Behavioural Discrimination of Polarized Light in the Damselfish *Chromis viridis* (Family Pomacentridae)

by

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Supervisor: Dr. Craig W. Hawryshyn

Abstract

The objectives of this study are to demonstrate the ability of the damselfish Green Chromis, *Chromis viridis*, to perceive ultraviolet polarized light and investigate their ability to resolve small differences in e-vector orientation of ultraviolet polarized light. I obtained direct evidence of such visual capabilities in *C. viridis* through behavioural experiments. 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 UV 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 feeding and communication.

Supervisor: Dr. C.W. Hawryshyn (Department of Biology)

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1. INTRODUCTION

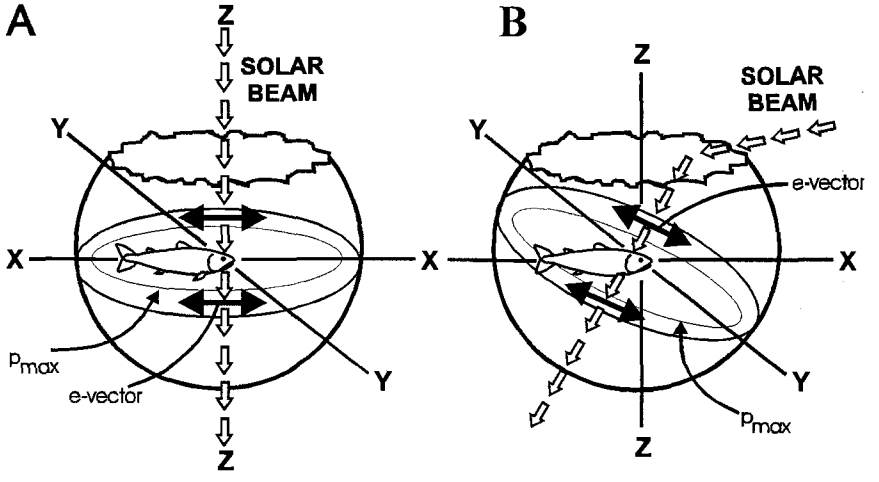
1.1 Natural Polarized Light

Natural polarized light arises in both the atmosphere and hydrosphere primarily due to scattering and differential reflection from surfaces (Waterman, 1954; Lythgoe and Hemmings, 1967; Waterman, 1984; Wehner, 1989). Direct sunlight is not polarized, i.e. solar radiation has an equal distribution of electric field orientations for all directions. Polarization in the sky occurs when solar radiation encounters small particles in the atmosphere and, through scattering, it becomes partially linearly polarized (Rayleigh scattering) (Waterman, 1954, 1984). Three basic characteristics define natural polarized light: e-vector orientation, intensity, and degree of polarization (Waterman, 1954). The e-vector orientation (plane of polarization) is orthogonal to the direction of the light flux. Scattering varies as the reciprocal of the fourth power of the wavelength (λ^{-4}); therefore, short wavelength light at 400 nm is scattered 10.5 times more strongly than long wavelength light at 720 nm (Waterman, 1984). The degree of polarization represents the ratio of polarized light intensity to total light intensity, and it ranges from zero (no polarization) in directions parallel to the light flux, to one (complete polarization), in a plane orthogonal to the light flux (Waterman, 1954, 1984). Polarized light is a function of the position of the sun with the greatest degree of polarization at an angle of 90° from the sun. When the sun is on the horizon at sunrise and sunset (crepuscular period), the maximum polarization is at zenith with the e-vector orientation

perpendicular to the solar meridian, whereas, when the sun is at zenith, the band of maximum polarization is on the horizon (Waterman, 1954).

Underwater polarization occurs mostly through scattering from water molecules and reflection at the air-water interface (Waterman, 1954; Lythgoe and Hemmings, 1967; Waterman, 1984; Wehner, 1989). The polarization field underwater differs from that in the atmosphere because, for an underwater observer, it occupies a sphere rather than a band as for the celestial hemisphere (Figure 1A and B). When the sun is at or near zenith, the observer is surrounded by a horizontal band of linearly polarized light (Figure 1A). This band is orthogonal to the sun's direction and parallel to the water surface (Novales Flamarique and Hawryshyn, 1997). When the sun is on the horizon, this band of polarization is tilted (Figure 1B). Maximal tilt, however, does not exceed the critical angle of about 48.6° because of refraction at the air-water interface.

Figure 1.
Polarized light field underwater. Figure adapted from Hawryshyn (2003).



An animal requires at least two differentially sensitive classes of photoreceptors to discriminate between two stimuli with different e-vector orientations independent of differences in stimulus intensity. Both the degree of polarization and intensity of the stimuli must be in the functional range of operation for the detector system (Hawryshyn, 1991; Bernard and Wehner, 1977). If an animal exhibits discrimination, one can surmise that it possesses polarization vision. Electrophysiological approaches examine neural outputs at different anatomical levels, and can be used to understand how animals extract the information needed for polarization detection. In fact, both 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). Using electroretinography (ERG), Hawryshyn et al. (2003) showed that three species of damselfish respond to ultraviolet polarized light stimuli. However, direct evidence of polarization vision can be obtained solely through behavioural discrimination experiments (e.g. Jacobs, 1981; Neumeyer, 1984, 1986).

1.2 Functional Significance of Polarization Vision

Visual systems possess polarization vision when they respond to e-vector orientations of polarized light independent of variation in intensity (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 as they are able to utilize

patterns of polarized light present in the celestial hemisphere as an external compass with which to orient themselves (Wehner *et al.*, 1975; Kirschfeld *et al.*, 1975; Brines and Gould, 1982; Wehner, 1984; Rossel and Wehner, 1984; Fent, 1986; Labhart and Meyer, 1999; Labhart, 1999). Similarly, aquatic animals within the photic zone are surrounded by complex patterns of polarized light (Novales Flamarique and Hawryshyn, 1997; Cronin and Shashar, 2001), and polarization vision is probably widespread in the marine environment as some invertebrates and fish are able to detect linearly polarized light (Lythgoe and Hemmings, 1967; Waterman and Hashimoto, 1974; Waterman, 1974; Saidel *et al.*, 1983; Hawryshyn and McFarland, 1987; Parkyn and Hawryshyn, 1993, 2000; Coughlin and Hawryshyn, 1995; 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, 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 communicative cues to other members of the same species (Denton and Rowe, 1994; Shashar *et al.*, 1996; Shashar and Hanlon, 1997; Marshall *et al.*, 1999), or may be additionally utilized for camouflage, e.g. many silvery fish produce linearly polarized light from scale reflections to reduce their

visibility to predators or even prey (Cott, 1940; Denton and Nicol, 1965; Denton, 1970; Lythgoe, 1979; Herring, 1994). Polarization vision can 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; Johnsen, 2001a; Johnsen, 2001b; Novales Flamarique and Browman, 2001).

1.3 Polarization Vision in Damselfish

Damselfishes belong to the family Pomacentridae and represent one of the largest groups of colourful fish inhabiting tropical and sub-tropical oceans. They usually live in shallow water around coral reefs, mangroves, and beds of sea grass (Allen, 1991). Damselfishes have a diversity of morphological characteristics, feeding habits, ecological niches, and behavioural specializations (Hobson, 1974; Williams, 1980; Thresher, 1983; Allen, 1991). Interactions of damselfish with predators, prey, competitors and mates rely on light and vision, and clear waters around coral reefs offer a complex photic environment for such daytime activities (McFarland, 1991). In fact, damselfish possess a high cone photoreceptor density in their retina (McFarland, 1991), which suggests exceptional visual acuity, i.e. the ability to resolve detail within its visual field. Moreover, Hawryshyn and colleagues (2003) showed that three species of damselfish (*Chromis viridis*, *Dascyllus melanurus*, and *D. trimaculatus*) possess varied and complex polarization sensitivity (PS) and four different

types of cone photoreceptors: UV-sensitive (UVS), medium wavelength-sensitive (MWS), short-wavelength-sensitive (SWS), and long wavelength-sensitive (LWS) cones.

2. Thesis objectives

The present study provides behavioural evidence for polarization vision in the damselfish Green Chromis, *C. viridis*, that links previous electrophysiological recordings (Hawryshyn *et al.*, 2003) with the behavioural capacity of *C. viridis* to perceive and discriminate between different e-vector orientations of polarized light. I assessed (i) the ability of *C. viridis* to discriminate between 0° and 90° e-vector orientations independent of brightness, (ii) the importance of the ultraviolet component of the stimulus to behaviour, and (iii) the minimum e-vector differences required for discrimination. My findings represent the first behavioural evidence of e-vector discrimination in a vertebrate, independent of the brightness content of the stimulus.

The first objective was to assess the ability of *C. viridis* to discriminate between 0° and 90° e-vector orientations. These experiments were repeated by 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.

I then examined the importance of the contribution of the ultraviolet sensitive (UVS-) cone mechanism to e-vector sensitivity by removing the ultraviolet portion of the stimulus, since chromatic adaptation of the UVS

cone mechanism in *C. viridis* removed polarization sensitivity (Hawryshyn *et al.*, 2003). Furthermore, in salmonids when UVS cones were not stimulated fish were incapable of orientating between e-vectors (Hawryshyn *et al.*, 1990; Degner and Hawryshyn, 2001; Parkyn *et al.*, 2004).

The third objective was to examine the fish's ability to resolve small differences between e-vectors. The possibility of varied discrimination ability of e-vectors required quantification of the minimum angular difference (Δ e-vector) between reference and comparison e-vectors that the fish could discriminate.

3. Materials and methods

3.1 Animals

Behavioural experiments were performed on Green chromis, *Chromis viridis* (Figure 2).

Animals were kept in a flow-through seawater aquarium system for six months prior to experiments. Each fish was kept in a separate aquarium (16cm × 8.5 cm × 10cm). Fish were fed daily with flake food (Formula One – flake food, Ocean Nutrition), and occasionally with freshwater mysis shrimp, *Mysis relicta* (Aqua Yums - Ocean Nutrition).

Temperatures, pH, and salinity were monitored twice daily, and kept at approximately 28°C, a pH of 7.8, and a salinity of 30-32 parts per thousand.

C. viridis spectral and polarization sensitivities are shown in Figure 3. The complexity of polarization sensitivity found in this species and its

relative ease of acquisition (aquarium stores) made *C. viridis* ideal model fish species for these behavioural experiments.

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.

Figure 2.
Green Chromis, *Chromis viridis*, family Pomacentridae.

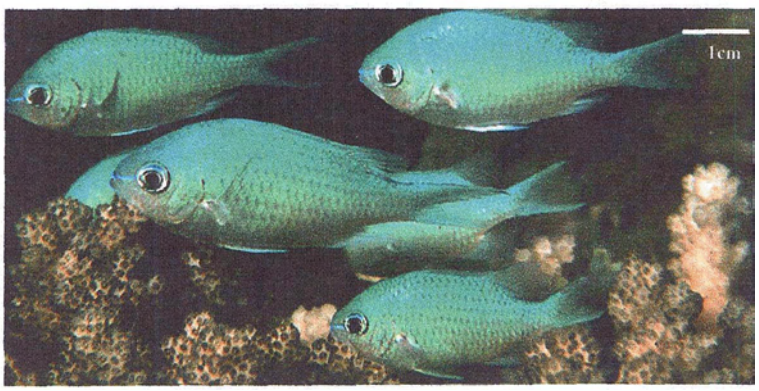
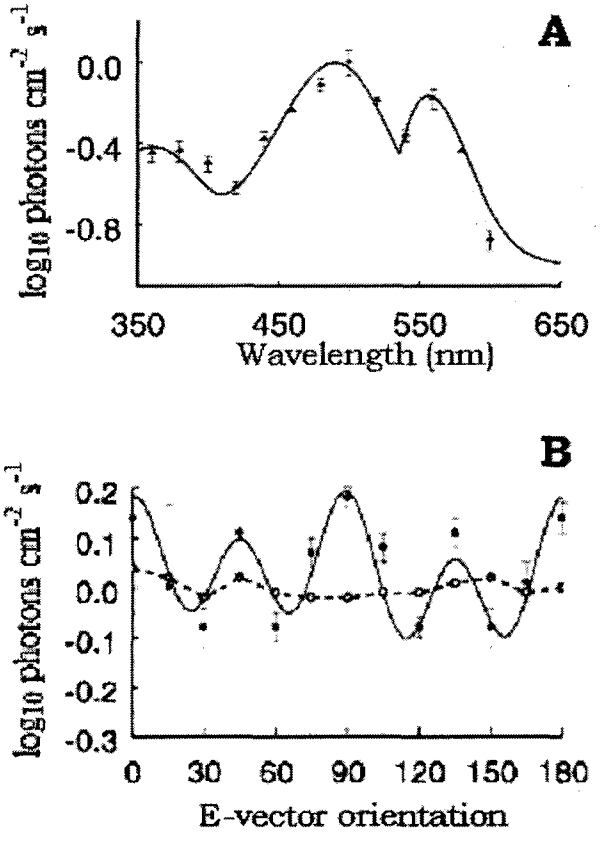


Figure 3.
3A. Mean spectral sensitivity of *C. viridis* recorded using electroretinograms. Sensitivity was determined using the inverse of threshold b-wave amplitude from bipolar cells as a function of intensity for each wavelength. Spectral stimuli were presented onto a white light background (adapted from Hawryshyn *et al.*, 2003).

3B. Mean polarization sensitivity of *C. viridis* recorded using electroretinograms. E-vector sensitivity was determined as for spectral sensitivity, except e-vector varied instead of wavelength. A UV stimulus of 360nm and a white light background were used. The solid line represents polarization sensitivity with a 45° periodicity and approximately 0.3 log unit depth of modulation with maximal sensitivities at 0°, 45°, 90°, and 135°. The dashed line represents polarization sensitivity under UV adaptation (adapted from Hawryshyn *et al.*, 2003).



3.2 Optical system

Light was generated using a 150 W Xenon lamp system, which offers a broad spectral source of high energy light throughout the entire visible spectrum, including UV light, which is fundamental for polarization vision (Hawryshyn *et al.,* 1990; Parkyn and Hawryshyn 1993; Degner and Hawryshyn, 2001; Hawryshyn *et al.,* 2003). A black metal wall (30cm high and 40 cm wide), with a hole, 5cm in diameter, was placed in front of the Xenon lamp at a distance of about 20 cm (Figure 4). The wall acted as a light baffling system to avoid any undesired light or reflection into the experimental area. The beam was projected though two UV transmissive lenses onto a beamsplitter, which transmitted 50 percent of the light to one optical window of the behavioural chamber, and 50 percent to a front surface mirror lens positioned at 45 degrees with respect to the beamsplitter and the window of the behavioural chamber (Figure 4). The reflected beam was redirected onto a front surface mirror and then projected to the second of the two optical windows (13cm spacing) on the front wall of the behavioural chamber. 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 intensity. Measurements of the light stimuli within the test tank were taken using an integrating radiometer (Photodyne Model 88XLA, Radiometer/Photometer - Optikon). Light passed through a diffuser to remove any inherent polarized light. Between the polarizer and the quartz windows in the test tank were two UV-grade polarizing filters. The diffuser and UV-grade polarizing filters were positioned in indexed holders (Figure 5), which could fit onto the

quartz windows of the behavioural chamber and the plane of polarization was manipulated as required.

Figure 4.
Optical system. Light from the 150 W Xenon lamp was projected though two UV transmissive biconvex lenses before hitting a beamsplitter, which transmitted 50 percent of the light to the right optical window of the behavioural chamber and 50 percent to a front surface mirror, which hit the left optical window of the behavioural chamber.

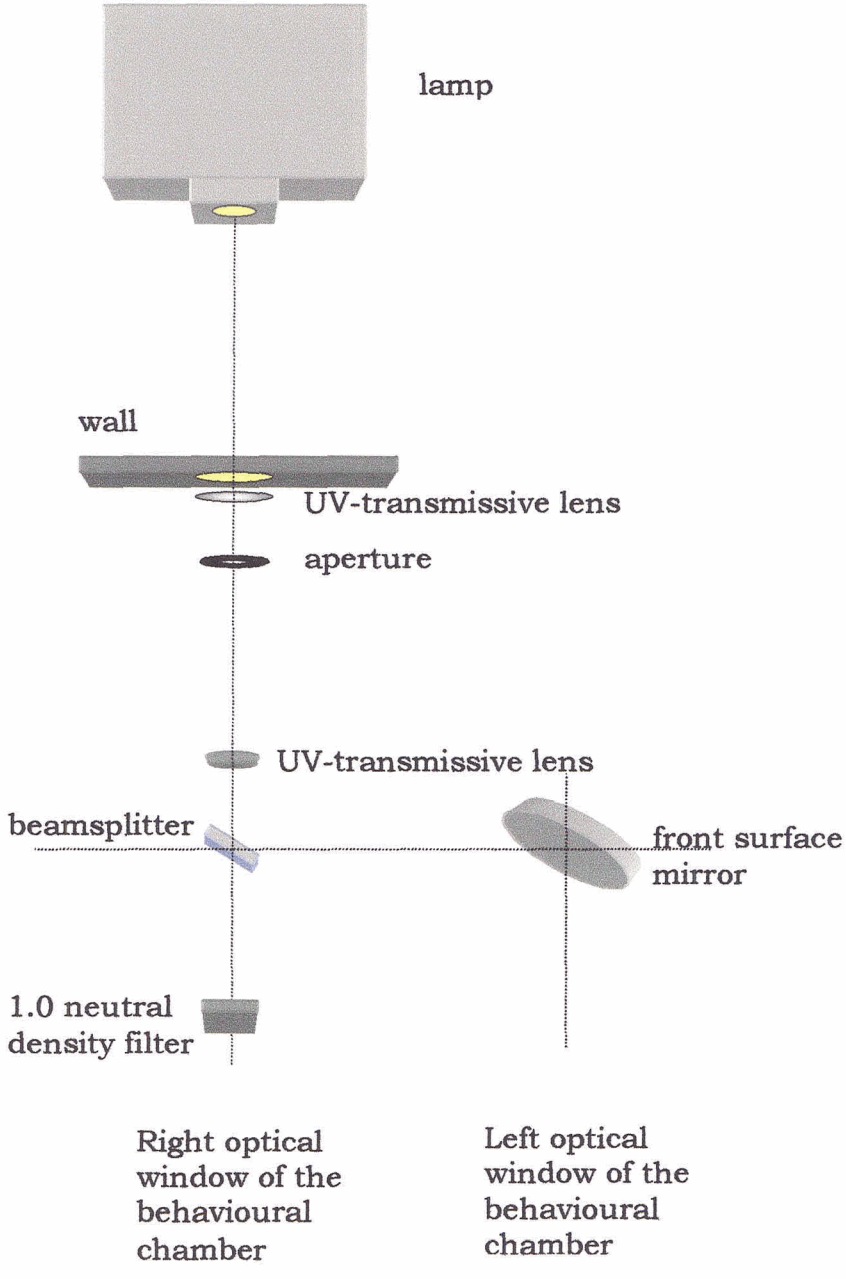
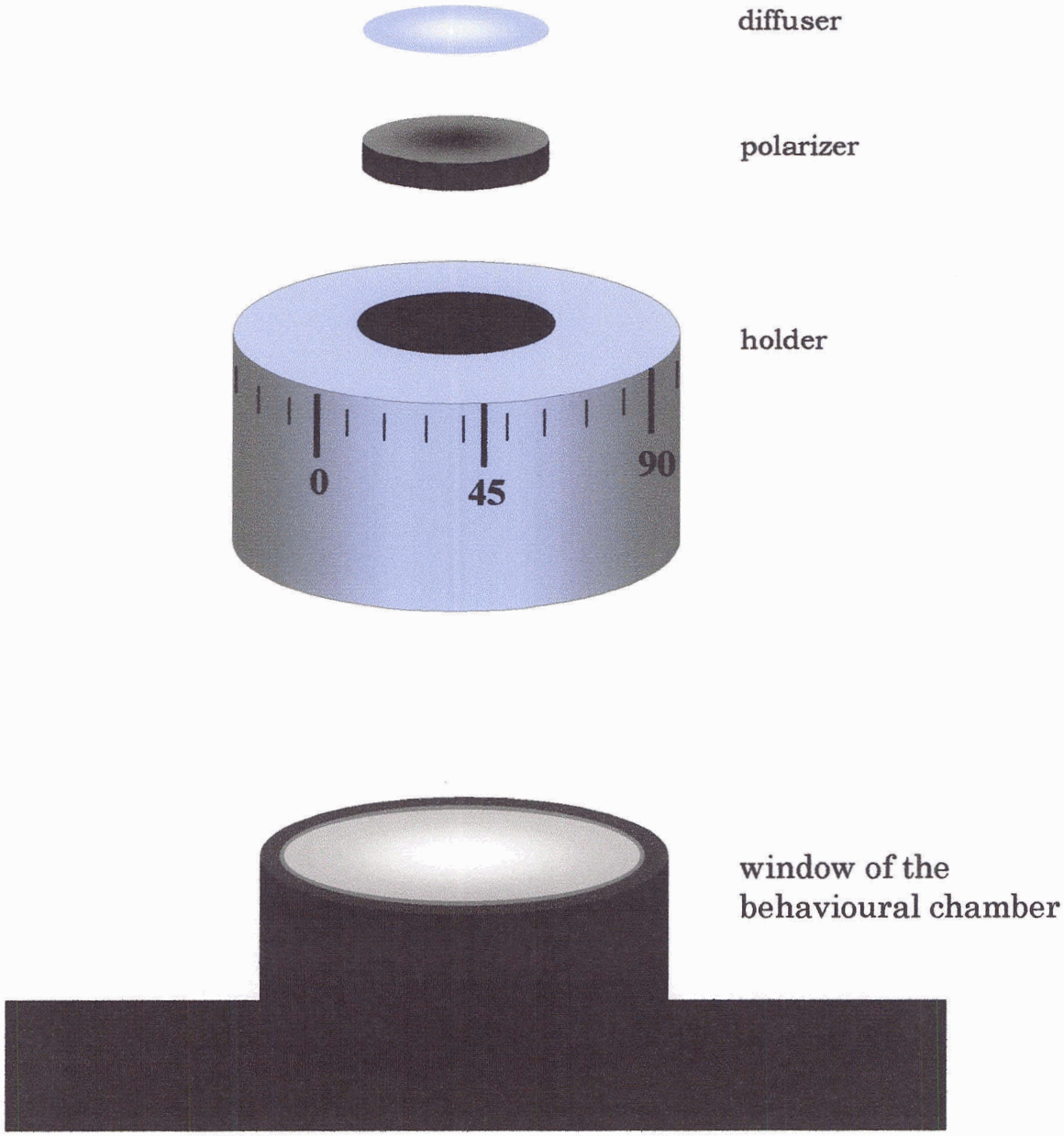


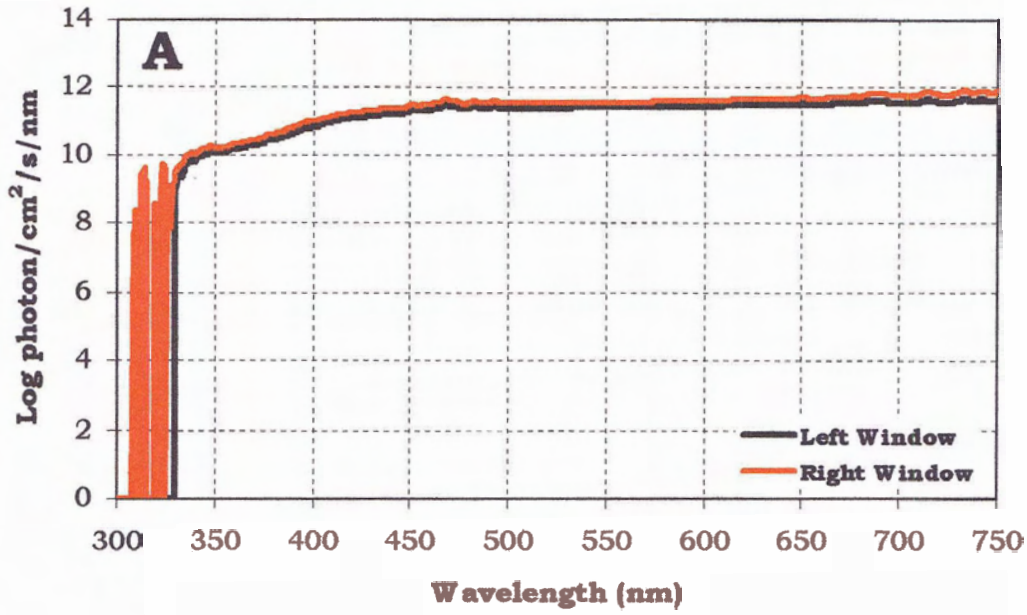
Figure 5.
The diffuser removed any inherent polarized light. The diffuser and UV-grade polarizing filters were positioned in indexed holders. Holders were placed onto the quartz windows of the behavioural chamber.

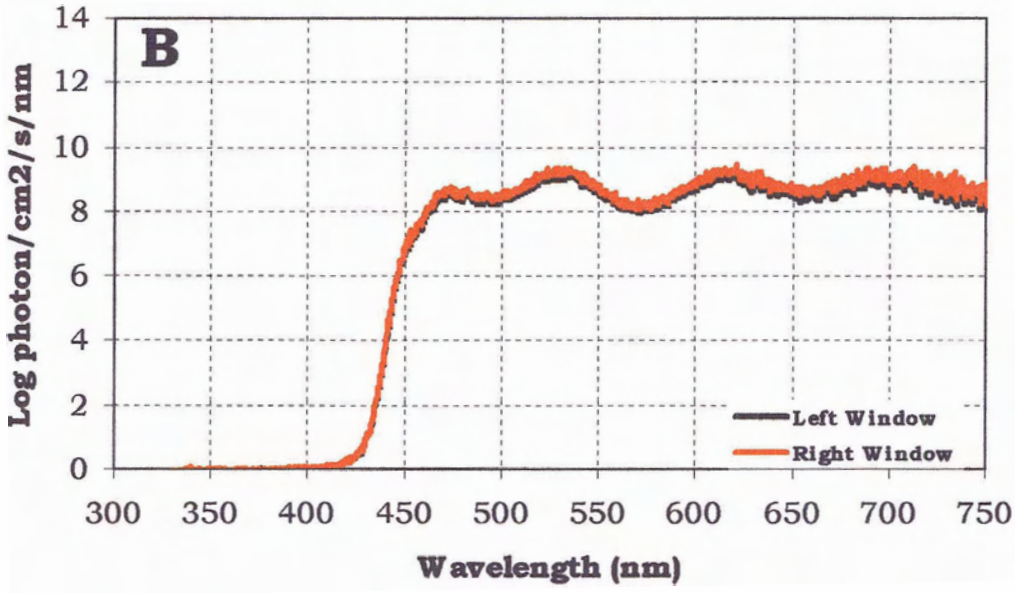


Light entering the two windows in the test tank were spectrally similar (Figure 6A), measured with a spectroradiometer (Ocean Optics USB2000 Fiber Optic Spectrometer with CC-3-UV cosine-corrected irradiance probe, P600-2-UV/VIS fiber optic cable, OOIIrrad Application Software for Irradiance Measurement version 2.01.0, Standard source - LI-COR Spectral Irradiance Lamp Model N. 1800-02L).

I conducted an experiment in which UV light content in the light field was blocked using a 450 nm long pass filter (Corion Spectra Physics (Figure 6B).

Figure 6.
6A. Spectroradiometer measurements (Ocean Optics USB2000), of the light entering the two windows in the test tank.
6B. Spectral background of the light entering the test tank when the UV light content was filtered using a 450 nm long pass filter.



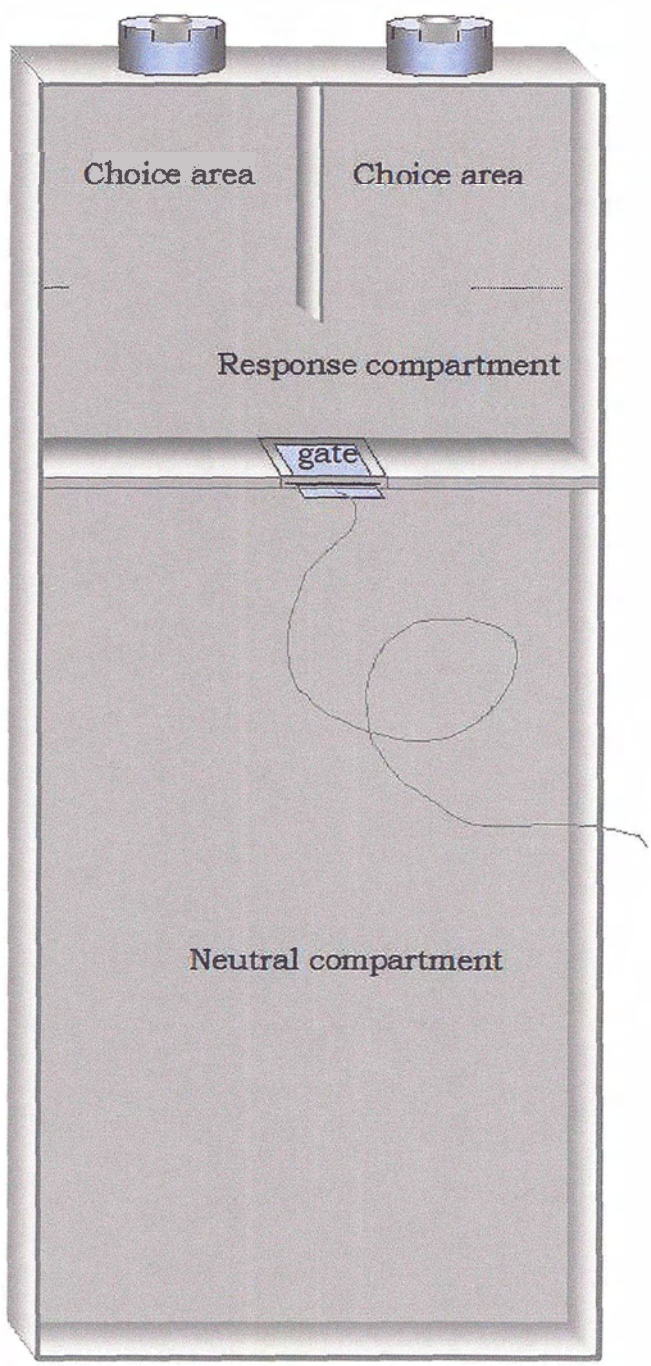


3.3 Behavioural chamber

The flow-through seawater aquarium system and behavioural chamber were connected to a seawater sump, 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 (Figure 7). The neutral compartment (60cm × 16cm × 30.7cm) 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 (30.7cm × 16cm × 44.8cm) had two round quartz optical windows (diameter 6.7cm), partially separated by a black Plexiglas barrier, through which light was passed.

Figure 7.
The behavioural chamber is divided in response and neutral compartment. The two compartments are separate by a gate. Fish had to enter the choice area entirely for a response to be scored.



3.4 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 and swim 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 this study, four fish were used out of 30, which is typical in behavioural discrimination experiments given the difficulty and length of experimentation required to assess visual performance (Neumeyer 1984, 2003; Degner and Hawryshyn, 2001).

Fish were divided in 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 minutes to allow it to acclimatize. 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 minutes, 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 motivational state is maintained throughout the session.

Once the fish consumed the food reward, it was guided back towards the neutral compartment, and the gate closed. This process was repeated at least ten times per day for each fish. The position of the positive and negative stimuli was randomised for each trial, in order to ensure 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 percent of the time based on 20 trials.

3.5 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. Trials began by allowing fish to acclimate for at least 30 minutes in the neutral compartment. At the end of this period, the gate separating the neutral from the response compartment was opened allowing the fish to enter the response compartment, where they were given a choice between the two e-vector orientations.

In the response compartment, fish had to enter the choice area entirely for a response to be scored (Figure 7). 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 seconds subsequent to the opening 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.

3.6 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 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. Ten trials were conducted each day for an individual fish for a total of 40 trials. Either the positive or the negative stimulus was varied by ± 1.0 ND, so that I could verify that discrimination was an indicator of choice based on e-vector orientation rather than brightness differences.

3.7 Eliminating UV light from the Polarization Stimuli

0° and 90° e-vector discrimination experiments were repeated using a 450 nm long pass filter (Corion Spectra Physics). This optical filter eliminates the ultraviolet portion of the spectrum from the light fields of

the two stimuli. Figure 6B illustrates that 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 90° e-vector orientation, and fish B, which was trained to select 0° e-vector orientation. A total of 40 trials was conducted for each fish.

3.8 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 (group one: B and D, and group two: A and C). Group one, which was trained to respond to the e-vector of 0°, was presented with comparison e-vectors between 5° and 45°, i.e. 0° e-vector orientation was tested against 45°, and then 40°, and so on. The comparison e-vectors were randomly presented, and both choice frequency and time to response were recorded. A total of 40 trials was conducted per fish for each comparison e-vector.

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

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

for colour vision and e-vector discrimination for polarization vision. For instance, Neumeyer's studies on goldfish colour discrimination revealed a varied performance across the spectrum that reflects the nature of cone photoreceptor interactions (Neumeyer, 1984). I had planned to evaluate Δ e-vector between 0° and 90° in 15° steps, however fish health problems limited my investigation.

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

4. Results

Green chromis (*C. viridis*) was successfully trained to select and discriminate between the horizontal and the vertical plane of ultraviolet 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 ultraviolet 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 varies 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 comparison e-vector (Δ e-vector) was between 20° and 25°. However, in fish trained to 45° as the reference e-vector, the Δ e-vector was 10°.

4.1 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 (Figure 8). A positive response was considered to be at least a 70 percent correct choice frequency.

The time to response 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 (Figure 9).

Figure 8.
Each session (S1-S10) of training are shown in the x-axis, plotted against choice frequency. The 4 panels show individual training sessions. Positive response was a correct choice frequency of 70 percent or above at least three times in a row.

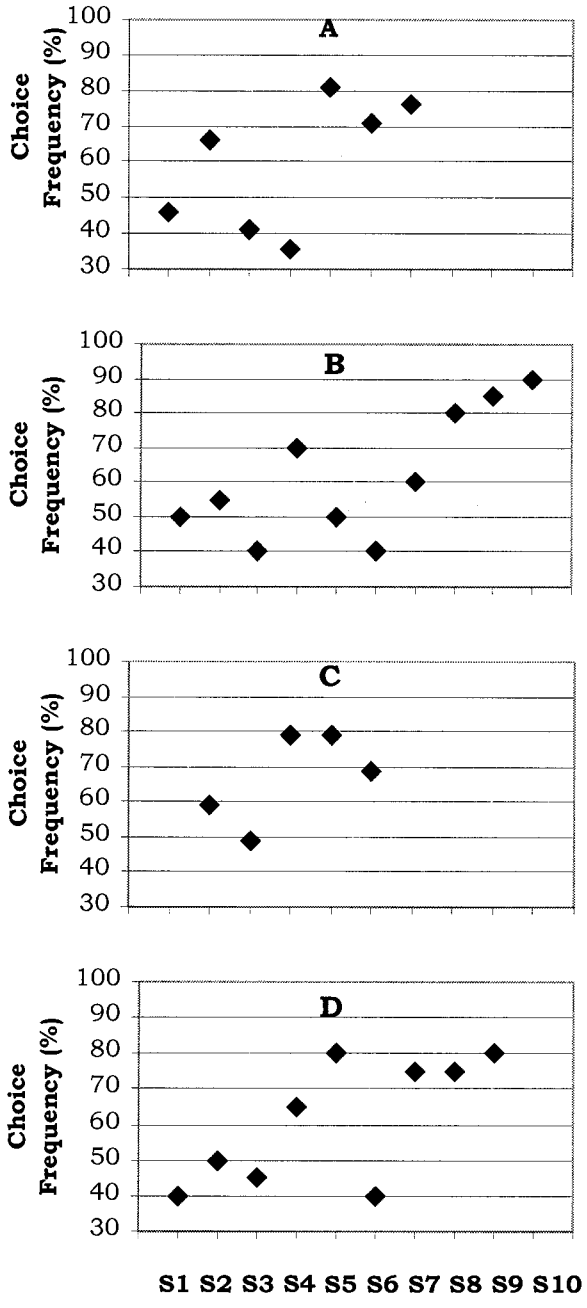
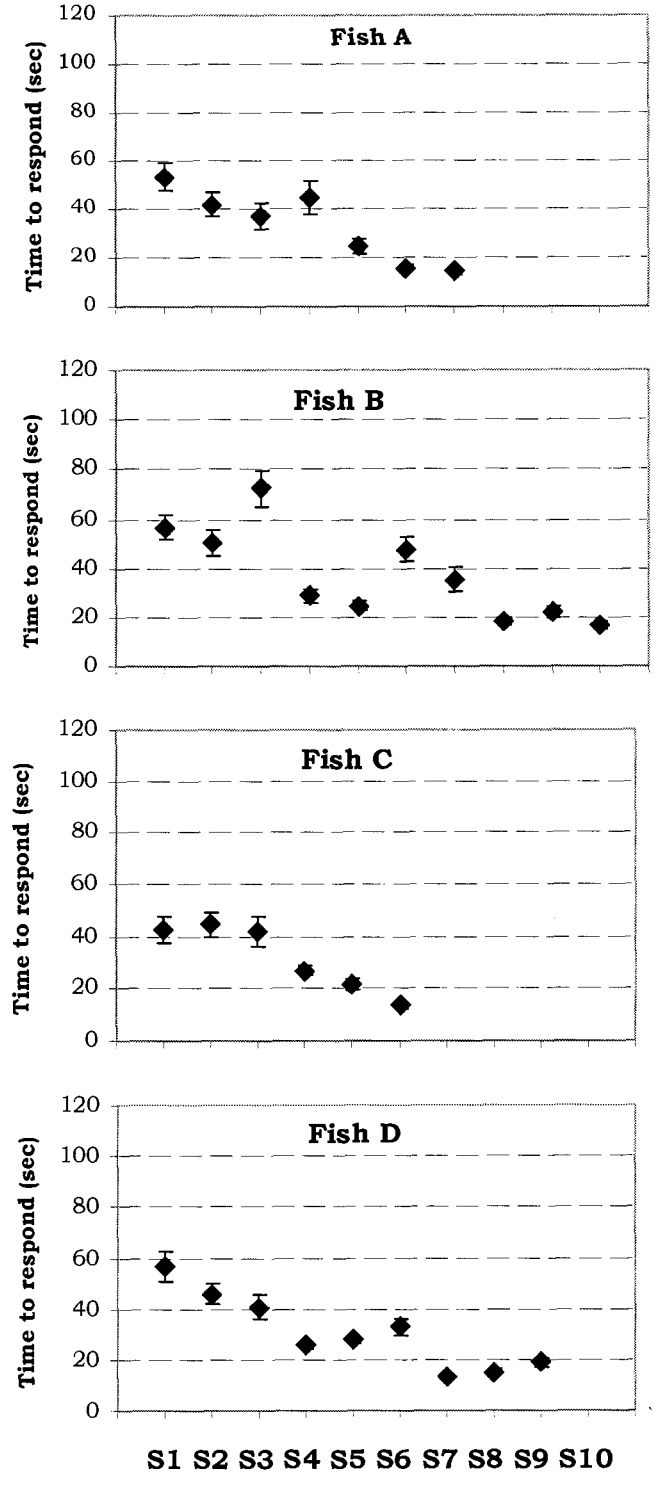


Figure 9.
Time of response ± standard error. The time to respond was recorded from the opening of the gate until fish chose an e-vector within the choice area. The time to respond diminished along with the training sessions in each fish.

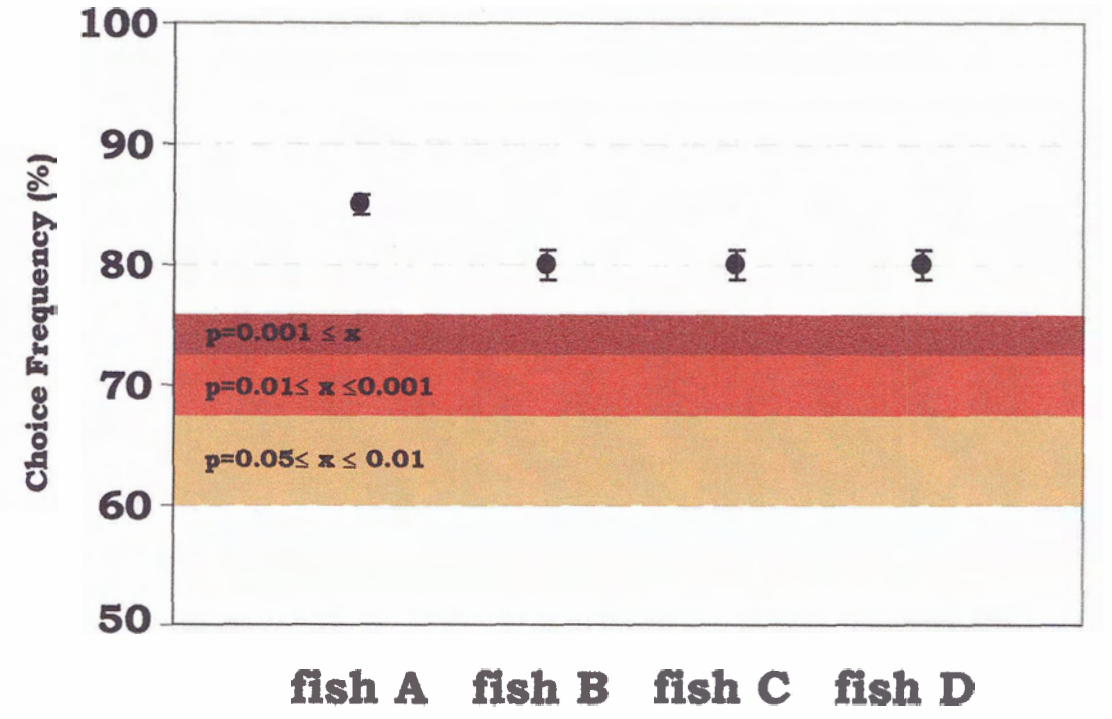


4.2 0° versus 90° E-vector Discrimination

Figure 10 shows that *C. viridis* could discriminate between 0° and 90° e-vector orientations ($p<0.001$, $N=4$). Each fish was tested based on 40 trials, and each point in Figure 10 represents the percentage of correct choice frequency (average ± standard error), when 0° and 90° e-vector orientations were presented. Fish A and C were trained to swim towards 90° e-vector orientation (85 percent and 80 percent correct choice frequency, respectively), whereas fish B and D were trained to select 0° e-vector orientation (80 percent correct choice frequency in both fish).

The average response of all four fish was at or above 80 percent correct choice frequency, indicating that fish could clearly distinguish between the two e-vector orientations. The ranges of significance levels ($p=0.001 \leq x$, $p=0.01 \leq x \leq 0.001$, and $p=0.01 \leq x \leq 0.05$) were calculated using the binomial distribution for forty trials, and they are shown in Figure 10.

Figure 10.
C. viridis could discriminate between 0° and 90° e-vector orientations ($p<0.001$, N=4). Each point is the percentage of correct choice frequency (average ± standard error), when 0° and 90° e-vector orientations were presented. Fish A and C were trained to swim towards 90° e-vector orientation (85 percent and 80 percent correct choice frequency, respectively). Fish B and D were trained to select 0° e-vector orientation (80 percent correct choice frequency in both fish).



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

Table 1. The left column shows the reference e-vector for each fish and the left/right presentation of the positive stimulus in 40 trials. The right column indicates the correct responses to the reference e-vector out of 40 trials, and the left/right presentation of the chosen positive stimulus.

	Reference e-vector ***Left vs. Right***	Correct responses/total ***Left vs. Right***
Fish A	90°	34/40
	19 – 21	*16 – 18*
Fish B	0°	32/40
	19 – 21	*16 - 16*
Fish C	90°	32/40
	22 – 18	*18 - 14*
Fish D	0°	32/40
	17 – 23	*14 - 18*

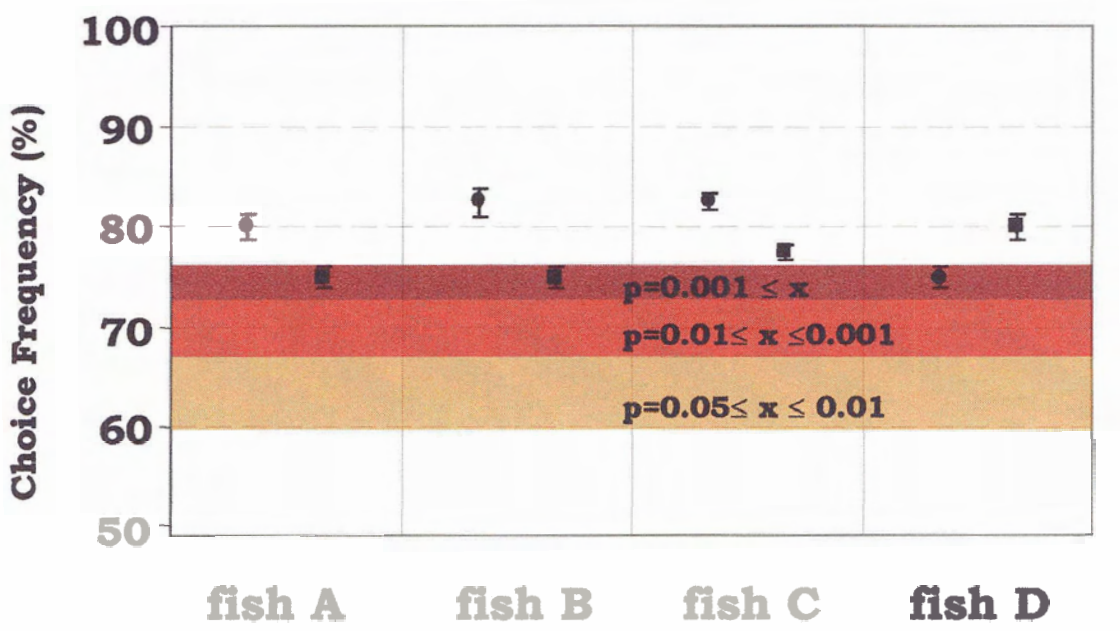
4.3 The Brightness Test

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 (Figure 11). A total of 80 trials per fish (N=4) were conducted. Each circle in Figure 10 represents the percentage of correct choice frequency (average ± standard error), when 0° was dimmer than 90°. Each square in Figure 11 represents the percentage of correct choice frequency (average ± standard error), when 90° was dimmer than 0°.

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 percent of correct choice (Figure 11). When the positive stimulus 90° was dimmer than the negative 0°, correct choice between the two stimuli occurred at relatively lower frequencies, i.e. 75 and 77.5 percent for fish A and C, respectively (Figure 11).

In contrast, fish B and D did not show any particular pattern. Fish B chose its reference e-vector 82.5 percent of the time when it was dimmer than the negative stimulus; whereas, when the negative stimulus 90° was dimmer than the positive stimulus, correct choice was 75 percent. Fish D showed an opposite pattern; when its reference e-vector (0°) was dimmer, fish chose it 75 percent of the times; whereas, when the negative stimulus was dimmer, fish selected correctly 80 percent (Figure 11).

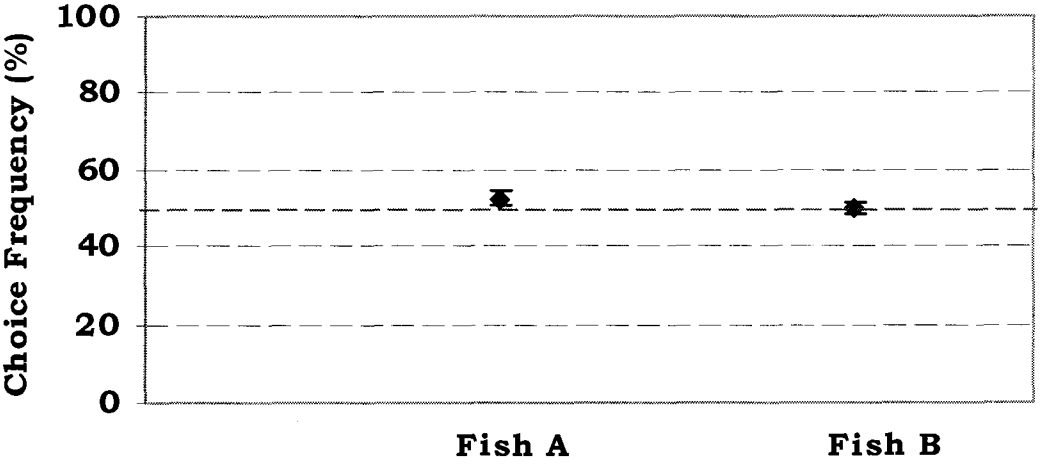
Figure 11.
The brightness test showed that the choice between the horizontal and the vertical plane of polarized light was made based on the orientation of the e-vectors, independent of the light intensity of the stimuli (80 trials per fish, N=4). Each circle represents the percentage of correct choice frequency (average ± standard error), when 0° was dimmer than 90°, whereas each square represents the percentage of correct choice frequency (average ± standard error), when 90° was dimmer than 0°.



4.4 Eliminating UV light from the Polarization Stimuli

Fish could no longer discriminate between their respective reference and comparison e-vectors when the ultraviolet part of the spectrum in both stimuli was filtered out using 450 nm long pass filter (Figure 11). A total of 40 trials was used to test each fish (N=2), and choice frequency was approximately 50 percent. This clearly indicated that the fish chose randomly between the two stimuli.

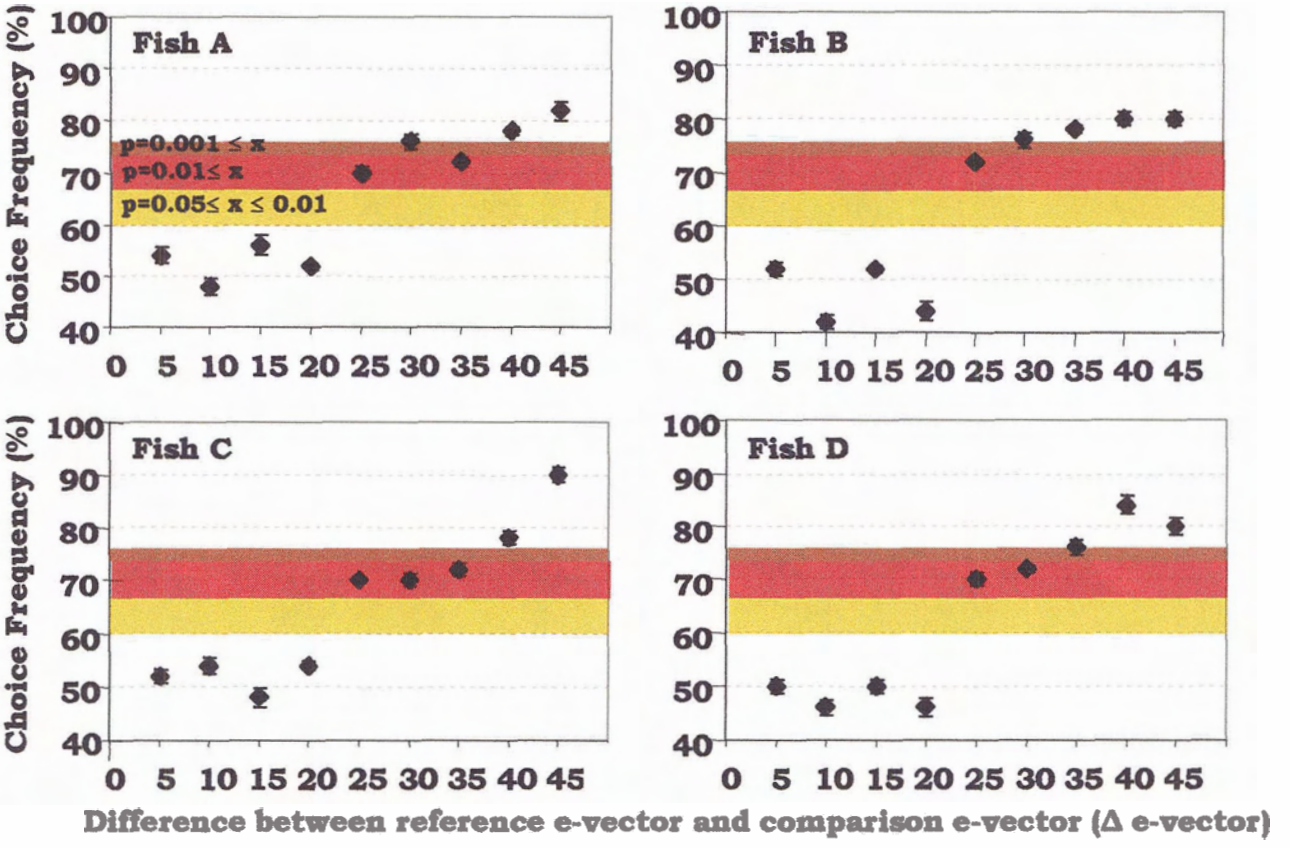
Figure 12.
Fish did not discriminate between e-vectors when the ultraviolet part of the spectrum in both stimuli was removed using 450 long pass filter (40 trials per fish, N=2).



4.5 Minimum Angular Difference in E-Vector Discrimination

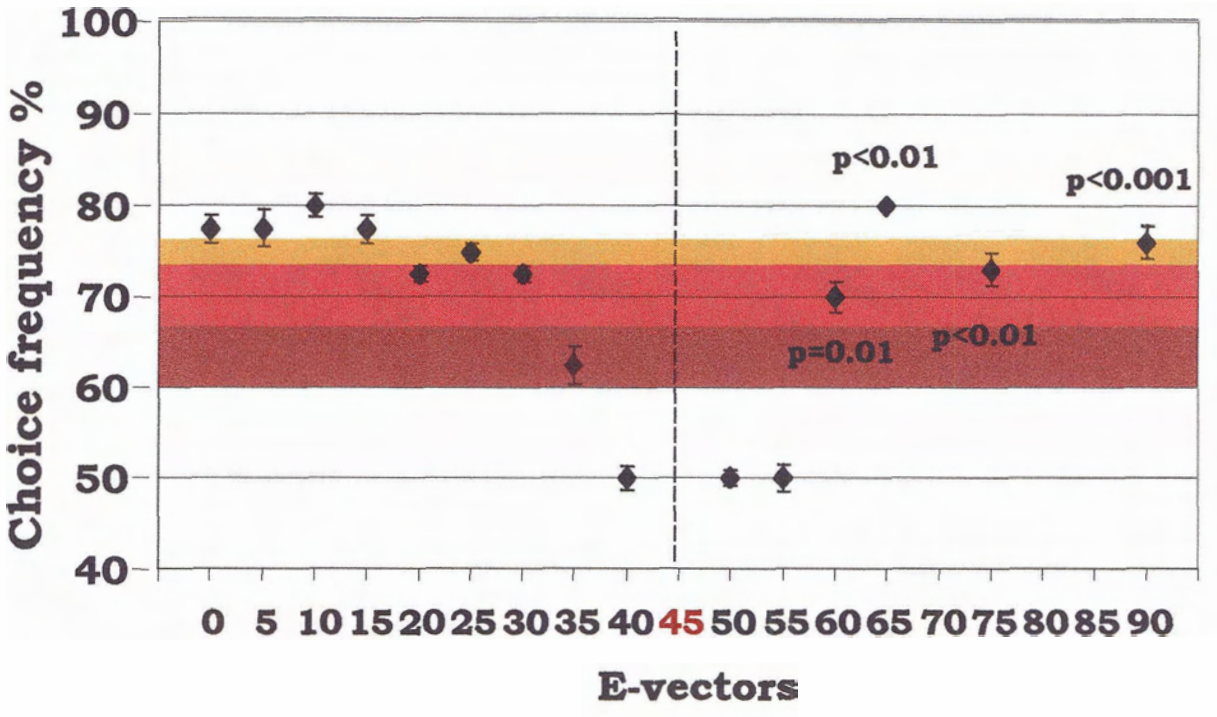
Figure 13 shows the percentage of choice frequency for each fish plotted *versus* the angular difference between the reference e-vector and the comparison e-vector (Δ e-vector). Each point in Figure 13 represents the percentage of correct choice frequency (average ± standard error) between the reference e-vector and the comparison e-vector. A total of forty 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° were the reference e-vector. Fish B and D could discriminate between 0° and 25° (72 percent and 70 percent, respectively), but could not discriminate between 0° and 20° (44 percent and 46 percent, respectively). Similarly, fish A and C could differentiate between 90° and 65° (70 percent in both fish), but were not able to discriminate between 90° and 70° (52 percent and 54 percent, respectively). Interestingly, the smallest Δ e-vector was approximately 25° for both reference e-vectors (0° and 90°) indicating an inherent symmetry around these polarization angles.

Figure 13.
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 (average ± standard error), between the reference e-vector and the comparison e-vector (40 trials per fish, N=4). The smallest Δ e-vector was approximately 25° in all four fish. The ranges of significance levels $p=0.001 \leq x$, $p=0.01 \leq x \leq 0.001$, and $p=0.01 \leq x \leq 0.05$, are indicated and were calculated using the binomial distribution for forty trials.



When Fish C was re-trained to respond to 45° e-vector orientation, the smallest Δ e-vector was approximately 15° (Figure 13). The left side of the Figure 13 shows the choice frequencies between the reference e-vector (45°) and the 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 percent correct choice), and between 45° and 60° (70 percent of correct choice), but not between 45° and 40° (50 percent correct choice) or between 45°and 55° (50 percent correct choice). The correct choice frequencies of the fish trained to 45° showed a notably higher resolution of discrimination than fish trained to 0° or 90°.

Figure 14.
The figure shows Δ e-vector plotted against the percentage of choice frequency 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 e-vectors of comparison up to 0°, and the right side shows the choice frequencies between 45° and the e-vectors of comparison up to 90°. The smallest Δ e-vector was approximately 10°.



5. Discussion

5.1 Ultraviolet Polarization Vision in C. viridis

A two-choice behavioural experiment using the learned response protocol was conducted in this study. 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.

My behavioural experiments demonstrated the ability of *C. viridis* to perceive and utilize polarized light cues confirming the ERG results. Most importantly, measurements of e-vector discrimination facilitate characterization of *C. viridis* polarization vision. *C. viridis* discriminated between 0° and 90° e-vector orientations of polarized light (Figure 10), and the discriminative capabilities were not compromised by manipulating the brightness content of the stimuli (Figure 11). Therefore, choice was made based on the e-vector orientations regardless of brightness differences between the two stimuli (1 log photons $\cdot$ cm^{-2} $\cdot$ s^{-1} difference in intensity). Note that the relative spectral distribution of the two stimuli was maintained. 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.

In contrast, fish could no longer discriminate between 0° and 90° e-vector orientations when the ultraviolet part of the spectrum was filtered out (Figure 12). The 450 nm long pass filter narrowed the spectral width of the stimuli (Figure 7), eliminated the UV portion of the spectrum from the polarized light field. The amount of energy absorbed by each cone class was calculated with and without the 450 nm long pass filter (Figure 5B). Using the long pass filter, the quantum catch of the UV-sensitive cones was effectively eliminated. The use of the 450 nm long pass filter demonstrates the critical role played by the ultraviolet portion of the spectrum for polarization vision; when UV-sensitive 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.,* 2004). In Hawryshyn *et al.* (2003) a UV-transmitting filter (Shott -UG-11) was used to produce UVS cone chromatic adaptation. This disabled polarization sensitivity (flat curve in Figure 3). Therefore, electrophysiological and behavioural experiments indicate that stimulation of the UVS cone mechanism is an essential requirement for e-vector discrimination.

Many vertebrates have corneas and lenses that filter ultraviolet light (Kennedy and Milkman, 1956, Muntz, 1972), but this is not the case for goldfish (Hawryshyn *et al.,* 1985), salmonids (Hawryshyn *et al.*, 1989) and damselfishes (McFarland and Loew, 1994; Losey *et al.,* 2003). The family Pomacentridae has significant ocular media transmission well into the UV-A portion of the spectrum (Losey *et al.*, 2003).

Perception of polarized light in the ultraviolet represents an interesting aspect of polarization vision in animals, since it has been shown that both in clear skies and underwater there is significantly lower UV radiation than in the longer wavelength portion of the spectrum (Cronin and Shashar, 2001; Barta and Horvath, 2004). However, it has been suggested that celestial ultraviolet polarized light is the most stable and detectable cue under clouds and forest canopies (Pomozi *et al.*, 2001; Barta and Horvath, 2004).

5.2 Mechanisms of Polarization Vision in C. viridis

Measurements of e-vector discrimination facilitate characterization of *C. viridis* polarization vision. Figure 12 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 approximately 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 values of Δ e-vector were approximately 15° (Figure 13). Changes in reference e-vector from either 0° or 90° to 45° further illustrates the complexity of polarization vision in *C. viridis* and points at the possible opponent interaction between the vertical and horizontal polarization detectors. This experimental finding also demonstrates the importance of experimental design required 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 polarization sensitivity in damselfish and salmonids mediates different behavioural activity, these two species could conceivably have the same polarization detection/cone mechanisms. ERG data (Hawryshyn *et al.*, 2003, Figure 2B), showed that *C. viridis* has complex polarization sensitivity with four peaks at 0°, 45°, 90° and 135° e-vector orientation. Peak polarization sensitivities at 0° and 90° may follow the two-channel system found in salmonids and cyprinids, where one detector was maximally sensitive to 0° e-vector orientation (or vertical plane of polarized light), and the other to 90° e-vector orientation (or horizontal plane of polarized light) (Hawryshyn and McFarland, 1987; Parkyn and Hawryshyn, 1993; Coughlin and Hawryshyn, 1995; Novales Flamarique and Hawryshyn 1997). Polarization sensitivity in salmonids has been investigated using compound action potential (CAP) recording from the optic nerve (Parkyn and Hawryshyn, 1993, 2000), while in damselfishes, polarization sensitivity was recorded through ERG (Hawryshyn *et al.*, 2003). Electroretinograms measure mainly bipolar cell activity occurring in the retina, whereas the information recorded at the level of the optic nerve represents the ultimate signal going to the brain (ganglion cell axons) subsequent to interneuronal processing in the retina.

Recently, ERG recordings have been conducted in rainbow trout (*Oncorhynchus mykiss*) that show the same four-peaked polarization sensitivity pattern at 0°, 45°, 90° and 135° as in *C. viridis* (Ramsden *et al.*, 2004). Such differences between ERG and CAP recordings in rainbow

trout reveal information about interneuronal interactions within the retina. Since optic nerve PS recordings have not been performed in *C. viridis* one cannot exclude the possibility that damselfish and salmonids have similar neural network for processing polarized light consisting of a two channel detection system. This study shows that e-vector discriminative capabilities increased when the test fish was trained to 45°. This is consistent with what one would expect from a two channel detection system (vertical and horizontal detector classes) since discrimination should improve at e-vector orientations within the zone of maximum overlap of the detector sensitivities not unlike what would be evident in wavelength discrimination.

5.3 Polarization Vision and Colour Vision

As indicated previously, e-vector discrimination could be considered analogous to hue discrimination in colour vision. Colour can be described by intensity, purity, and wavelength as polarization can be described by intensity, degree of polarization, and of 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, 1986). 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 angular region where the sensitivity of the two detectors show the greatest degree of overlap. The peak sensitivity at 45° could result from the interaction between the two channels, i.e. the detector of the vertical plane of polarized light (0°), and the detector of the horizontal plane of polarized light (90°)(see Figure 2B). The neural interaction between the two channels likely explains the higher discriminative capabilities around 45° e-vector orientation.

5.4 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 an environment where horizontal polarized light is predominant (Cronin and Shashar, 2001), other e-vector orientations would elicit contrast between targets and background. For *C. viridis,* which form large schools above coral reefs feeding on zooplankton carried by currents (Hobson, 1974), foraging

success is strongly dependent on the visibility of prey. 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 ultraviolet vision and polarization sensitivity 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, 2001a,b; 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, 2001a,b; 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 important visual tasks, such as 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). Social behaviour often involves intricate communication abilities as a result of coevolution of efficient signal design and visual sensitivity (McDonald *et al.*, 1995 a and 1995 b). Damselfishes possess chromatophores, which provide many combinations of body colouration and patterns, and they also possess iridophores (Fujii, 1993). Iridophores consist of a lamellar structure of thin guanine crystals, which reflect light through interference occurring in the stacks of guanine

crystals (Denton and Nicol, 1965; Fujii, 1993). As well, light reflected from iridophore crystals can produce polarization (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; Shashar and Hanlon, 1997; Shashar *et al.*, 2001). Moreover, *C. viridis* can rapidly change polarization patterns on their bodies 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; Fujii, 2000). For *C. viridis*, which is able to discriminate small differences in e-vector orientation, 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, 1977; Shashar *et al.* 2001). Therefore, reflections off the fish body could provide polarized light signals at e-vector orientations that would be contrasting to those surrounding the fish.

6. Conclusions

Investigation of the functional significance of polarization vision is a relatively new research topic. While polarization sensitivity has been intensively studied in terrestrial arthropods (Rossel and Wehner, 1984; Fent, 1986; Rossel, 1983; Labhart and Meyer, 1999; Labhart, 1999, Dacke *et al.*, 1999) and marine invertebrates (Saidel *et al.*, 1983; Shashar and Cronin, 1996; Marshall *et al.*, 1999; Shashar *et al.*, 2002), marine vertebrates have largely been ignored, as only goldfish, clupeids, cichlids, salmonids, and recently, damselfishes have been investigated (Waterman, 1974; Waterman and Hashimoto, 1974; Waterman and Aoki, 1974; Kawamura *et al.*, 1981; Hawryshyn and Bolger, 1990; Coughlin and Hawryshyn, 1995; Novales-Flamarique and Hawryshyn, 1998; Parkyn and Hawryshyn, 2000; Degner and Hawryshyn, 2001; Hawryshyn *et al.*, 2003).

This research provides the first behavioural evidence of e-vector discrimination in vertebrates. It represents an important step for understanding the dynamic nature of the damselfish visual system and provides a foundation for interpreting the ecological role in coral reefs.

This study demonstrated that *C. viridis* is able to select and discriminate between the horizontal and the vertical plane of ultraviolet polarized light independent of the stimuli brightness content. However, e-vector discrimination capability disappeared when the ultraviolet portion of the light stimuli was removed, indicating that the presence of UV light is critical for e-vector discrimination. Also, fish were able to distinguish between relatively small e-vector orientations of polarized light, and Δ e-vector varied based on the learned reference e-vector.

My research has provided evidence to justify future work in damselfish. For instance, investigation of behavioural outputs of polarization vision in *C. viridis*, underlying physiological and neural mechanisms of polarization vision, and how fish use their e-vector discriminative capabilities. Also, this research has provided experimental design and methodology for behavioural examination of polarization sensitivity in other organisms.

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