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RESEARCH ARTICLE

Null point of discrimination in crustacean polarisation vision

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ABSTRACT

The polarisation of light is used by many species of cephalopods and crustaceans to discriminate objects or to communicate. Most visual systems with this ability, such as that of the fiddler crab, include receptors with photopigments that are oriented horizontally and vertically relative to the outside world. Photoreceptors in such an orthogonal array are maximally sensitive to polarised light with the same fixed e-vector orientation. Using opponent neural connections, this two-channel system may produce a single value of polarisation contrast and, consequently, it may suffer from null points of discrimination. Stomatopod crustaceans use a different system for polarisation vision, comprising at least four types of polarisation-sensitive photoreceptor arranged at 0, 45, 90 and 135 deg relative to each other, in conjunction with extensive rotational eye movements. This anatomical arrangement should not suffer from equivalent null points of discrimination. To test whether these two systems were vulnerable to null points, we presented the fiddler crab *Uca heteropleura* and the stomatopod *Haptosquilla trispinosa* with polarised looming stimuli on a modified LCD monitor. The fiddler crab was less sensitive to differences in the degree of polarised light when the e-vector was at −45 deg than when the e-vector was horizontal. In comparison, stomatopods showed no difference in sensitivity between the two stimulus types. The results suggest that fiddler crabs suffer from a null point of sensitivity, while stomatopods do not.

KEY WORDS: Polarisation distance, Fiddler crab, Mantis shrimp, Discrimination threshold

INTRODUCTION

The polarisation of light provides an independent channel of visual information that some animals use to discriminate objects or for communication (Shashar et al., 1998; Wehner and Labhart, 2006; Glantz, 2007; Chiou et al., 2011; How et al., 2012; Temple et al., 2012; Marshall et al., 2014b). Visual systems that detect and identify objects using polarised light rely on comparative processing of the signals generated by polarisation-sensitive photoreceptors distributed across parts of the visual field. This is a process somewhat analogous to colour vision and adds to the well-known uses of the polarisation of light for navigation, orientation and habitat localisation (Wehner, 1976; Schwind, 1983; Labhart and Meyer, 1999). Animals may use this channel to improve the detection of objects through veiling light or glare (Schechner and Karpel, 2005; Alkaladi et al., 2013), to enhance the contrast of transparent prey (Shashar et al., 1998; Tuthill and Johnsen, 2006; but see Johnsen et al., 2011), to break camouflage (Shashar et al., 2000; Jordan et al., 2012; Temple et al., 2012) or to allow the detection of polarised body patterns for communication (Shashar et al., 1996; Marshall et al., 1999).

Object-based polarisation vision systems that have been studied to date are restricted to cephalopods and crustaceans. In these examples, the photoreceptors that act as polarised light detectors in the eye are aligned horizontally and vertically (Tasaki and Karita, 1966; Shaw, 1969; Waterman, 1981), forming two channels of polarisation sensitivity. Furthermore, these animals maintain precise alignment of their eyes relative to the visual world around them (Zeil et al., 1986; Talbot and Marshall, 2011), thus ensuring that the polarisation-sensitive receptors remain horizontal and vertical relative to the visual scene. Stomatopods are an exception. These crustaceans possess an anatomically complex array of polarisation receptors with e-vector sensitivities arranged at 0, 45, 90 and 135 deg relative to each other (Marshall et al., 1991; Kleinlogel et al., 2003). They also have receptors in rows 5 and 6 of the mid-band of the eye that are sensitive to left- and right-handed circularly polarised light (Chiou et al., 2008; Roberts et al., 2009). In addition to this anatomical complexity, these animals regularly rotate their eyes around the axis of view (Cronin et al., 1988; Land et al., 1990), thereby altering the orientation of the polarisation detectors relative to the outside world. Therefore, such a system is likely to have very different properties from the more common two-channel polarisation vision system.

An understanding of the underlying anatomy of polarisation vision opens the possibility for modelling an animal's sensitivity to polarised light cues. One approach, inspired by analogous systems in colour vision (Vorobyev and Osorio, 1998), is to simulate the proposed neural architecture of early visual processing. For simple systems, two orthogonally oriented polarisation channels are assumed to have opponent connections (similar to dichromatic colour vision models) and produce a single level of neural activity (Bernard and Wehner, 1977). Subsequent comparison of this activity between two different parts of the visual field (one viewing an object and one viewing the background) results in a measure of polarisation contrast, termed 'polarisation distance' (How and Marshall, 2014). Presumably, this output is modulated at some stage by intensity variations, so that intensity signals and polarisation signals are fused into a single measure of contrast; however, this falls beyond the scope of the present study. Such models allow us to predict how well animals will perform in a discrimination task, assuming that they are using a given neurosensory apparatus.

Previous studies using this modelling approach identified the problem of potential ambiguities and null points in two-channel polarisation vision systems (Bernard and Wehner, 1977; How and Marshall, 2014). For example, polarised light cues with e-vector axes oriented at +45 or −45 deg stimulate horizontal and vertical polarisation receptors equally, and so are indistinguishable from each other and from unpolarised light of the same intensity. This is widely accepted as a problem for two-channel polarisation vision, yet, with the exception of some associative learning experiments with cephalopods (Moody and Parriss, 1961; Shashar and Cronin,

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1996), this has not been investigated in detail at the behavioural level. To this end, we used a modified LCD monitor (Glantz and Schroeter, 2006; Pignatelli et al., 2011; How et al., 2012; Temple et al., 2012) to present polarised looming stimuli to the two-channel polarisation vision system of the fiddler crab *Uca heteropleura* (Smith 1870) and to the dynamic, multi-channelled polarisation vision system of the stomatopod *Haptosquilla trispinosa* (Dana 1852). These looming stimuli differed in the degree of polarisation, while the overall light intensity remained constant. We compared the response probability of these two species to stimuli that fell either within or outside a predicted null point of discrimination for a two-channel polarisation vision system.

RESULTS

Fiddler crabs *U. heteropleura* responded to polarised looming stimuli in a number of ways, similar to those reported for *Uca vomeris* (How et al., 2012), including cessation of all movement (freeze), drawing of the legs or claw closer to the body (tuck), a jerk or twitch, and a sprint. A small number of crabs responded to the zero contrast stimulus (Fig. 1A, dotted line) as a result of coincidental changes in behaviour during stimulus presentation. Therefore, these estimate the probability of false positive responses during the experiment. A significant response was recorded for crabs viewing the horizontally polarised stimuli down to differences in degree as low as 8% [linear mixed effects model (LMER) for 8% stimulus versus background: family binomial, n=22 crabs, d.f.=1, χ^2=18.7, P<0.001; Fig. 1A, circles, Table 1]. However, when the degree contrast stimuli were presented at −45 deg, the probability of response was lower, only becoming significant at contrasts over 23% (LMER for 23% stimulus versus background: family binomial, n=22, d.f.=1, χ^2=9.0, P=0.0028; Fig. 1A, triangles, Table 1).

Results from the polarisation distance model suggest that the stimuli presented to fiddler crabs with an e-vector of 0 deg generate a higher level of contrast in the two-channel polarisation vision system than stimuli presented at −45 deg (Fig. 1B, compare circles with triangles). However, if we incorporate varying levels of eye alignment error into the model, we see an overall drop in the difference between the 0 deg and −45 deg stimuli (Fig. 1B, grey lines). This is reflected in the modelled just noticeable difference (JND) values for polarisation distance (Table 1). For the case of perfect eye alignment and order within the photoreceptors, the polarisation JND values are an order of magnitude higher for the 0 deg stimuli (0.07) versus the −45 deg stimuli (0.006). An error in eye alignment of ~10 deg is required for the polarisation distance JNDs for both 0 and −45 deg stimulus types to be roughly equal (0.05 and 0.04, respectively).

Table 1. Just noticeable difference (JND) values for the two stimulus orientation conditions (0 and −45 deg) presented as recorded degree contrast and calculated polarisation distance

JND value	0 deg stimuli	−45 deg stimuli
Degree contrast (%)	8	23
Polarisation distance	0.07	0.006
Polarisation distance (5 deg error)	0.06	0.02
Polarisation distance (10 deg error)	0.05	0.04
Polarisation distance (15 deg error)	0.04	0.06

The 5, 10 and 15 deg error values represent the calculated polarisation distance for incremental errors in eyestalk misalignment.

In comparison with fiddler crabs, the stomatopod *H. trispinosa* showed a very different pattern of response to the polarised stimuli presented in this study. Firstly, counter to expectations, *H. trispinosa* rarely responded to the looming stimulus by retreating into its burrow, as it would to an approaching predator. Rather than a locomotion-based behavioural change, the animals responded by making saccadic gaze shifts with one or both eyestalks towards the looming stimuli. These could be distinguished from the majority of general eye movements by their high speed and directionality – movements that are commonly observed in target tracking stomatopods (Cronin et al., 1988; Marshall et al., 2014a). Secondly, there was no statistical difference in the stomatopods' response probability between the two stimulus types (0 and −45 deg) (ANCOVA, F=0.208, P=0.66; Fig. 1A, bottom, compare fitted lines). Finally, the sensitivity of stomatopods to small degree contrasts was poor in comparison to that of the fiddler crabs. High response probabilities were not observed for *H. trispinosa* until the contrast in the degree of polarisation was over 50%, far greater than the 8% difference discriminated by *U. heteropleura*.

DISCUSSION

This study provides a strong indication of a behavioural null point in polarisation sensitivity. The fiddler crab *U. heteropleura* was far

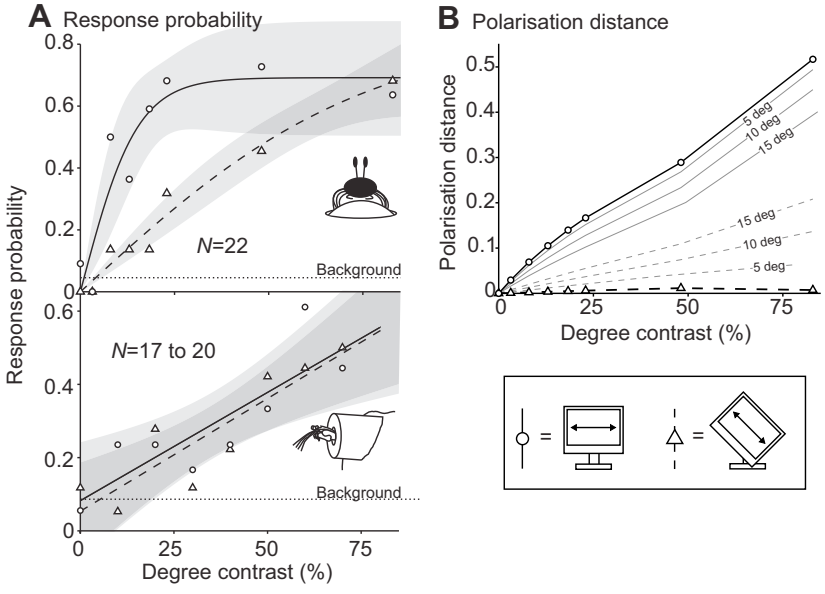

Fig. 1. Response probability to polarised stimuli. (A) Response probability of the fiddler crab *Uca heteropleura* (top) and the stomatopod *Haptosquilla trispinosa* (bottom) to horizontal (circles) and −45 deg (triangles) stimulus e-vector orientations (see diagram, bottom right). Curves represent the fitted hyperbolic tangent (top) or straight line (bottom) for each set of data. Both fitted lines were generated using the MATLAB curve fitting toolbox. Grey shading represents the 95% confidence interval for the fitted curves. Background dotted line indicates the probability of false positive responses. (B) Polarisation distance modelled for the fiddler crab stimuli and for each monitor orientation. Grey lines indicate the effect of incremental levels of crab eye misalignment relative to the monitor (5, 10 and 15 deg).

more sensitive to differences in the degree of polarised light when presented at e-vectors of 0 deg than at −45 deg, two stimulus types that are predicted to elicit the maximal and minimal amount of visual contrast, respectively. The level of diminished response at −45 deg, compared with a complete null response, is entirely consistent with a degree of misalignment between or across the eyes. In comparison, the stomatopod *H. trispinosa* – with its multi-channel and rotationally dynamic polarisation vision system – did not show evidence for a null point of discrimination at an e-vector angle of −45 deg. These results also provide evidence that crustaceans respond not just to differences in the e-vector orientation of polarised light (Marshall et al., 1999; Glantz and Schroeter, 2007; How et al., 2012), but also to small differences in the degree of polarised light.

What might be the functional consequences of this polarisation null point for fiddler crabs in their natural environment? At present, it is unknown exactly how the polarisation and intensity channels are integrated, so it is difficult to interpret the full functional relevance of their polarisation sensitivity. In related crustaceans, the polarisation system is thought to act as a contrast enhancer for the intensity channel, and may also be related to movement detection (Glantz, 2001; Glantz and Schroeter, 2006), so it is in this context that we frame our discussion. One of the main polarisation cues in the fiddler crab's mudflat environment is likely to be found in the area of damp mudflat in the direction of the sun. Here, most reflected light will be roughly horizontally polarised (Zeil and Hofmann, 2001; How and Marshall, 2014). The horizontal/vertical receptor organisation of the fiddler crab could therefore be adapted for detecting small changes in the degree of polarisation in this region. This could be used for enhancing the contrast of conspecific crabs against the mudflat surface, and could therefore be thought of as a 'matched filter' for this environment (Wehner, 1987; Zeil and Hofmann, 2001; Alkaladi et al., 2013; How and Marshall, 2014). However, there are several sources of non-horizontally polarised cues. For example, the sky polarisation field spans the entire celestial hemisphere (although it will often be obscured by clouds or large objects on the horizon) and the e-vectors of this polarised light are oriented in parallel to a circular band 90 deg from the sun's position (Labhart and Meyer, 2002). Therefore, for certain solar positions the polarised light field will be oriented at ±45 deg across large parts of the sky. This could have an impact on the use of polarised light for enhancing the contrast of airborne predators in these parts of the sky. However, it is unclear whether polarised light plays a role in predator surveillance in the wild. There have also been suggestions that fiddler crabs may use their acute polarisation vision for detecting subtle variations in the cuticle surface of conspecifics (Zeil and Hofmann, 2001). Light reflected from damp crab cuticle is likely to occupy a whole range of e-vector angles, many of which may fall at or near the null point of discrimination.

In conjunction with previous modelling attempts (How and Marshall, 2014), our current results imply that the fiddler crab's polarisation vision system is most sensitive to small differences in the degree of polarised light when the e-vector angle is close to horizontal. This suggests that the horizontal/vertical arrangement of the polarisation receptors is optimally adapted for discriminating small differences in the degree of polarised light (caused, for example, by a conspecific crab) against the horizontally polarised mudflat background (Zeil and Hofmann, 2001; How and Marshall, 2014). However, there is another related explanation for this kind of arrangement. The fiddler crab *U. vomeris* was recently found to have differences in the anatomical arrangement between the two polarised light channels, suggesting that, in the equatorial region of the eye, each ommatidial unit is more sensitive to vertically polarised light than to horizontally polarised light (Alkaladi et al., 2013). This would have the consequence of screening out some of the horizontally polarised glare reflected from the mudflat, analogous to the effect of wearing polarised sunglasses. This asymmetrical sensitivity to vertically polarised light is only possible if the receptors have a horizontal/vertical arrangement relative to the outside world. It must, however, be noted that this anatomical organisation would not affect the location of the polarisation null point, as long as the sensitivity maxima remain horizontally and vertically oriented.

An alternative explanation that must be addressed is that the fiddler crabs may be responding differently to the two stimulus types according to how realistic they seem. The horizontal stimulus set simulates a polarisation degree contrast viewed against a horizontally polarised background, a situation that may be common in the fiddler crabs' mudflat environment. It may be argued that the −45 deg stimulus set represents a less natural situation (although see discussion above), leading to a reduced response from the fiddler crabs. This hypothesis leads to the prediction that vertically oriented stimuli (even more unusual in the fiddler crab's environment) should elicit even lower response probabilities than the −45 deg set. We present preliminary data from a reduced sample (n=6 crabs) that provide a good indication that this is not the case (supplementary material Fig. S1). Fiddler crabs presented with vertically oriented degree contrasts responded with a similar probability as they did to horizontally oriented stimuli.

As a final note on the experimental methods used for the fiddler crab part of this study, we recorded an interesting discrepancy between our behavioural results and the predictions of the polarisation distance model. Our results initially suggested that fiddler crabs were more sensitive to polarised objects at the −45 deg null point than the polarisation distance model predicts. The polarisation distance JND values modelled for these −45 deg stimuli were 10 times lower than for the 0 deg stimuli (0.006 and 0.07, respectively; Table 1), implying that the fiddler crabs should not have been able to detect these stimuli. However, when we took into account the possible effect of slight misalignment between the crab eyes and the monitor (Fig. 1B, grey lines), this discrepancy could largely be explained. An alignment error of ~10 deg resulted in modelled polarisation distance values that were approximately equal for 0 and −45 deg JND points (0.05 and 0.04, respectively; Table 1), and this level of misalignment was certainly possible in the experimental apparatus. In their natural environment, fiddler crabs use the horizon as a visual cue to maintain the alignment of their eyestalks (Zeil et al., 1986). In our experiment, the false horizon presented to the test animal was only displayed on three of the four surrounding monitors and the close distance of these monitors (220 mm) suggests that small misplacements of the virtual horizon could have large effects on its perceived location. Future research of this kind should take extra precautions to reduce these types of alignment errors.

A second explanation, which cannot be discounted, for this higher-than-expected sensitivity to stimuli at −45 deg is that the fiddler crab may use some form of local comparison between imperfectly oriented polarisation receptors in adjacent regions of the eye to enhance contrast to such cues. The current polarisation distance model only attempts to represent visual input from two single sets of opponent receptors, and so may miss any effects of regional processing across the eye (Glantz and Schroeter, 2007). Future anatomical, electrophysiological and behavioural studies will be needed to investigate this fully.

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In comparison to fiddler crabs, the stomatopod *H. trispinosa* showed no evidence of a null point in polarisation sensitivity at an e-vector of −45 deg: these animals responded with equal probability to polarised stimuli at 0 deg and at −45 deg. This is not surprising given that anatomical evidence shows that these animals have a complex polarisation anatomy. Although these species do possess two-channel polarisation vision systems across the majority of the eye, the receptors are oriented differently in distinct regions. Firstly, orthogonal receptors in the eye's dorsal hemisphere tend to be shifted in orientation by 45 deg from orthogonal receptors in the ventral hemisphere, providing two pairs of differently oriented polarisation channels in regions with overlapping visual fields (Marshall et al., 1991). Secondly, these species make frequent eye rotations around the axis of view, thus altering the orientation of the polarisation receptors relative to the outside world (Land et al., 1990). Thirdly, stomatopods do not maintain a stable body orientation as they crawl or swim in their complex three-dimensional habitat, and changes in body position do not seem to be compensated by adjustments in eye alignment (as is the case for fiddler crabs). For example, *H. trispinosa* will often rest in a tilted, or occasionally upside-down, position in the entrance of the burrow, thus affecting the orientation of their polarisation vision system relative to the outside world.

There are several proposed explanations for the extreme mobility of stomatopod eyes, including their use for target acquisition and tracking, and for scan movements associated with the colour and polarisation sensitivity in the mid-band region (Cronin et al., 1988; Land et al., 1990; Thoen et al., 2014; Marshall et al., 2014a). Such movements have strong implications for their polarisation vision system, which, as a result, does not appear to suffer from the null points experienced by stabilised two-channel receptor arrangements. This could be of particular relevance for communication in stomatopods. Many species (including *H. trispinosa*) use linearly polarised body patterns (Chiou et al., 2011) and, given that stomatopods are highly mobile animals, these patterns could potentially be viewed from a variety of orientations. A rotatable multi-channel polarisation system is likely to be more efficient at detecting these signals than fixed two-channel arrays, which may miss those signals falling in or near the null point of discrimination. Eye anatomy and movement may therefore have evolved, in part, to allow the detection of linearly polarised signals regardless of their orientation. There is also the tantalising possibility that stomatopods may use eye rotations around the visual axis as a method of gain control, for optimising the polarisation contrast between an object of interest and the background. However, this has yet to be demonstrated.

Finally, it is interesting to note that fiddler crabs and stomatopods differed markedly in their overall sensitivity to the polarised light cues. Fiddler crabs showed clear responses to differences in the degree of polarised light as low as 8%. In comparison, stomatopods only responded well to stimuli with contrasts greater than 50%. Also, the two different species differed clearly in the types of responses that these stimuli elicited. Fiddler crabs exhibited clear anti-predator behaviour, including sprints away from the stimulus, freezing and/or tucking in the limbs. Stomatopods, in contrast, simply glanced at the looming stimulus as if it was a prey object of interest. The difference in sensitivity could be explained by the apparent difference in behavioural context between fiddler crabs and stomatopods. The former were clearly attempting to avoid predation and so were highly motivated to respond to looming stimuli, while the latter would simply have been looking for food items from the safety of the home burrow. Another possibility is that the results demonstrate an actual difference in the behavioural sensitivity of these two animal groups to polarised cues. Fiddler crabs are known to respond to very weak intensity cues in the dorsal part of the visual field, corresponding to the retinal position of small avian predators such as terns (Smolka and Hemmi, 2009; Smolka et al., 2011; Smolka et al., 2013). Given that polarisation is likely to act as a contrast enhancer for the intensity channel, it would seem logical that these animals should also respond well to small differences in polarised light. In contrast, stomatopods may use their polarisation sensitivity primarily for detecting signals on the cuticle of conspecifics, body patterns that tend to be very strongly polarised. Perhaps the relatively low polarisation sensitivity observed in the stomatopod is a consequence of some level of filtering of the polarisation information, a process that may be important for simplifying subsequent processing steps necessary for this unique scan vision system. Similar information reduction steps also seem to occur early in the stomatopod colour vision system (Thoen et al., 2014).

MATERIALS AND METHODS

Male fiddler crabs (n=22) of the species *U. heteropleura* were collected from the mudflats at the Pacific entrance to the Panama Canal (8°56′56.3″N, 79°34′24.3″W) in April 2013 and were transported to an outdoor flow-through aquarium facility at the Naos Island Laboratories of the Smithsonian Tropical Research Institute. The crabs were housed in clear perforated plastic containers for a period of 1–5 days and were fed daily with fish flakes (TetraMin, Tetra, Germany). Individual crabs were presented with polarised light stimuli using methods similar to those used previously (How et al., 2012). A wire hinge was attached to the animal's carapace with cyano-acrylate glue, and connected to a metal hanger, which tethered the animal on top of a 200 mm diameter Styrofoam ball (Fig. 2A, treadmill). The treadmill was suspended on a cushion of air provided by a hairdryer

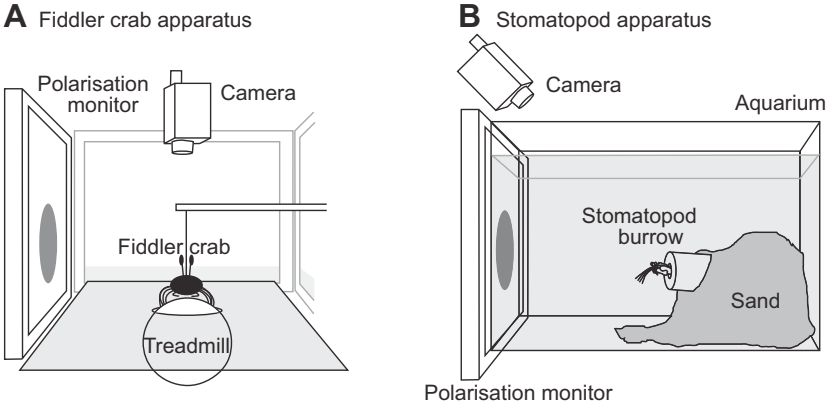

Fig. 2. Experimental apparatus for the presentation of polarisation stimuli. (A) Fiddler crab apparatus [according to How et al. (How et al., 2012)]. The animal was tethered above a treadmill and stimuli were presented on a modified LCD monitor (left). (B) Stomatopod apparatus. Unrestrained animals occupying a burrow within an aquarium partition were presented with stimuli on a modified LCD monitor pressed against the wall of the aquarium (left).

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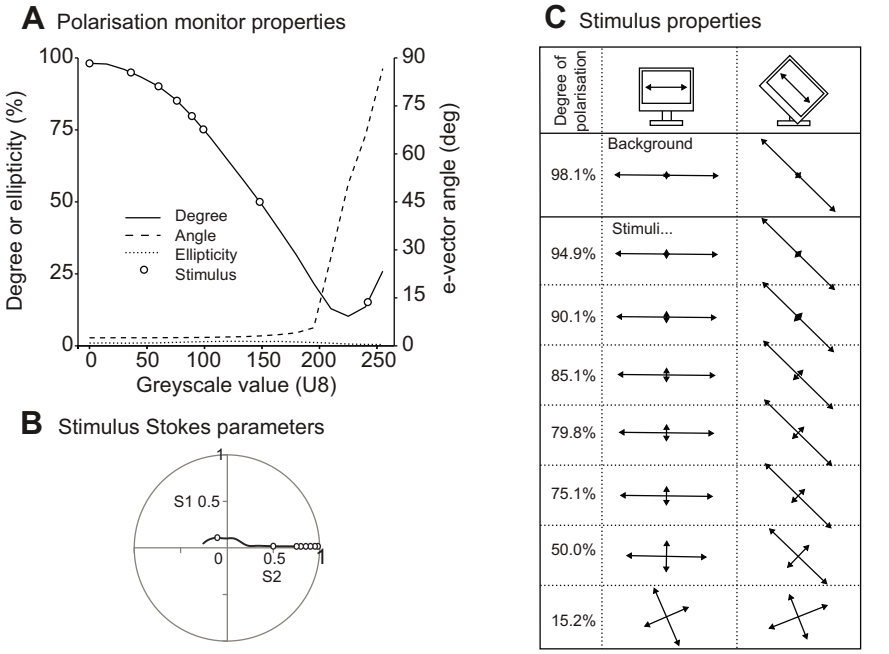

Fig. 3. Polarisation properties of the modified LCD monitor. (A) Degree of polarised light (solid line, *y*-axis left) changed gradually with greyscale value addressed to the monitor (*x*-axis), while e-vector angle (dashed line, *y*-axis right) and ellipticity (dotted line, *y*-axis left) remained constant (at ~0 deg and ~0%, respectively) between 0 and 200 U8 values of the greyscale range. White circles represent the exact stimulus set used in the fiddler crab experiment. (B) Representation of monitor output in Stokes coordinates (S1 and S2). Note that the *z*-axis (Stokes parameter S3 – ellipticity) remained close to zero so a 2D projection in the equatorial plane of the Poincaré sphere captured the relevant information. Solid line, full range of monitor output; white circles, fiddler crab stimuli. (C) Illustration of the polarisation properties of all stimuli used in the fiddler crab experiment and the background against which they were presented. The relative length and angle of the black arrows represent the degree and angle of polarised light, respectively. These are illustrated for the two stimulus sets: horizontal (0 deg; left) and diagonal (−45 deg; right). The stimuli presented to the stomatopods had similar polarisation properties, varying only in the degree values chosen. Left-hand column provides the actual degree of polarisation value.

(Venezia 2400, VS Sassoon, P&G and Conair) allowing the crab to walk freely in any direction (causing the treadmill to rotate beneath it). The treadmill was surrounded by four LCD monitors (1905fp, Dell, Round Rock, TX, USA), three of which were unmodified and displayed a white sky background and a dark brown floor with a flat horizon, level with the top of the treadmill. The fourth monitor had the front polarising filter removed so that the monitor output could vary in polarised light only. This LCD monitor uses an in-plane switching architecture; as the greyscale level addressed to the monitor varies from black [U8=0 (on an 8 bit scale)] through to grey (U8=200), the degree of linearly polarised light declines, while intensity, e-vector angle (0 deg) and ellipticity (~0%) remain constant (Fig. 3A). This allowed us to present the animals with a range of dynamic visual stimuli varying only in the degree of polarised light (Fig. 3). It must be noted that unmodified LCD monitors also generate polarised light. In the case of the three Dell monitors used in this experiment, this was 100% polarised and oriented in a vertical direction, but remained unchanged for the duration of the experiment and so had no influence on the fiddler crabs' response.

Adult male and female stomatopods of the species *H. trispinosa* were collected from Lizard Island lagoon on the Great Barrier Reef, Australia (14°40′40.8″S, 145°26′48.1″E) in July 2013. Animals were housed separately in artificial burrows under natural illumination in a flow-through aquarium system at the Lizard Island Research Station. Individuals were transferred to a 250×150×200 mm (length × width × height) partition within a test aquarium, and the animal's burrow was placed centrally facing the small glass front of the partition (Fig. 2B). The aquarium was constructed of non-tempered glass, and did not affect the transmission of polarised light in any way. Animals were allowed to acclimatise for >10 min. Then, prior to testing, a polarised LCD monitor (1905fp, Dell – same as above) was placed against the front glass panel.

To test whether the threshold response of crabs and stomatopods to the degree of polarised light depended on its e-vector axis, we presented the animals with a range of stimuli on the monitor in the standard position or with the monitor rotated by −45 deg (Fig. 3B). These are hereafter referred to as the 0 and −45 deg stimulus sets. We use the term 'degree' to refer to the proportion of polarised light, and 'degree contrast' as the difference in the amount of polarised light between stimulus and background. In this manuscript we use the percent scale to represent both of these measures, varying from 0 to 100%. For fiddler crabs, each animal was presented with each stimulus condition twice, and monitor orientation was alternated so that half the animals received the 0 deg stimulus set first, and the other half received the −45 deg stimulus set first. The stimulus consisted of a looming circle that expanded over 1 s from 0 to 145 mm diameter at a distance of 220 mm from the crab (thus occupying 36.5 deg of the visual field when fully expanded). The contrast in the degree of polarised light between the looming stimulus and the background was set to the following values, which were presented in a fully randomised order: 0, 3.2, 8.0, 13.0, 18.3, 23.0, 48.1 or 82.9% (note that the last stimulus contrast also varied in e-vector angle; Fig. 3A,C).

The procedure for the stomatopod experiment was similar, differing only in the following ways. Because these animals were unrestrained, there were frequent instances of the stomatopod being in an incorrect position for viewing the stimulus (e.g. tucked inside the burrow). Therefore, the 0 and −45 deg stimuli were presented to a different set of animals to maintain statistical independence between the treatments and for ease of analysis. A single stimulus set was presented to each of 56 different individuals twice, so that half viewed two presentations of the 0 deg stimulus set and half viewed two presentations of the −45 deg set. This proceeded in alternating order to avoid any diurnal or long-term temporal changes in stomatopod behaviour. The actual stimulus was similar to that presented to the fiddler crabs, consisting of an 80 mm loom expanding over 1 s, viewed at a distance of 125 mm (thus occupying a visual angle of 35.5 deg). The exact values of degree contrast presented at random to the stomatopod also differed slightly from those chosen for the fiddler crab experiment: 0, 10, 20, 30, 40, 50, 60 or 70%.

All stimuli were generated in MATLAB (R2012b, MathWorks, Natick, MA, USA) and responses were filmed with either a webcam (model C210, Logitech, Romanel-sur-Morges, Switzerland) or a digital video camera (HDR-SR11E, Sony, Tokyo, Japan). The responses of animals to the looming stimuli were subsequently scored from the digital video recordings by visually detecting changes in the animal's behaviour. This was undertaken in a fully blind process, in which all videos were randomised before analysis. Animals that responded to either of the repeat stimuli were scored 1, and those that responded to neither were scored 0. The score was then averaged across all animals to produce a response probability value for each stimulus/background condition. Because some of the scored behaviours occurred independently of the stimulus, we faced the potential problem of recording false positive responses. To overcome this, we used the response probability to the zero contrast stimuli as an estimate of the background probability of the behaviour.

For the fiddler crab results, a linear mixed model approach was used to determine which data points differed significantly from background levels. This was implemented in R (v.3.0.1, CRAN May 2013) using the 'lmer' function from the lme4 package. The model included animal identity as a random factor to account for any variance and possible biases due to response differences between individuals. We used the link function 'logit' and family 'binomial', and the significance of each stimulus response was determined by comparing the model that included the background level of

response against the model without the background level of response. For the stomatopod results, an analysis of covariance (ANCOVA) was performed to test for any effect of stimulus orientation on the response probability of the stomatopods. This method was used rather than the mixed model approach because of the statistical independence of the two treatments, and was implemented in R using the 'lm' function.

For each fiddler crab stimulus condition, an estimate of the level of contrast available to a two-channel polarisation vision system was calculated using the How–Marshall polarisation distance model (How and Marshall, 2014). Briefly, the model assumes that the animal uses a two-channel polarisation vision system with receptors organised horizontally and vertically relative to the outside world. These receptors then have opponent connections to a simulated visual interneuron, and the relative levels of activity between interneurons viewing the stimulus and the background are compared to produce a single estimate of contrast (see How and Marshall, 2014). For this specific case, we used a polarisation sensitivity (S_p) of 10 for the model. However, the results would equally apply for lower S_p values.

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Competing interests
The authors declare no competing financial interests.

Author contributions
M.J.H. and N.J.M. contributed to conception and design. M.J.H. and J.C. contributed to the execution of the experiments, and all authors contributed to interpretation of the results, and drafting and revision of the article.

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