striped pattern of their bodies to provide confusing motion cues. It has been proposed that irregular locomotion, animal orientation and erratic movement are also key in creating spurious motion signals (Cuthill *et al.* 2019; Hogan *et al.* 2016), and it is likely that confusion is more effective when there is movement in different directions (Von Mühlenen and Müller 1999). Future studies should investigate how animals can alter their behaviour to maximise the motion dazzle effect by broadening the scope of behaviours assessed.

## Conclusions

To our knowledge, this is the first study to demonstrate that striped animals alter their behaviour depending on the background to maximise the protection offered via motion dazzle. This is a baseline study, assessing uniform grating sizes in a controlled setting without consideration of ecological factors. However, nature is not uniform and it has been suggested that motion dazzle requires movement of both prey and predator and is likely to depend on the background environment (Franklin 2022). Several ecological factors including complexity in environment, attenuating properties of water and the presence of other similar damselfish could all influence the effects of motion dazzle. The humbug damselfish provides an accessible system with which to explore these questions of motion camouflage. It is likely that motion dazzle is not a ‘one-size-fits-all’ scenario which can lead to conflicting research findings.

Here we found where the background is more uniform (or in discriminable to a viewer) that the humbugs may rely on the confusing motion cues created by internal stripes but where the background is high contrast that they can rely more on disruption of edge detection, and confusing motion cues induced by both the background and body patterning. We suggest that future studies consider dazzle camouflage based on three components: 1) movement of the striped animal, 2) the background environment (complexity, movement and lighting) and 3) the viewer’s visual system and capacity. These findings will provide greater insights into the evolution of behaviour and colouration. This is exciting research that increases our understanding of the motion dazzle phenomenon and demonstrates the need for greater understanding of the interaction between pattern and motion.

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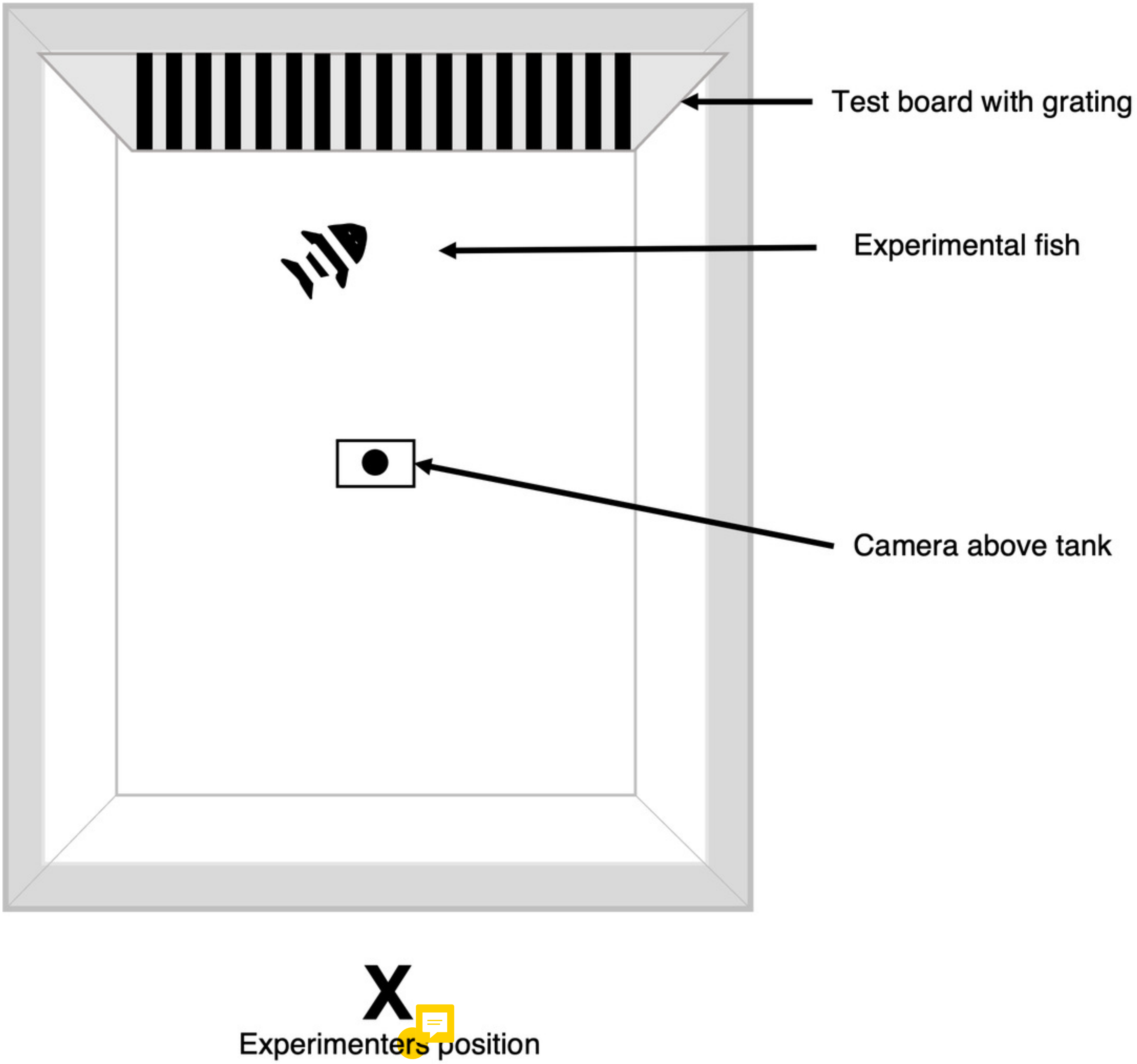
# Figure 1

Experimental Arena

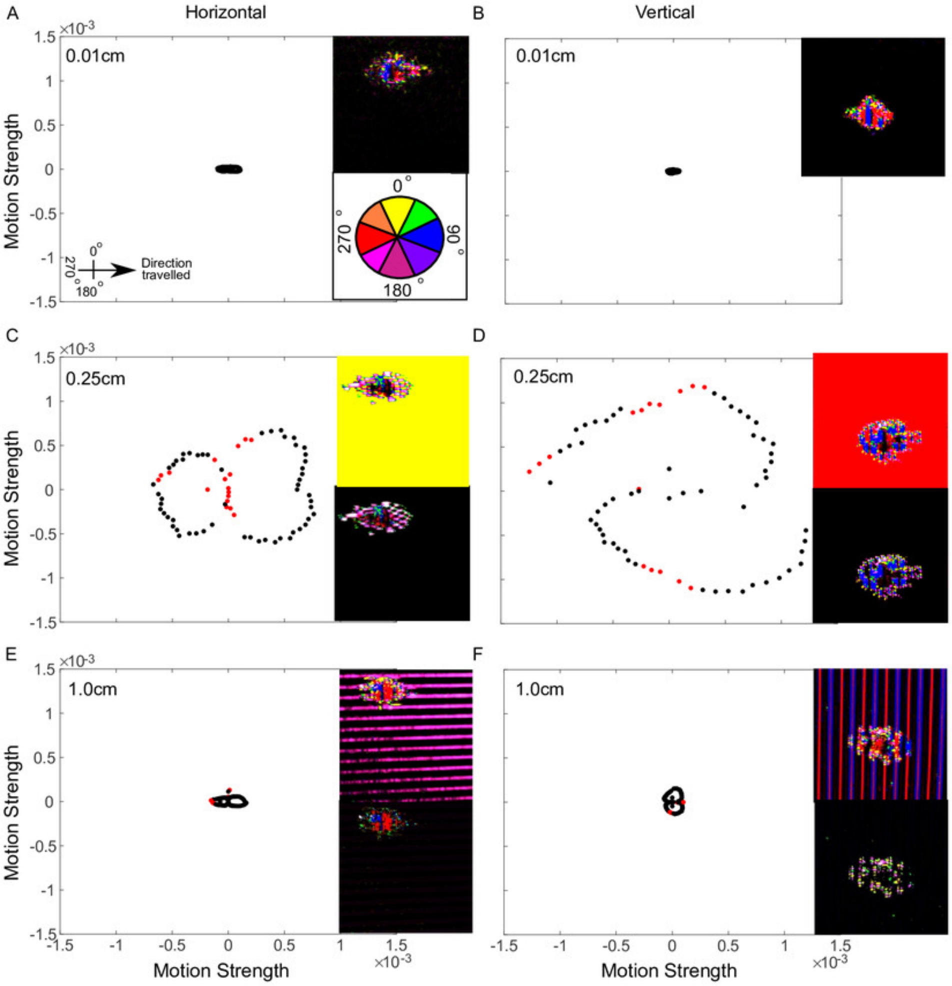
Schematic of the experimental arena used for the humbug behavioural trials



# Figure 2

Direction and strength of motion cues from the 2DMD model

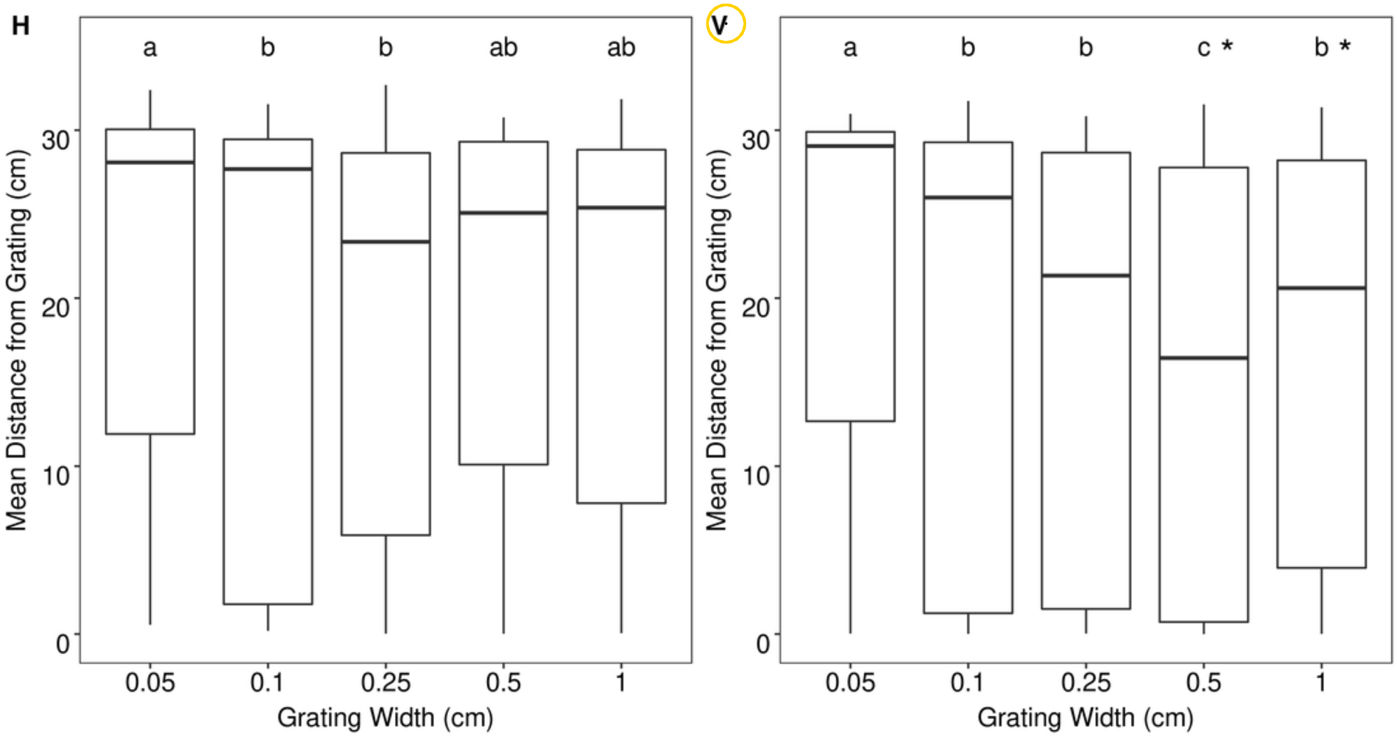
Direction and strength of motion cues over 360 degree directions from the 2DMD model of a the humbug viewed against background gratings sizes of 0.01cm (A,B), 0.25cm (C, D) and 1cm (E,F). Panels (A, C, E) show motion strength for horizontal gratings and (B, D, F) for vertical gratings. Red open dots indicate angle vectors that were significantly different between the horizontal and vertical grating of the same spatial size and black dots were not significantly different. Units are an arbitrary scaling value. Inlay show example frames of motion direction over pixel location, where pixel colour corresponds to the motion direction in the colour wheel (inlay A).


A
Horizontal
B
Vertical
0.01cm
0.01cm
0°
90°
180°
270°
Direction travelled
C
D
0.25cm
0.25cm
E
F
1.0cm
1.0cm
Motion Strength
Motion Strength
×10⁻³
-1.5
-1
-0.5
0
0.5
1
1.5

# Figure 3

## Distance From Grating

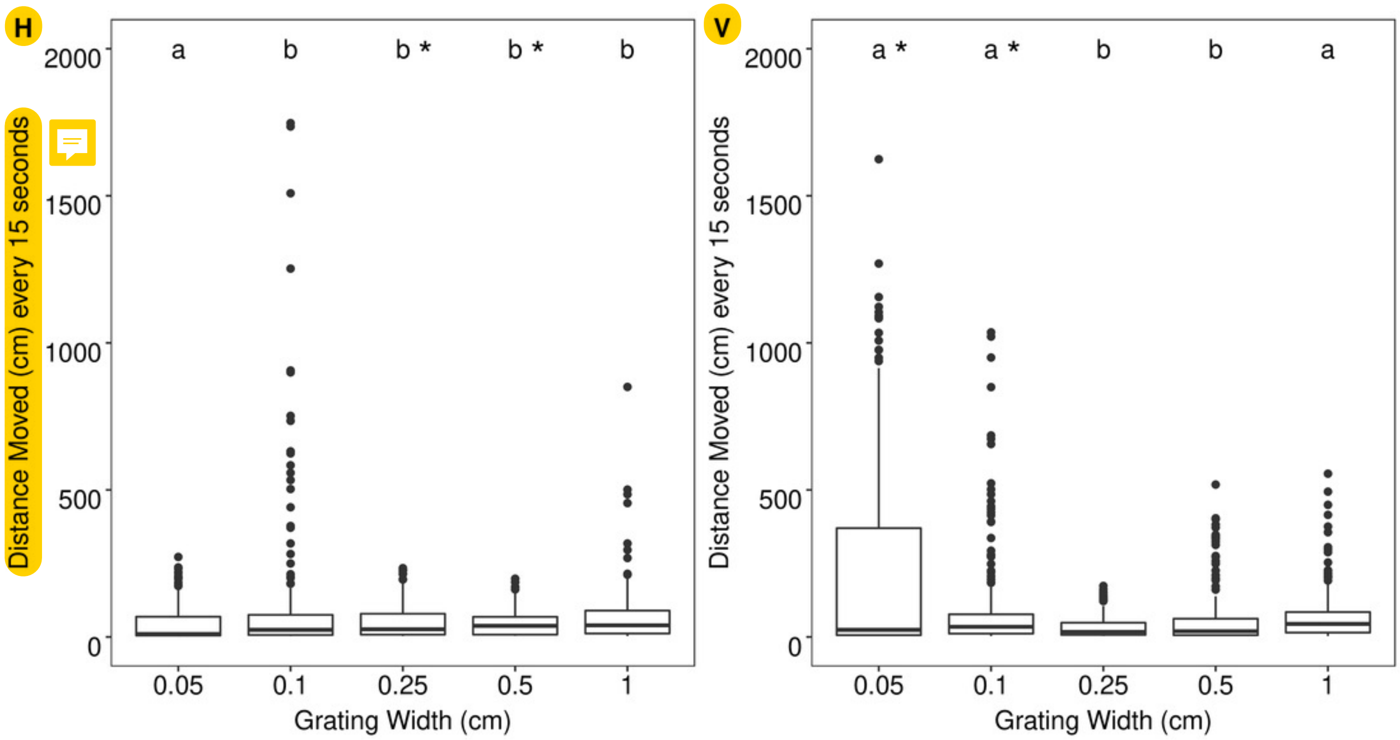
Boxplot showing the median distance that humbugs were positioned in relation to the different gratings. *Indicate significant differences (P < 0.05) between horizontal gratings compared to the vertical grating of the same size. Letters indicate significant differences (P < 0.05) between gratings of the same orientation.



# Figure 4

Distance Moved

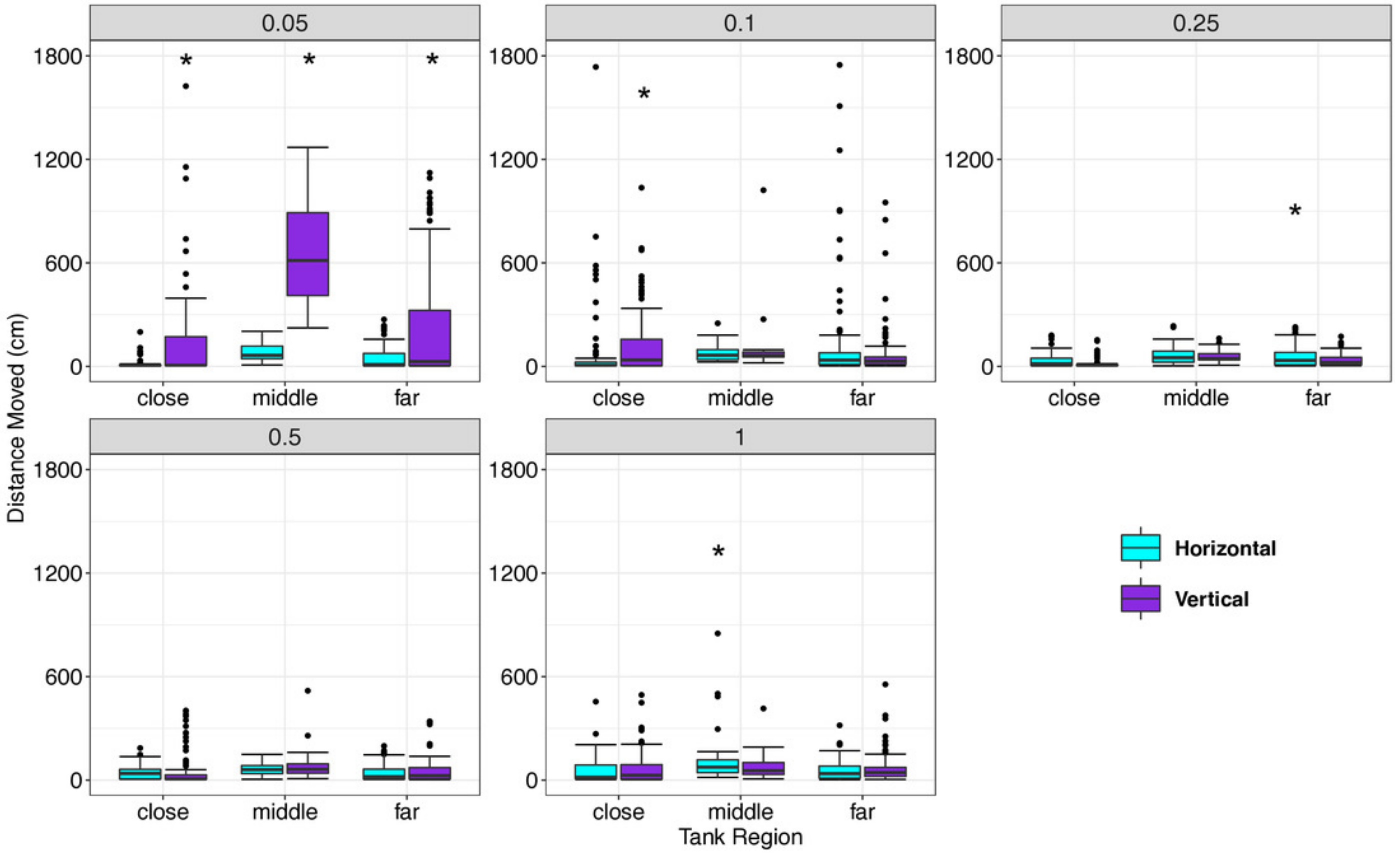
Boxplot showing the median distance that humbugs moved in relation to the different gratings. *Indicate significant differences between horizontal gratings compared to the vertical grating of the same size (*P < 0.05, **P < 0.005). Letters include differences between gratings of the same orientation.



# Figure 5

Distance Moved in Different Tank Regions

Mean (+/- SE) for the mean distance that humbugs moved in response to the different gratings. *Indicate significant differences between horizontal gratings compared to the vertical grating of the same size



# Table 1(on next page)

Distance Moved Summary

Average distances moved (cm) every 15 seconds in response to the different grating widths and orientations.

| | **Grating size (cm)** | **N** | **mean** | **sd** | **se** |
|---|---|---|---|---|---|
| H | 0.05 | 240 | 42.84 | 56.47 | 3.64 |
| | 0.1 | 240 | 99.06 | 240.41 | 15.52 |
| | 0.25 | 240 | 49.65 | 54.36 | 3.51 |
| | 0.5 | 240 | 46.37 | 42.83 | 2.76 |
| | 1 | 240 | 64.66 | 89.2 | 5.76 |
| V | 0.05 | 240 | 224.67 | 332.4 | 21.46 |
| | 0.1 | 240 | 91.06 | 166 | 10.72 |
| | 0.25 | 240 | 33.95 | 35.63 | 2.3 |
| | 0.5 | 240 | 53.87 | 86.47 | 5.58 |
| | 1 | 240 | 70.01 | 85.49 | 5.52 |