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Behavioural discrimination of polarized light in the damselfish *Chromis viridis* (family Pomacentridae)

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Summary

In this study, we demonstrate the capacity for damselfish (green chromis, *Chromis viridis*) to discriminate between different e-vector orientations of ultraviolet polarized light. We examined the ability of green chromis to resolve small differences in e-vector orientation of ultraviolet polarized light. Fish were successfully trained to swim towards an e-vector orientation of polarized light using a behavioural chamber. *C. viridis* was able to discriminate between the horizontal and the vertical plane of ultraviolet polarized light independent of brightness content of the stimuli. However, e-vector discrimination capability disappeared when the ultraviolet portion of the light stimuli was removed, indicating that the presence of ultraviolet light was critical for e-vector discrimination. Fish could also distinguish between relatively small e-vector orientations of ultraviolet polarized light. Functional implications for high e-vector discriminative capabilities could be used in functional domains such as feeding and communication.

Key words: fish behaviour, visual behaviour, polarization sensitivity, e-vector, discrimination, fish vision.

Introduction

Visual systems responding to e-vector orientations of polarized light, independent of variation in intensity, possess polarization vision (Lythgoe and Hemmings, 1967; Fent, 1986; Hawryshyn, 1992; Degner and Hawryshyn, 2001). Polarization vision has been found in terrestrial animals, including birds, honeybees and ants (e.g. Wehner et al., 1975; Brines and Gould, 1982; Wehner, 1984; Rossel and Wehner, 1984; Fent, 1986; Labhart and Meyer, 1999), and in some invertebrates and fish (e.g. Waterman and Hashimoto, 1974; Saidel et al., 1983; Hawryshyn and McFarland, 1987; Parkyn and Hawryshyn, 1993, 2000; Shashar and Cronin, 1996). These animals may extract useful information from polarized light, produced by scattering in the surrounding media and/or by reflection from targets within it, to accomplish different visually mediated behavioural tasks (Lythgoe and Hemmings, 1967; Waterman, 1975, 1984; Hawryshyn et al., 1990; Shashar and Cronin, 1996; Shashar et al., 1998). In fact, celestial polarization patterns can be exploited as navigational cues (Brines, 1980; Brines and Gould, 1982; Hawryshyn et al., 1990; Goddard and Forward, 1991; Parkyn et al., 2003). Also, reflection of polarized light produced by integumental iridophores on an animal's body surface may provide intraspecific communicative cues (Cott, 1940; Denton and Rowe, 1994; Shashar and Cronin, 1996; Shashar and Hanlon, 1997; Marshall et al., 1999). Polarization vision can be used to increase the visual contrast of specific targets (Lythgoe and Hemmings, 1967; Shashar and Cronin, 1996; Tyo et al., 1996; Shashar et al., 1998; Wehner, 2001), as demonstrated in plankton feeders, which are capable of detecting zooplankton that appear transparent in the water column (Shashar et al., 1998; Novales Flamarique and Browman, 2001).

Planktivores, such as the damselfish *Chromis viridis*, form stationary aggregations along the reef to feed on zooplankton delivered by currents from the open ocean or during tidal events (Hobson, 1974, 1991; Williams, 1980; Thresher, 1983). Damselfish possess high cone photoreceptor density in their retina (McFarland, 1991), which suggests exceptional visual acuity. Also, using electroretinogram (ERG) recordings, Hawryshyn et al. (2003) showed that three species of damselfish (*Chromis viridis*, *Dascyllus melanurus* and *Dascyllus trimaculatus*) possess varied and complex polarization sensitivity (PS) and four different types of cone photoreceptors: ultraviolet (UV)-sensitive (UVS), medium-wavelength-sensitive (MWS), short-wavelength-sensitive (SWS) and long-wavelength-sensitive (LWS) cones.

The present study provides behavioural evidence for polarization vision in damselfish (green chromis, *C. viridis*). Our first objective was to assess the ability of *C. viridis* to discriminate between 0° and 90° e-vector orientations. These experiments were repeated, changing the brightness content of the positive and the negative stimuli alternately, to ensure that fish were choosing stimuli based on e-vector orientation rather than light intensity differences between the two stimuli. We then examined the importance of the contribution of UVS cone mechanism to e-vector sensitivity by eliminating the UV

portion of the linearly polarized stimulus. Finally, we examined the ability of test fish to resolve small angular differences between e-vectors.

Materials and methods

Animals

Behavioural experiments were performed on green chromis, *Chromis viridis* Cuvier 1830. Animals were kept in a flow-through seawater aquarium system for six months prior to experiments. Each fish was kept in a separate aquarium (16 cm × 8.5 cm × 10 cm). Fish were fed daily with flake food (Formula One flake food; Ocean Nutrition, Aqua Pet Americas, Salt Lake City, UT, USA) and, occasionally, with freshwater mysis shrimp, *Mysis relicta* (Aqua Yums; Ocean Nutrition). Temperature, pH and salinity were monitored twice daily and kept at approximately 28°C, a pH of 7.8 and a salinity of 30–32‰. Care and treatment of fish were in accordance with the University of Victoria Animal Care Committee, under the auspices of the Canadian Council for Animal Care.

Optical system

Light was generated using a 150 W xenon lamp system, which offers a broad spectrum of light, including the UV-A portion of the spectrum. A black metal wall (30 cm high and 40 cm wide), with a hole, 5 cm in diameter, was placed in front of the xenon lamp at a distance of ~20 cm (Fig. 1). The wall acted as a light baffling system to avoid any undesired light or reflection into the experimental area. The beam was projected through two UV-transmissive lenses onto a beam-splitter, which transmitted 50% of the light to one optical window of the behavioural chamber and 50% to a front-surface mirror to the second optical window (13 cm spacing; Fig. 1). Finally, a 1.0 neutral density filter was placed in front of the right-channel window in order to ensure that the light striking both channels was equal in photon irradiance. Measurements of the light stimuli within the test tank were taken using an integrating radiometer (Photodyne model 88XLA radiometer/photometer; Optikon, Waterloo, ON, Canada). Light passed through a diffuser (tracing paper) to remove any inherent polarized light. Between the polarizer and the quartz windows in the test tank were two UV-grade polarizing filters (Optics for Research, Caldwell, NJ, USA). The diffusers and UV-grade polarizing filters were positioned in indexed holders, which could fit onto the quartz windows of the behavioural chamber, and the plane of polarization was manipulated as required.

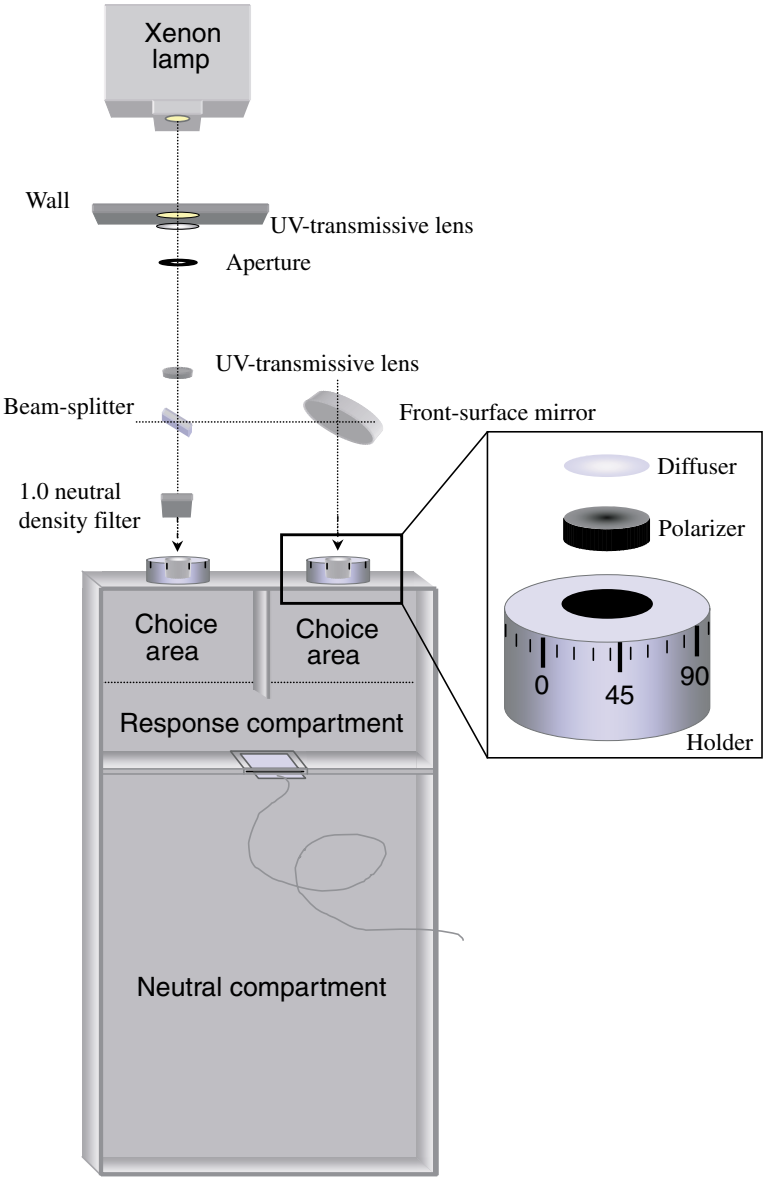

Fig. 1. Optical system. Light from the 150 W xenon lamp was projected though two UV-transmissive biconvex lenses before hitting a beam-splitter, which transmitted 50% of the light to the right optical window of the behavioural chamber and 50% to a front-surface mirror, which hit the left optical window of the behavioural chamber.

Light entering the two windows in the test tank was spectrally similar (Fig. 2), measured with a spectroradiometer [Ocean Optics (Dunedin, FL, USA) USB2000 Fiber Optic Spectrometer with CC-3-UV cosine-corrected irradiance probe; P600-2-UV/VIS fiber optic cable (Ocean Optics); OOIIrrad Application Software for Irradiance Measurement version 2.01.0 (Ocean Optics); standard source – LI-COR spectral irradiance lamp model N. 1800-02L (LICOR Biosciences, Lincoln, NE, USA)].

We conducted an experiment in which UV light content in the light field was blocked using a 450 nm long-pass filter (Corion Spectra Physics, Franklin, MA, USA; see Fig. 2).

Behavioural chamber

The flow-through seawater aquarium system and behavioural chamber were connected to a seawater sump,

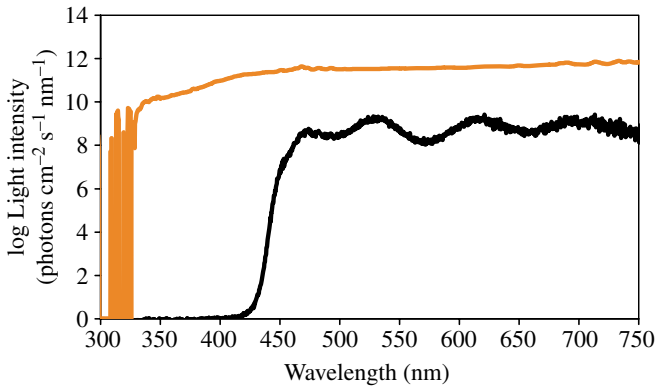

Fig. 2. Spectroradiometer measurements (Ocean Optics; USB2000) of the light entering the two windows in the test tank (orange line). Spectral background of the light entering the test tank when the UV light content was filtered using a 450 nm long-pass filter (black line).

where the water was heated, sterilized and filtered. Prior to experiments, the behavioural chamber was filled with seawater. Seawater from the sump fed both the aquaria and the behavioural chamber. This maintained consistent physical and chemical properties of water in the two areas. For each fish in the training or testing scenario, the behavioural chamber was drained, cleaned and refilled with fresh seawater from the sump. During training and testing, a heater and an air stone were placed on the rear wall of the neutral compartment of the behavioural chamber to maintain oxygenation and water temperature.

The behavioural chamber had two compartments: the response and the neutral compartments (Fig. 1). The neutral compartment (60 cm × 16 cm × 30.7 cm) was used to acclimatize the fish to the experimental tank and separate the fish from the response compartment by a gate. The gate was manually operated. The front of the response compartment (30.7 cm × 16 cm × 44.8 cm) had two round quartz optical windows (diameter 6.7 cm) partially separated by a black Plexiglas barrier, through which light was passed.

Training

An operant conditioning protocol was used to train fish to respond to polarization stimuli. Learned-choice tests included a training session, where fish were trained to select toward a particular e-vector orientation of UV polarized light. Such experimental design can provide evidence of animal's visual capabilities; however, because of the difficult training tasks, sample size was limited. In fact, training began using 15 fish; six fish died and five fish failed to learn the task. Four fish were used in this study, which is typical in behavioural discrimination experiments, given the length of experimentation required to assess visual performance for an individual fish (Neumeyer, 1984, 2003; Degner and Hawryshyn, 2001; Parkyn et al., 2003).

Fish were divided into two groups; one group was trained to select and swim toward the vertical e-vector orientation (0°, relative to the gravitational axis; positive stimulus 0°, negative stimulus 90°), and the second group was trained to the horizontal e-vector orientation (90°; positive stimulus 90°, negative stimulus 0°). Training began by placing the fish in the neutral chamber for 30 min to allow it to acclimate. During this time, the gate separating the response compartment from the neutral compartment was closed. In addition, the light stimuli were turned off, while the external fan was left running to familiarize the fish to the background acoustic noise. At the end of 30 min, stimulus light was turned on, the gate was raised and the fish entered the response chamber, where it was exposed to the light patches having two different e-vector orientations. During initial training, fish were gently guided with a rod toward the positive stimulus. A small piece of mysis shrimp was provided following the selection of the correct e-vector, i.e. when fish entered the choice area containing the correct e-vector. To facilitate fish training, a positive partial reinforcement method was used, where correct e-vector selections were reinforced with food every five trials (Hawryshyn et al., 1990). Responses are much harder to extinguish when stimulus acquisition used partial rather than continuous reinforcement (Williams, 1989; Pearce et al., 1997; Sangha et al., 2002) and there is better control over motivational state.

Once the fish had consumed the food reward, it was guided back towards the neutral compartment, and the gate was closed. The position of the positive and negative stimuli was randomised for each trial, in order to ensure that choice was based on orientation of the e-vector rather than a bias towards a particular location. Training continued until each fish responded correctly (i.e. choosing the correct e-vector orientation) at least 70% of the time, based on 20 trials.

In all experimental trials, test fish made a choice responding correctly or incorrectly.

0° versus 90° e-vector discrimination experiments

Two groups of fish trained to either the vertical or to the horizontal e-vector were presented with a choice between 0° and 90° stimuli (N=4).

In the response compartment, fish had to enter the choice area entirely for a response to be scored (Fig. 1). The time required to enter the choice area (either positive e-vector or negative e-vector) was recorded using a timer. Trials were considered valid when the choice area was occupied for at least 120 s subsequent to the opening of the gate. Ten trials were conducted for each test day for an individual fish, with a total of 40 trials used to calculate the fish's choice performance.

Brightness test

To ensure that fish were choosing stimuli based on e-vector orientation rather than light intensity differences between the two stimuli, brightness tests were conducted (Jacobs, 1981). The light intensity of both positive and negative stimuli was manipulated using a one neutral density filter (1.0 ND). A total of 80 trials were conducted for each fish (N=4), where 40 trials were carried out by placing the neutral density filter in front of the 90° window (90°+1 ND), and the other 40 trials with the filter in front of the

0° window (0°+1 ND). The neutral density filter was randomly placed in front of the positive or negative stimulus. 10 trials were conducted each day for an individual fish.

Eliminating UV light from the polarization stimuli

0° and 90° e-vector discrimination experiments were repeated using a 450 nm long-pass filter. This optical filter eliminates the UV portion of the spectrum from the light fields of the two stimuli. Fig. 2 illustrates the spectral distribution of the light field when the 450 nm long-pass filter was used. Two fish were used; fish A, which was trained to select the 90° e-vector orientation, and fish B, which was trained to select the 0° e-vector orientation. Forty trials were conducted for each fish.

Minimum angular difference in e-vector discrimination

Experiments were conducted using the same two groups of fish used for the 0°/90° e-vector discrimination test (N=4). Group one, which was trained to respond to the 0° e-vector, was presented with comparison e-vectors between 5° and 45°. The comparison e-vectors were randomly presented, and both choice frequency and time to respond were recorded. Forty trials were conducted per fish for each comparison e-vector.

This protocol was repeated with the second group, where the reference 90° e-vector fish were randomly presented with e-vectors of comparison between 85° and 45°. The angular difference between the positive e-vector and the comparison e-vector was determined (termed Δ e-vector).

One fish (fish C) was re-trained to select 45° e-vector orientation to investigate the possibility that Δ e-vector may change with different reference e-vectors. Such comparisons are necessary since, like colour vision, photoreceptor mechanism interaction can affect discrimination performance.

The fish trained to 45° died before all sessions of comparison could be completed. Forty trials were conducted comparing 45° with each of 0°, 5°, 10°, 15°, 20°, 25°, 30°, 35°, 40° and 55°. Thirty trials were conducted comparing 45° with each of 50°, 60°, 75° and 90°, and 20 trials were conducted comparing 45° with 65°.

Statistical analysis

In all experiments, 10 trials were conducted for each test day for an individual fish, and the correct choice frequency recorded. Four days of experiments for each fish provided a total of 40 trials per fish to calculate the fish's choice performance. The correct choice frequencies of each test day were averaged, and the standard error calculated.

Statistical analyses for all four fish required the use of the binomial distribution model of Bernoulli trials since the outcome of all the experiments was either a correct or incorrect choice. Therefore, the probability was calculated using the binomial probability formula:

$$P\ (x \text{ successes in } n \text{ trials}) = \binom{n}{x} p^{x}\ (1-p)^{n-x},$$

where n was the number of trials, x was the number of successes, p was the probability of success in one trial (i.e. 50% correct choice), $1–p$ was the probability of failure in one trial, and $n–x$ was the number of failures.

Results

Green chromis (*C. viridis*) was successfully trained to select and discriminate between the horizontal and the vertical plane of UV plus visible spectrum polarized light. The discrimination capability between the two orthogonally oriented e-vector orientations was confirmed using a brightness test. However, when the UV portion of the stimulus field was removed, e-vector discrimination performance deteriorated. Choice behaviour experiments between the reference e-vectors and various comparison e-vectors were conducted. Interestingly, the ability to resolve e-vector differences between two stimuli varied with the angular orientation of the reference e-vector. When fish were trained to the reference e-vectors, 0° or 90°, the minimum separable angular difference between the reference e-vector and the

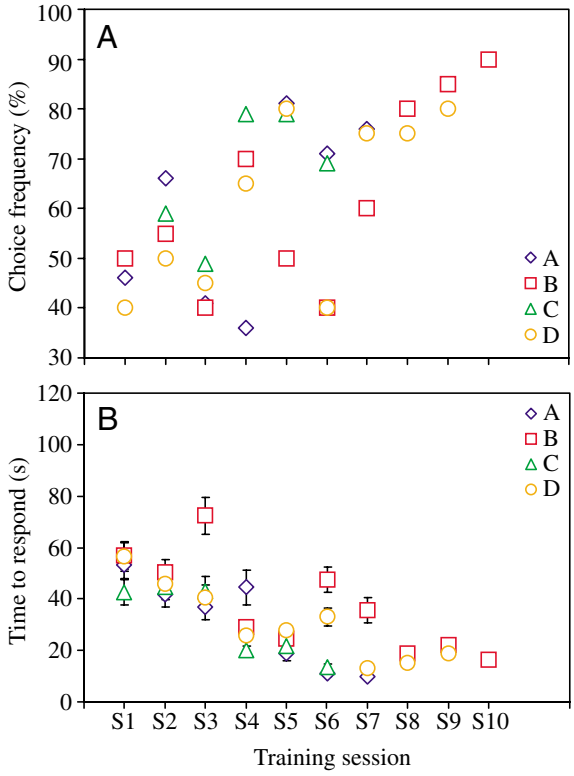

Fig. 3. (A) Each session (S1–S10) of training, which included 20 trials, is shown on the x-axis, plotted against choice frequency. Fish A (diamond) required four sessions before discriminating the reference e-vector. Fish B (square) required seven sessions, whereas fish C (triangle) required three, and fish D (circle) required six sessions. Positive response was a correct choice frequency of 70% or above. (B) Time to respond. The time to respond was recorded from the opening of the gate until fish chose an e-vector within the choice area. The time to respond (mean ± S.E.M.) diminished with the training sessions for each fish.

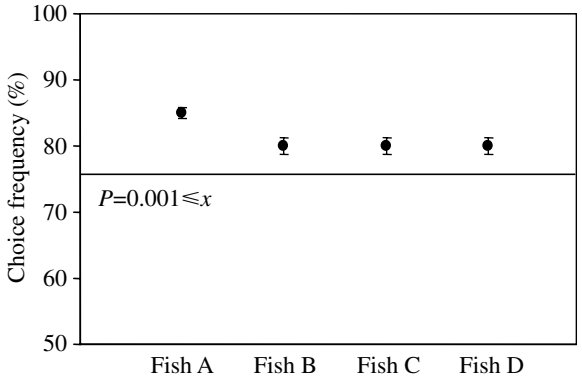

Fig. 4. *C. viridis* could discriminate between 0° and 90° e-vector orientations ($P<10^{-4}$ in each case). Each point represents the percentage of correct choice frequency (mean ± S.E.M.) when 0° and 90° e-vector orientations were presented. The horizontal line represents the choice frequency value for a significance level of $P=0.001\leqslant x$ for 40 Bernoulli trials calculated using the binomial probability function. Fish A and C were trained to swim towards 90° e-vector orientation (85% and 80% correct choice frequency, respectively). Fish B and D were trained to select 0° e-vector orientation (80% correct choice frequency in both fish).

comparison e-vector (Δ e-vector) was 25°. However, when fish were retrained to 45° as the reference e-vector, the Δ e-vector was between 10° and 15°.

Training sessions and time to respond

Several trials were conducted for each fish before reaching a consistently positive response of respective reference e-vectors. Each training session for each fish included 20 trials. Fish were considered trained to criterion and were designated as test fish for experiments when they were capable of responding positively for at least three consecutive sessions (Fig. 3A). A positive response was considered to be at least a 70% correct choice frequency.

The time to respond was recorded during both training sessions and experiments. During the experimental training, the time to respond after the opening of the gate diminished with consecutive training sessions in each fish, and in particular, it decreased considerably when fish began to select the correct stimulus (Fig. 3B).

0° versus *90° e-vector discrimination*

All four of the *C. viridis* tested were capable of distinguishing 0° from 90° e-vectors over 40 trials (Fig. 4). Fish A and C were trained to swim towards 90° e-vector orientation (85% and 80% correct choice frequency, respectively), whereas fish B and D were trained to select 0° e-vector orientation (80% correct choice frequency in both fish). The probability of correct choices occurring by chance is $<10^{-4}$ in each case. A one-sample *t*-test of the four frequencies against a null hypothesis of random choice was also highly significant ($t=25$, d.f.=3, $P<10^{-4}$).

During the trials, the positions of the reference e-vector and the comparison e-vector were changed randomly. To satisfy our concern that the fish might have a lateral bias for choice, the choice frequencies for either left or right presentation were compared with the left/right distribution of the e-vector of reference. Table 1 shows that choice frequencies were not biased by side preference.

Table 1. *Choice frequencies for either left or right presentation compared with the left/right distribution of the reference e-vector*

	Reference e-vector (left *vs* right)	Correct responses/total (left *vs* right)	Incorrect responses/total (left *vs* right)
Fish A	90° (19–21)	34/40 (16–18)	6/40 (3–3)
Fish B	0° (19–21)	32/40 (16–16)	8/40 (3–5)
Fish C	90° (22–18)	32/40 (18–14)	8/40 (4–4)
Fish D	0° (17–23)	32/40 (14–18)	8/40 (3–5)

The left column shows the reference e-vector for each fish and, in parentheses, the left/right presentation of the positive stimulus in 40 trials. The central and right columns indicate the correct and incorrect responses, respectively, to the reference e-vector out of 40 trials and, in parentheses, the left/right presentation of the chosen positive stimulus.

The brightness test

The brightness test confirmed that the choice between the horizontal and the vertical plane of polarized light (90° and 0° e-vector orientation, respectively) was made based on the orientation of the e-vectors, independent of the light intensity of the stimuli (Fig. 5). A total of 80 trials per fish ($N=4$) was conducted. Each circle in Fig. 5 represents the percentage of correct choice frequency (mean ± S.E.M.) when 0° was dimmer than 90°. Each square in Fig. 5 represents the percentage of correct choice frequency when 90° was dimmer than 0°.

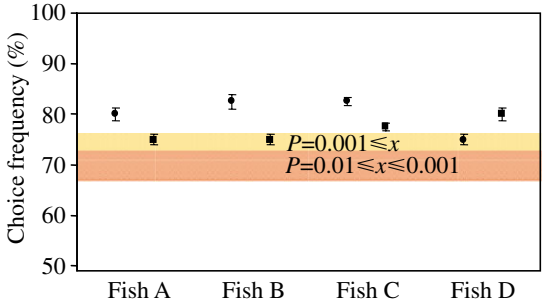

Fig. 5. The brightness test showed that the choice between the horizontal and the vertical plane of polarized light was made based on the orientation of the e-vectors, independent of the light intensity of the stimuli (80 trials per fish, $N=4$). Each circle represents the percentage of correct choice frequency (mean ± S.E.M.), when 0° was dimmer than 90°, whereas each square represents the percentage of correct choice frequency (mean ± S.E.M.) when 90° was dimmer than 0°. The horizontal shaded bands represent the range of choice frequency values that correspond to significance levels of $P=0.001\leqslant x$ and $P=0.01\leqslant x\leqslant 0.001$, calculated using the binomial probability function for 40 Bernoulli trials. The reference e-vector for fish A and C was 90°, whereas the reference e-vector for fish B and D was 0°.

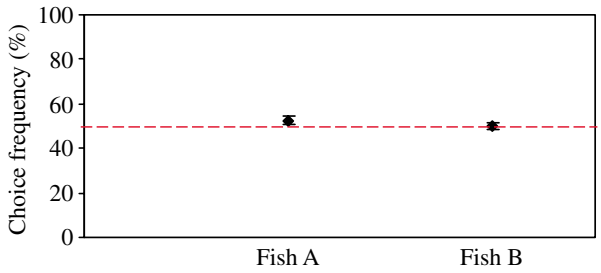

Fig. 6. Eliminating the UV part of the spectrum in the polarization stimuli impairs e-vector discrimination. The UV part of the spectrum in both stimuli was removed using a 450 nm long-pass filter (40 trials per fish, *N*=2). Each point represents the percentage of correct choice frequency (mean ± S.E.M.) between the reference and comparison e-vectors. The horizontal dashed line indicates 50% choice, i.e. random choice between the two stimuli.

Choice frequencies of fish A and C, towards their reference e-vector, were relatively high when the negative stimulus 0° was one log unit dimmer; in fact, both points were at or above 80% of correct choice (Fig. 5). When the positive stimulus 90° was dimmer than the negative 0°, correct choice between the two stimuli occurred at slightly lower frequencies, i.e. 75 and 77.5% (but statistically significant) for fish A and C, respectively (Fig. 5).

By contrast, fish B and D did not show any particular pattern. Fish B chose its reference e-vector 82.5% of the time when it was dimmer than the negative stimulus; when the negative stimulus (90°) was dimmer than the positive stimulus, correct choice was 75%. Fish D showed an opposite pattern; when its reference e-vector (0°) was dimmer, fish chose it 75% of the time; when the negative stimulus was dimmer, fish selected correctly 80% of the time (Fig. 5).

Eliminating UV light from the polarization stimuli

Fish could no longer discriminate between their respective reference and comparison e-vectors when the UV part of the spectrum in both stimuli was filtered out using a 450 nm long-pass filter (Fig. 6). A total of 40 trials was used to test each fish (*N*=2), and choice frequency was approximately 50%. This clearly indicated that fish chose randomly between the two stimuli.

Minimum angular difference in e-vector discrimination

Fig. 7 shows the percentage of choice frequency for each fish plotted *versus* the angular difference between the reference e-vector and the comparison e-vector (Δ e-vector). Each point in Fig. 7 represents the percentage of correct choice frequency (mean ± S.E.M.) between the reference e-vector and the comparison e-vector. A total of 40 trials were conducted for each comparison (*N*=4). The smallest Δ e-vector that damselfish were able to discern was approximately 25°, when 0° or 90° was the reference e-vector. Fish B and D could discriminate between 0° and 25° (72% and 70%, respectively) but could not discriminate between 0° and 20° (44% and 46%, respectively). Similarly, fish A and C could differentiate between 90° and 65° (70% in both fish) but were not able to discriminate between 90° and 70° (52% and 54%, respectively). Interestingly, the smallest Δ e-vector was approximately 25° for both reference e-vectors (0° and 90°), indicating an inherent symmetry around these e-vector orientations.

When fish C was re-trained to respond to 45° e-vector orientation, the smallest Δ e-vector detected was approximately 10° (Fig. 8). The left side of Fig. 8 shows the choice frequencies between the reference e-vector 45° and the

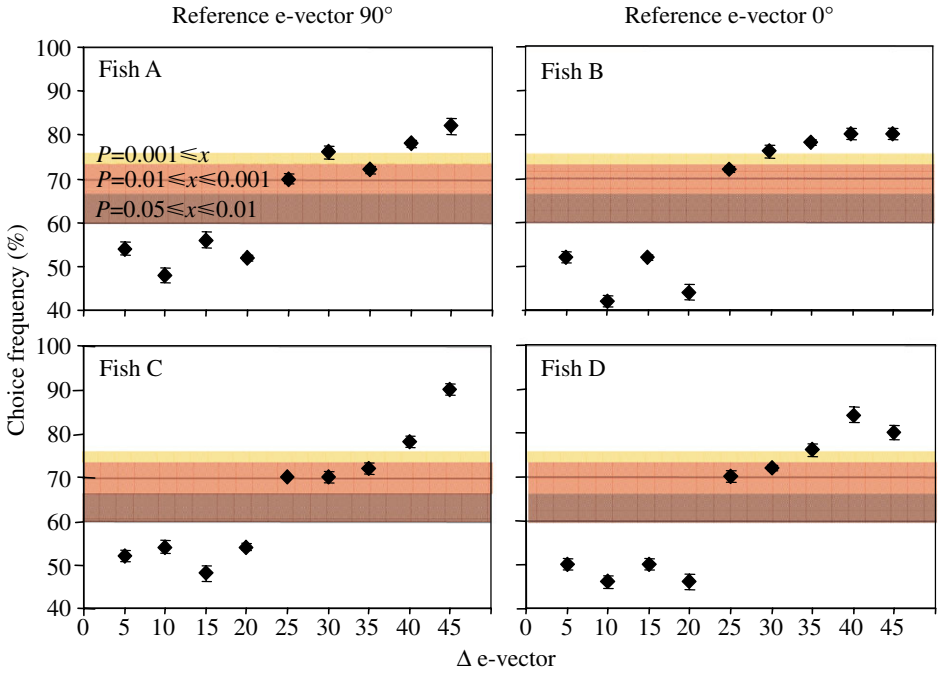

Fig. 7. The angular difference between the reference e-vector and the comparison e-vector (Δ e-vector) is plotted against the percentage of choice frequency for each fish. Each point represents the percentage of correct choice frequency (mean ± S.E.M.) between the reference e-vector and the comparison e-vector (40 trials per fish, *N*=4). The horizontal shaded bands represent the range of choice frequency values that correspond to significance levels of $P \leq 0.001 \leqslant x$, $P=0.01 \leqslant x \leqslant 0.001$ and $P=0.05 \leqslant x \leqslant 0.01$, calculated using the binomial probability function for 40 Bernoulli trials. The smallest Δ e-vector was approximately 25° in all four fish.

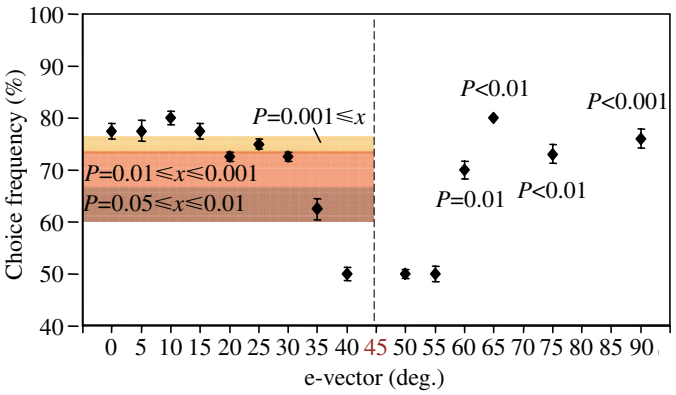

Fig. 8. The percentage of choice frequency (mean ± S.E.M.) plotted against e-vector orientation when fish C was re-trained to swim towards 45° e-vector orientation. The left side of the figure shows the choice frequencies between the reference 45° and the comparison e-vectors of up to 0°, and the right side shows the choice frequencies between 45° and the comparison e-vectors up to 90°. The horizontal shaded bands represent the range of choice frequency values that correspond to significance levels of $P=0.001 \leqslant x$, $P=0.01 \leqslant x \leqslant 0.001$ and $P=0.05 \leqslant x \leqslant 0.01$, calculated using the binomial distribution model of Bernoulli trials for 40 trials. Except for the 55° e-vector comparison, points and their associated significance levels on the right side of the figure are for less than 40 trials. The smallest Δ e-vector was approximately 10°.

comparison e-vectors of up to 0°. The right side of the figure shows the choice frequencies between 45° and the comparison e-vectors of up to 90°. Fish could distinguish between 45° and 35° (62.5% correct choice), and between 45° and 60° (70% of correct choice), but not between 45° and 40° (50% correct choice) or between 45°and 55° (50% correct choice). The correct choice frequencies of the fish trained to 45° showed a notably higher resolution of discrimination.

Discussion

UV polarization vision in C. viridis

Psychophysical and electrophysiological observations have been extensively used to study polarization detection (e.g. Waterman and Aoki, 1974; Kawamura et al., 1981; Coughlin and Hawryshyn, 1995; Hawryshyn and McFarland, 1987). However, direct evidence of polarization vision can be obtained solely through behavioural discrimination experiments (e.g. Jacobs, 1981; Neumeyer, 1984, 1986). We conducted a two-choice behavioural experiment using the learned response protocol. The advantage of using learned responses *versus* unlearned responses was the greater degree of control for testing discrimination between many different stimuli. This allows the experimenter to largely remove motivation as a confounding factor. Secondly, using trained fish avoids problems associated with habituation, which more than likely occurs in unlearned response experiments (Jacobs, 1981). Thirdly, innate response levels may be influenced by spurious cues.

This research demonstrated the ability of *C. viridis* to perceive and utilize polarized light cues, confirming the ERG results of Hawryshyn et al. (2003). *C. viridis* discriminated between 0° and 90° e-vector orientations of polarized light, and the discriminative capabilities were not compromised by manipulating the brightness content of the stimuli. Therefore, choice was made based on the e-vector orientations regardless of brightness differences between the two stimuli (1 log photons cm^{-2} s^{-1} difference in intensity). The light intensity of the negative and the positive stimuli was randomly varied during the same session of 10 trials. This approach avoids the problem that test fish may learn to avoid stimuli brighter or dimmer than the positive stimulus.

By contrast, fish could no longer discriminate between 0° and 90° e-vector orientations when the UV part of the spectrum was filtered out. The use of a 450 nm long-pass filter narrowed the spectral width of the stimuli, with the purpose of eliminating the UV portion of the spectrum from the polarized light field. The use of the 450 nm long-pass filter demonstrates the critical role played by the UV portion of the spectrum for polarization vision; when UVS cones were not stimulated, fish were incapable of discriminating between e-vectors. Similar conclusions were found in salmonids (Hawryshyn et al., 1990; Degner and Hawryshyn, 2001; Parkyn et al., 2003). In Hawryshyn et al. (2003), a UV-transmitting filter (Shott; UG-11; Corion Spectra Physics) was used to produce UVS cone chromatic adaptation. This disabled polarization sensitivity (PS). Therefore, stimulation of the UVS cone mechanism is an essential requirement for e-vector discrimination.

Perception of polarized light in the UV part of the spectrum represents an interesting aspect of polarization vision in animals. Polarization has been shown to be significantly lower in the UV spectrum in comparison with the longer wavelength spectrum under clear skies and underwater (Cronin and Shashar, 2001; Barta and Horvath, 2004). However, it has been suggested that celestial UV polarized light is the most stable and detectable cue under clouds and forest canopies (Pomozi et al., 2001; Barta and Horvath, 2004).

Mechanisms of polarization vision in C. viridis

Measurements of e-vector discrimination facilitate characterization of *C. viridis* polarization vision. Fig. 8 shows the smallest angular difference between the reference e-vector and the comparison e-vector (termed Δ e-vector) when the reference e-vector was either 0° or 90°. Low values of Δ e-vector indicate good discrimination acuity. Below 25° Δ e-vector, fish could not distinguish between the two stimuli, with choice frequencies approximating randomness. Interestingly, when the reference e-vector was 45°, the lowest value of Δ e-vector was 10–15° (Fig. 8). Changes in reference e-vector from either 0° or 90° to 45° further illustrates the complexity of polarization vision in *C. viridis* and point to the possible opponent interaction between the vertical and horizontal polarization detectors. This experimental finding also demonstrates the importance of experimental design for discrimination experiments, not unlike the considerations used

in colour discrimination experiments. The differential discriminative capabilities of fish tested at different reference e-vectors provide an important foundation for understanding the neural processing underlying polarization vision.

While it is likely that PS in damselfish and salmonids mediates different behavioural activity, these two species could conceivably have the same polarization detection/cone mechanisms. PS in salmonids has been investigated using compound action potential (CAP) recording from the optic nerve, and a two-channel system was found, where one detector was maximally sensitive to 0° e-vector orientation and the other to 90° e-vector orientation (Hawryshyn and McFarland, 1987; Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1995; Novales-Flamarique and Hawryshyn, 1997). ERG data (Hawryshyn et al., 2003) showed that *C. viridis* has complex PS, with four peaks at 0°, 45°, 90° and 135° e-vector orientation. Recently, ERG recordings have been conducted in rainbow trout (*Oncorhynchus mykiss*), revealing the same four-peaked PS pattern at 0°, 45°, 90° and 135° as in *C. viridis* (S. D. Ramsden, L. Anderson, M. Mussi, T. J. Thairnberger, M. Kamermans and C. W. Hawryshyn, unpublished).

Polarization vision and colour vision

As indicated previously, e-vector discrimination could be considered analogous to wavelength discrimination in colour vision. Colour can be described by intensity, purity and wavelength, as polarization can be described by intensity, degree of polarization and e-vector orientation (Bernard and Wehner, 1977). As for colour vision, polarization vision is characterized by the ability to discriminate between two lights of the same brightness but of different e-vector orientation or degree of polarization (Bernard and Wehner, 1977). Wavelength discrimination responses could be compared to e-vector discrimination. Wavelength discrimination was used to characterize the goldfish colour vision system (Neumeyer, 1992). Neural interactions were found between cone mechanisms; the spectral sensitivity curve obtained from the behavioural experiments showed their maxima at different points with respect to the relative spectral absorbance of the photopigments (Neumeyer, 1984). Cone responses were modified by inhibitory interactions between cone mechanisms, and this opponency between cone mechanisms was described by a linear subtractive interaction (Neumeyer, 1984).

Similarly, the high discrimination capabilities of *C. viridis* at 45° could be explained assuming that two mechanisms or channels were interacting and that discrimination was most effective in the region of angular disparity where the sensitivity of the two detectors shows the greatest degree of overlap.

Functional significance of polarization vision in C. viridis

Polarization vision in *C. viridis*, with its e-vector discriminative capabilities, especially around 45°, could find its use in a number of different visually mediated behaviours. In the underwater environment, horizontal polarized light is predominant (Cronin and Shashar, 2001), and thus other e-vector orientations would elicit visual contrast between targets and background. It has already been suggested (Lythgoe and Hemmings, 1967; Loew et al., 1993; McFarland and Loew, 1994; Shashar et al., 1998; Johnsen and Widder, 2001; Hawryshyn et al., 2003) that special visual adaptations, such as UV vision and PS, have likely evolved to increase visibility of transparent plankton. In fact, polarization vision can reveal camouflage of transparent prey through scattering of polarized light from the prey exoskeleton (Johnsen, 2001, 2002; Novales-Flamarique and Browman, 2001). The birefringence of calcium carbonate exoskeletons of plankton can rotate the plane of polarization and make them conspicuous to a polarization-sensitive visual system (Giguére and Dunbrak, 1990; Shashar et al., 1998; Johnsen, 2001, 2002; Novales-Flamarique and Browman, 2001). Therefore, higher discriminative capabilities at intermediate e-vectors between 0° and 90° might be advantageous for prey detection.

Also, *C. viridis* could advantageously utilize such polarization vision for optical signalling. In fact, swimming modalities characteristic of schooling behaviour and mate choice (nuptial displays) can produce changes in colouration in *C. viridis* behaviour (Allen, 1991). Damselfishes possess chromatophores and iridophores, which provide many combinations of body colouration and patterns (Fujii, 1993). Iridophores reflect light through interference occurring in the stacks of guanine crystals (Denton and Nicol, 1965; Fujii, 1993; Herring, 1994), and light reflected from iridophore crystals can be polarized (Denton and Nicol, 1965; Kasukawa et al., 1987; Fujii, 1993). Both the plane and the degree of polarization produced by reflective iridophores can change dramatically depending on the movements of the fish (Denton and Nicol, 1965; Denton and Rowe, 1994; Shashar et al., 1996, 2001; Shashar and Hanlon, 1997). Moreover, through active movements of their motile iridophores (Fujii and Oshima, 1986; Kasukawa and Oshima, 1987; Kasukawa et al., 1987; Fujii et al., 1989; Oshima et al., 1989, Fujii, 1993, 2000), *C. viridis* could change polarization patterns on their bodies. For *C. viridis*, display of polarization patterns across the body surface would represent a powerful resource for signalling and a reliable communication channel. This is particularly true since the scales of fish produce a distinct polarization reflection that is different from the polarization characteristics of the underwater light field (Denton, 1970; Rowe and Denton, 1997; Shashar et al., 2001). Therefore, reflections off the fish body could provide polarized light signals at e-vector orientations that would contrast those surrounding the fish.

Conclusions

Investigation of the functional significance of polarization vision is a relatively new research topic. Our research provides the first behavioural evidence of e-vector discrimination in vertebrates, representing an important step for understanding the dynamic nature of the damselfish visual system. Here, we show that *C. viridis* is able to select and discriminate between the horizontal and vertical planes of UV

linearly polarized light independent of the stimuli brightness content. The capacity for e-vector discrimination disappeared when the UV portion of the light stimuli was removed, indicating that the presence of UV polarized light is critical for e-vector discrimination. Furthermore, fish were able to distinguish between relatively small e-vector orientations of polarized light, and Δ e-vector varied based on the reference e-vector.

Our study offers compelling justification for future work in damselfish. Areas of particular interest include: the investigation of behavioural outputs of polarization vision in *C. viridis*; the examination of underlying physiological and neural mechanisms of polarization vision; and the functional significance of the e-vector discriminative capabilities. Our research provides an effective framework for experimental design and methodology of behavioural studies that examine PS in organisms.

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