



# Acquisition of polarized-light orientation in salmonids under laboratory conditions

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We examined orientation responses of juvenile salmonids to a polarized-light stimulus under laboratory conditions. Juvenile rainbow trout, *Oncorhynchus mykiss*, steelhead (anadromous), *O. mykiss*, and brook char, *Salvelinus fontinalis*, were trained using an operant conditioning methodology to orient relative to the axis of a linear-polarized light field. On average, rainbow trout responded correctly relative to the orientation of the light stimulus approximately 70% of the time within five training sessions. The proportion of correct responses increased further under an intermittent reinforcement schedule. We released trained and untrained fish individually in a circular tank and quantified orientation responses using a digital image-tracking system. Experimentally naïve rainbow trout had no directional tendency, in contrast to trained rainbow trout, steelhead and brook char, which oriented relative to the plane of polarized light. Trained fish showed no orientation response when a diffuser was used to depolarize the light source. Rainbow trout trained to orient parallel to a polarization axis in the laboratory were tested under natural skylight before sunset. These fish oriented parallel to the bearing of maximally polarized light in the celestial hemisphere.

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Although speculation on the mechanisms mediating migration in fish has been documented for over a hundred years (Buckland 1880), the first scientific study of this phenomenon was undertaken early in the 20th century (Craigie 1926). Sensory mechanisms and cues implicated in migratory behaviour of fish include audition (Kleerekoper & Chagnon 1954), chemotaxis (Craigie 1926; Hasler & Wisby 1951; Wisby & Hasler 1954; McInerney 1964; Johnson & Hasler 1980; Hasler & Scholz 1983; Stabell 1992; Nevitt et al. 1994), electrolocation (Heiligenberg 1974; Kalmijn 1982; Metzner & Viete 1996), magnetic sense (Quinn 1980; Kalmijn 1982; Quinn & Brannon 1982; Taylor 1986; Klimley 1993), rheotaxis (Arnold & Cook 1984; Quinn & Groot 1984), thermal gradients (Ekman 1932; Neave 1964; Groot & Quinn 1987) and visually based cues, such as landmarking and various forms of sun-compass orientation (Aronson 1951; Hasler et al. 1958; Braemer 1960; Winn et al. 1964; Dill 1965, 1971; Groot 1965; Hasler 1971; Forward et al. 1972; Waterman & Forward 1972; Forward

& Waterman 1973; Kleerekoper et al. 1973; Churmasov & Stepanov 1977; Kawamura et al. 1981; Levin & Belmonte 1988; Hawryshyn & Bolger 1990; Hawryshyn et al. 1990).

With the discovery that several species of fish orient to linearly polarized light, it was proposed that they also might use celestial polarized light cues as a guidance mechanism during migration or other movements (Dill 1965, 1971; Groot 1965; Waterman & Forward 1970; Forward et al. 1972; Forward & Waterman 1973; Hawryshyn & Bolger 1990; Hawryshyn et al. 1990). The utility of polarized light as a cue for orientation behaviour has been demonstrated for invertebrates (von Frisch 1949; Waterman 1981; Wehner 1983, 1997; Goddard & Forward 1991), amphibians (Taylor & Adler 1973; Phillips et al. 2001) and birds (Kreithen & Keeton 1974; Able 1982, 1989; Moore & Phillips 1988; Phillips & Waldvogel 1988; Phillips & Moore 1992; but see Kramer 1950; Montgomery & Heinemann 1952; Coemans et al. 1990; Vos Hzn et al. 1995).

Migratory salmonids (e.g. trout, char and salmon) are of particular interest because of both their economic value and their high degree of philopatry or spawning-site fidelity during reproductive migrations (over 85%; Quinn 1990). Although physiological characterization of polarized-light sensitivity in salmonid fish has been presented elsewhere (Parkyn & Hawryshyn 1993, 1999, 2000; Coughlin & Hawryshyn 1995; Novales Flamarique

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& Hawryshyn 1997), only a few studies have examined the effect of polarization sensitivity on the orientation behaviour of these fish (Hawryshyn & Bolger 1990; Hawryshyn et al. 1990; Degner & Hawryshyn 2000).

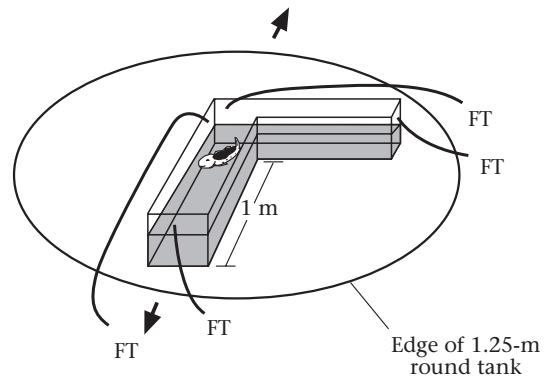
Ultraviolet-A (UV) radiation (300–399 nm for the purposes of this paper), linearly polarized UV and visible light (400–700 nm) are transmitted by the lens and ocular media of both rainbow trout, *Oncorhynchus mykiss*, and brook char, *Salvelinus fontinalis* (Hawryshyn et al. 1989; Parkyn 1998). UV has been implicated in the detection of polarization in fish (Hawryshyn & McFarland 1987; Kunz & Callaghan 1989; Hawryshyn & Bolger 1990; Hawryshyn et al. 1990; Hawryshyn 1992; Parkyn & Hawryshyn 1993, 2000; Coughlin & Hawryshyn 1995; Novales Flamarique & Hawryshyn 1997; Novales Flamarique et al. 1998). However, the role of UV polarization sensitivity was unrecognized when earlier field-based studies of polarized-light orientation in fish were conducted (e.g. Groot 1965; Dill 1971; Forward et al. 1972; Forward & Waterman 1973), although Kramer (1950) suggested that UV cues might be an important factor for orientation behaviour in birds.

The objectives of the present study were three-fold: (1) to test whether hatchery-reared rainbow trout innately orient to plane-polarized light; (2) to compare orientation responses of potamodromous or land-locked juvenile rainbow trout, anadromous or ocean-going steelhead, *O. mykiss*, and potamodromous brook char under controlled laboratory conditions, using an operant behaviour methodology, to assess whether the degree of anadromy affects the ability to orient to polarized light; and (3) to examine orientation responses of laboratory-trained juvenile rainbow trout relative to a natural polarized light field. Although rainbow trout have been shown to orient to polarized light in laboratory conditions (Kawamura et al. 1981; Hawryshyn et al. 1990; Degner & Hawryshyn 2000), this study represents the first interspecies comparison and examination of this phenomenon in either *S. fontinalis* or migratory *O. mykiss*. Physiological studies have shown that juvenile rainbow trout, steelhead and brook char have polarization sensitivity (Parkyn & Hawryshyn 1993, 2000). The present study addressed whether this physiological sensitivity is indicative of the ability of these fish to behaviourally orient to plane-polarized light.

## METHODS

### Origins and Maintenance of Study Animals

Juvenile rainbow trout (stock origin=Badger Lake, British Columbia, Canada), steelhead (stock origin=Cowichan River, British Columbia), and brook char (stock origin=Aylmer Lake, British Columbia, originally introduced from Quebec in 1908; Clemens & Wilby 1949) were held in the fish culture facility at the University of Victoria, for a minimum of 4 weeks before testing. During this interim, fish were maintained at 15°C on a 12:12 h light:dark cycle and fed every second day with commercial fish food pellets (Biodiet Grower, Warrington, Oregon, U.S.A.). All fish were trained and tested within



**Figure 1.** Training tank for laboratory experiments, illustrating positions of tubing used for delivery of food rewards. Solid arrow heads indicate maximal plane of polarized light. FT=Tygon feeding tube.

the 12-h daylight portion of the photoperiod. Subjects were housed individually in 20-litre aquaria. The University of Victoria Animal Care Committee, in accordance with the Canadian Council for Animal Care, approved all experiments.

### Training Protocol

Fish were trained to orient either perpendicular or parallel to the axis of a linear polarizer (Hawryshyn & Bolger 1990). Observations were conducted on juvenile rainbow trout ( $\bar{X} \pm \text{SE}$  wet weight =  $4.8 \pm 0.3$  g) to determine the effectiveness of the training protocol.

Training of the fish involved the use of an L-shaped training-tank (1 × 1 m) constructed from UV/visible light-transmitting acrylic (OP-1, CYRO Plastics, Mississauga, Ontario, Canada), placed within a larger, 2.5-m diameter, round tank with 1-m walls (Fig. 1). The water in the training tank was changed before placement of a fish for training and was maintained at a 15-cm depth. This was done both to keep the water temperature relatively constant at 15°C and to reduce the effects of olfactory cues on behaviour. Fish were deprived of food for 3 days before the first training session. A single fish was placed in a clear plastic cylinder positioned at the vertex of the L-shaped training tank. After a 5-min acclimation period, we removed the cylinder and recorded the channel initially selected by the fish following release. A fish was considered to have selected a channel if it either remained in one of the two channels of the training tank for more than 30 s, or swam to the end of a channel. Fish that performed a correct response (e.g. swimming down the arm of the tank that was parallel to the plane of polarized light projected on the tank) received a food reward for each correct response, for up to 10 food rewards in a single training session. Food rewards consisted of a pellet (Biodiet Grower) delivered through one of four Tygon tubes at the two ends of the tank and on opposite walls of the training tank at its vertex (Fig. 1). These feeding tubes extended to the outer perimeter of the larger round tank. Food pellets were delivered by sending a short pulse of air down the tube, which caused

**Table 1.** Percentage polarization transmission of linearly polarized light projected through an acrylite diffuser (OP-1, CRYO Plastics), measured using a photometer and an analyser (Polaroid HNP'B)

Wavelength (nm)	% Polarized light (without diffuser)	% Polarized light (with diffuser)
380	93.6±1.7	0.57±0.4
460	94.4±1.2	0.68±0.2
540	95.4±2.6	0.62±0.3
600 LP+650 SP	97.1±1.2	0.33±0.7

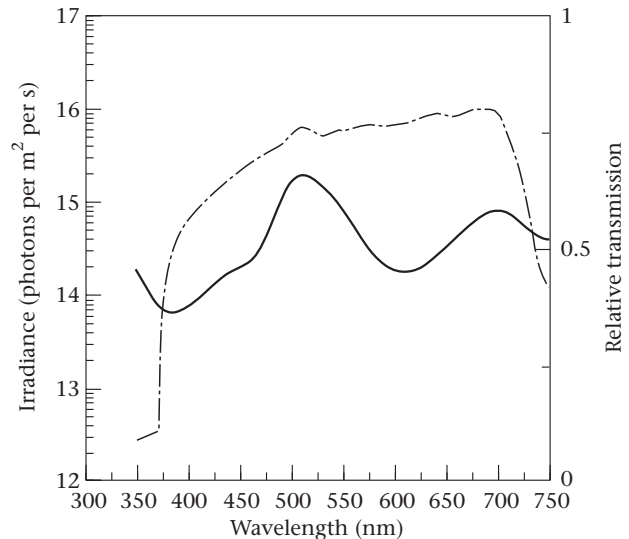
Wavelengths correspond to 380-, 460- and 540-nm narrowband interference filters, and a 600-nm long-pass interference filter (LP) in combination with a 650-nm short-pass filter (SP) for the red region of the spectrum. Percentages are given as±SE.

the pellet to land on the surface of the water near the end of the tank where the tube inserted. When the fish swam back to the vertex of the tank, a second pellet was delivered as a reward. Fish that did not train by actively foraging were eliminated from the trials after three training sessions and were not included in the training calculations. Typically, unresponsive fish remained at the release site for the duration of the trial and did not respond to food rewards.

After three successful training sessions, on a fixed-ratio reinforcement schedule, we initiated a variable-ratio or intermittent reinforcement schedule (Mazur 1986). These fish were rewarded randomly with a single pellet, after performing one to six correct behaviour responses, for a total of up to 10 pellets. Fish were maintained on this training schedule for seven more training sessions. Following completion of the training schedule, we tested the fish in a larger arena (2.5-m tank).

### The Polarized-light Source

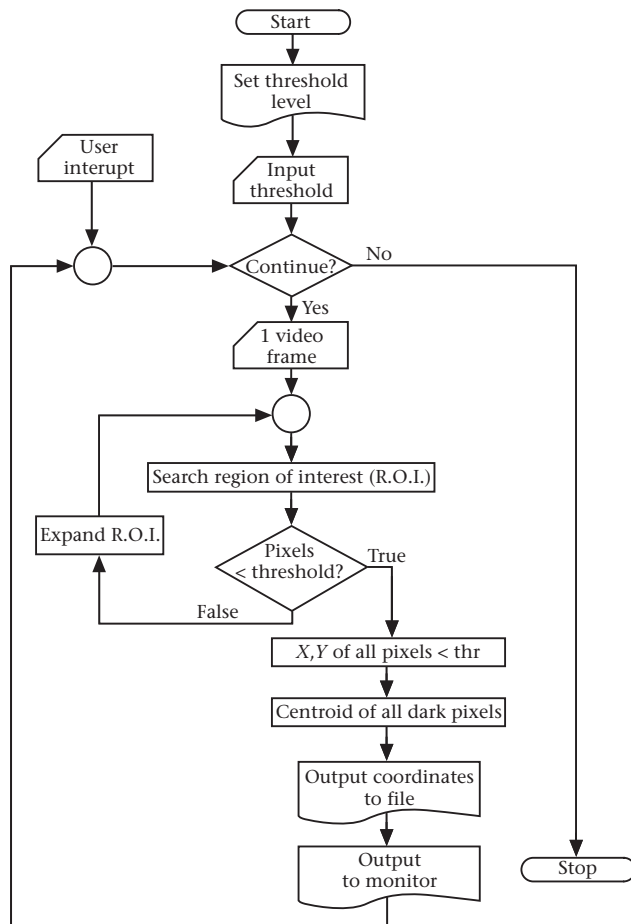
The light source, a DC voltage-regulated 1000-W tungsten-halogen lamp (Oriel) with UV-transmitting optics was suspended 6 m above the centre of the tank. A black baffle eliminated stray light from the lamp housing. A near-UV/Visible transmitting water cooled-filter (Oriel) attached to the end of the projection lens assembly removed infrared radiation to prevent damage to the polarizing filter. Light from the lamp was adjusted to project a diverging envelope across a 50-cm diameter sheet of UV/visible light-transmitting acrylite (OP-1, CRYO Plastics) 1 m below the lamp aperture. The acrylite was frosted on both sides and was used as a diffuser to remove inherent polarization in the optical system. To ensure that depolarization by the acrylite was independent of wavelength, we examined transmission at four spectral points of polarized light using interference filters, a polarizer and an analyser (Table 1). The light was depolarized almost completely and the percentage polarization of diffused light did not differ as a function of wavelength (Kruskal–Wallis analysis of variance, ANOVA, chi-square approximation:  $\chi^2_{0.05,3}=6.1$ ,  $P=0.11$ ). A 100-mm diameter HNP'B UV-visible linear-polarizing filter (Polaroid Corporation, Wayland, Massachusetts, U.S.A.) was situated below the diffuser plate (Fig. 2). A



**Figure 2.** Downwelling spectral irradiance of a DC voltage-regulated 1000-W quartz halogen tungsten (QHT) light source coupled with an OP-1 acrylic diffuser (CYRO Plastics) and Polaroid HNP'B polarizing filter used in the experiments, as measured using a Licor Li-1800 UW spectroradiometer with a cosine collector (dashed line). Relative spectral transmittance of an HNP'B polarizing filter as measured by an S2000 Ocean Optics spectrometer (solid line).

biweekly check of the polarizing filter with a monochromatic light source ensured that at least 85% of the light was polarized for a series of wavelengths (range 360–700 nm). The filter was discarded and replaced if it failed to meet these criteria. The resultant region of illumination (diameter=3.0 m) was slightly larger than the area of the test tank, and the diameter of the polarized-light region was approximately 0.60 m. The small size of the polarized-light region relative to the diameter of the testing tank may have reduced previously described brightness artefacts resulting from the interaction of the polarized light source and the wall of the tank (Jander & Waterman 1960), because such differences could not be measured.

The orientation of the polarizer was changed randomly between both training and testing trials. For free-swimming, stream- and lake-dwelling fish, landmark orientation (piloting) on underwater and shoreline features of a familiar area may provide important cues for orientation (Hasler 1971). In the present study, we reduced the possibility of this occurring by randomizing the position of the polarizer between individual training episodes and before testing under indoor conditions. Both the position of the training tank and the polarizer were adjusted to maintain the same relationship between the orientation of the tank and the orientation of the polarized-light cue. A black curtain surrounding the testing tank functioned as a blind. The experiments were conducted in a light-tight room painted flat black, and the tank surface was coated with a spectrally flat white paint. A pseudocolour digital-image of the tank surface was made using a PIP 512 Digital Image Processor (Matrox) and was used to identify and remove potential bright spots in the tank. Measurements of light intensity



**Figure 3.** A flow diagram of the F-chase tracking algorithm showing the steps used by a Matrox PIP-512 video processor to process video frames for the determination of a series of contiguous  $X$ ,  $Y$  coordinates of a fish released in the training tank.

across the tank (Hawryshyn et al. 1990) indicated that the intensity of light differed approximately 1 log unit from its centre to the periphery.

### Videotracking of Fish Position

Fish were placed into the same clear plastic cylinder that was used during training and were allowed to acclimate in the cylinder for 5 min before release into the test tank. We recorded the position of the fish during experimental trials on VHS videotape, from a position 4 m above the fish, using a Sony video camera equipped with an Optex ultra-wide-angle lens. The videotape was then preprocessed by background subtraction using an Image-1 Digital Image Processing System (Universal Imaging Corporation, Downingtown, Pennsylvania, U.S.A.). Using this technique, a pretrial video frame (fish absent) was subtracted from subsequent video-image frames from the testing sequence on the tape (fish present). Thus, the background was largely removed from the video image to allow accurate determination of the  $X$ ,  $Y$  position of the fish. We then analysed the enhanced video output using the F-chase algorithm (Fig. 3) from the

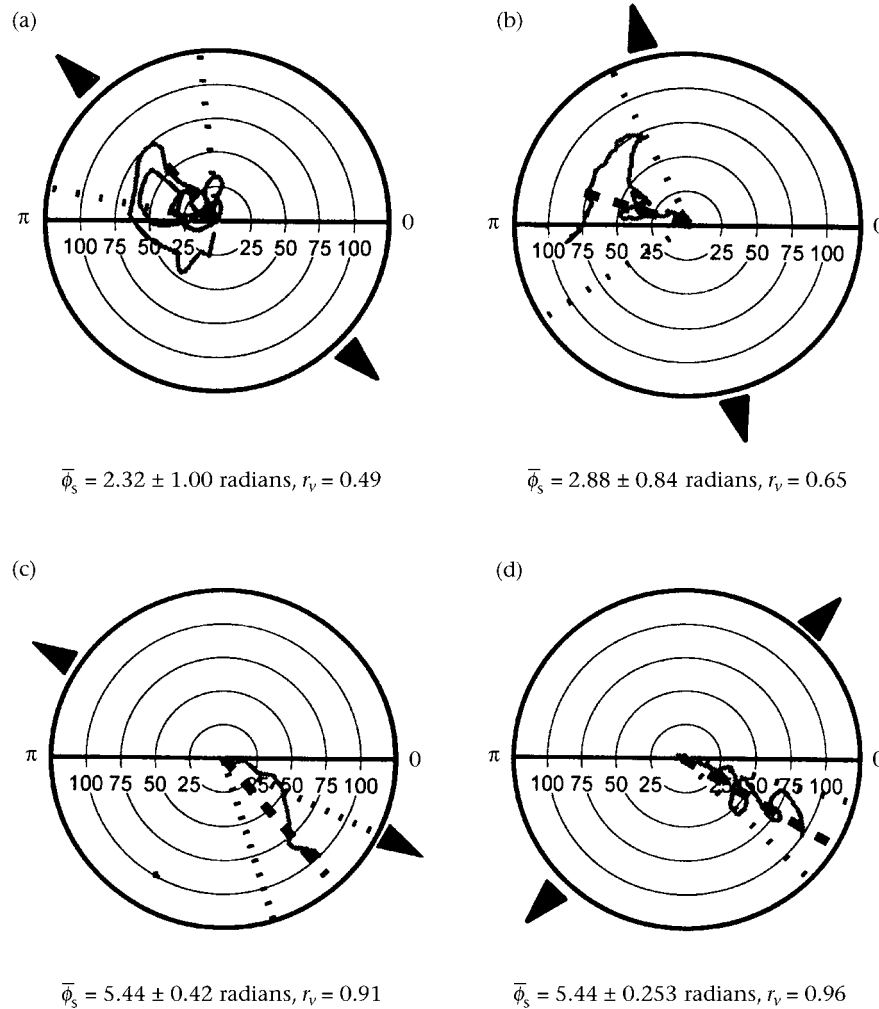
PIP 512 Digital Image Processor. At the start of an experiment, the experimenter inputted a threshold contrast level. The F-chase program used this value to determine the coordinates of all pixels (the fish) in the video frame that were darker than this threshold. This was repeated for subsequent video frames until the experimenter stopped the experiment, either when the fish had moved to within 25 cm of the periphery of the tank or 5 min had elapsed since release. The  $X$ ,  $Y$  coordinates and time stamp of the fish's instantaneous position at 0.03-s intervals during a trial were written to a computer file for statistical analysis.

### Statistical Analyses and Testing

Following determination of the  $X$ ,  $Y$  coordinates of the fish after release, we calculated the mean angular orientation ( $\bar{\varphi}_s$ ) from the repeated measures of an individual fish. To determine this value, we converted the  $X$ ,  $Y$  coordinates (in pixels) of an individual fish's position to polar coordinates ( $\bar{\varphi}_s$ , radius). The rate of data collection was high; about 9000 data points were collected over the course of a trial for a fish that responded slowly. Some data points represented no change in position. Serial-repeated data points were excluded from the analysis so as not to bias the determination of  $\bar{\varphi}_s$ , the mean angular orientation of a fish and the vector of directionality ( $r_v$ ). Standard circular statistics were used in the analyses (Mardia 1972; Batschelet 1981; Zar 1996). All statistical tests in the present study were performed with an  $\alpha$  level of 0.05. Examples of tracking records of four fish are illustrated in Fig. 4 to demonstrate the output from the F-chase  $X$ ,  $Y$  tracking program and the basic summary statistics used.

In the first experiment, we individually tested 50 juvenile rainbow trout (parr) to determine whether they had an innate response to plane-polarized light (size range 3.2–9.8 g;  $\bar{X} \pm \text{SE} = 6.8 \pm 1.4$  g). The fish had not been trained and thus were naïve with respect to the test conditions. The movements of individual fish were recorded on video until they reached within 25 cm of the periphery of the tank or until 5 min had elapsed from the time of their release. Fish that did not move during the trials were not included in calculations. Because we could not make a priori predictions on the direction of the angular responses of the naïve fish, we used a Rayleigh test (Batschelet 1981; Zar 1996) in the first set of experiments to examine whether the group of untrained fish had an innate directional response to the plane-polarized stimulus.

In the second set of experiments, rainbow trout parr of the same size were trained as before to orient either perpendicular or parallel to the plane-polarized-light source. The two groups of fish would be expected to have orientation responses perpendicular to each other if they used polarized light as the cue for their directional responses (Hawryshyn et al. 1990). We performed an additional test to determine whether, in the absence of polarized light, the orientation responses of these trained fish differed significantly from a uniform distribution. In this instance, *O. mykiss* trained to orient parallel to



**Figure 4.** (a–d) Individual tracking records of four rainbow trout recorded using the F-chase program. Axis scale represents the distance from the centre of the tank (cm). Fish were tracked to 100 cm from the centre of the tank. The heavy dashed line represents the mean angular orientation of an individual fish,  $\bar{\phi}_s$ ; the arc between the two dotted lines represents the circular standard deviation,  $s_g$ . The radial distance of the dashed line is proportional to the size of  $\bar{r}_v$ , the mean vector of directionality (0–1 scale). Solid arrow heads outside the circle indicate orientation of polarized-light stimulus.

the plane of polarized light were tested with a UV-transmitting Plexiglas diffuser to diffuse light from the polarizing filter. A rejection of the null hypothesis of uniform distribution would suggest that the fish were using an orientation cue other than polarized light (e.g. brightness cues from the light source).

Following determination of  $\bar{\phi}_s$  for each fish, we standardized the angular orientation responses relative to zero radians within the two test groups of parallel- versus perpendicular-trained fish. This was done to facilitate comparison of trials, because the orientation of the polarizer was randomized between different experimental replicates to ensure that responses were not a function of avoidance or attraction to an external cue. We determined the mean angle of orientation for replicate trials,  $\bar{\phi}_g$ , for both parallel-trained and perpendicular-trained fish using the values of  $\bar{\phi}_s$  from individual fish (Batschelet 1979, 1981; Zar 1996), and we applied a *V* test (Batschelet

1981) to determine whether the fish had a uniform or clumped distribution around an a priori defined angle (e.g. the angular orientation of the plane of polarization during testing). When the data had an axial (bidirectional) distribution, we used a standard doubling of angles procedure (Durand & Greenwood 1958; Groot 1965; Batschelet 1981). Following application of the *V* test to test distributions of rainbow trout, we used a Watson's  $U^2$  test (Zar 1996) to address whether perpendicular- and parallel-trained fish had a similar distribution. To facilitate the comparison between the training regimes, we transformed the angular data from the perpendicular-trained fish by subtracting  $\pi/2$  from the response angles.

In the third and fourth experiments, juvenile steelhead ( $\sim 3$ – $5$  g) and brook char ( $\sim 7$ – $10$  g) were also trained to orient parallel to the plane of polarization using the methodology outlined earlier. We determined the

orientation responses of these trained, juvenile steelhead and brook char and then compared them with rainbow trout using a multisample Wheeler & Watson test (Mardia 1972; Zar 1996).

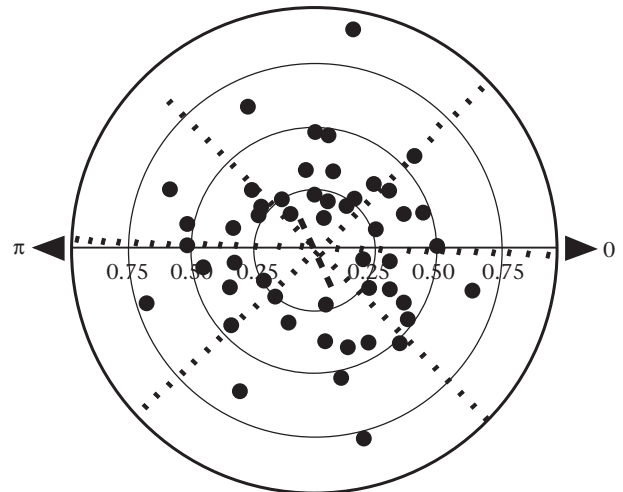
### Outdoor Tests of Laboratory-trained Fish

Rainbow trout trained to orient parallel to the plane of polarized light in the controlled laboratory experiments also were tested under ambient outdoor light to determine their orientation responses under natural lighting conditions. The test tank was identical to the tank in the laboratory experiment. This test was used to determine whether fish trained under a simple polarized-light field in the laboratory could orient using natural plane-polarized light, because these natural conditions may contain confounding polarization as well as other sky-light cues. However, spectral cues were reduced because a fish at the centre of the testing tank, near the bottom, could minimally view the sky down to 38°, but a fish at the edge of the tank could view the opposite region of the sky down to 22°. A forest, however, obscured the horizon in the south and west regions of the sky, such that the minimum viewable angle was 29° for a fish at the polar opposite of the tank. Spectral cues from the horizon were thus reduced but may not have been entirely eliminated. Under natural conditions, fish undoubtedly depend on many features of their environment to serve as cues for orientation, including features below and above the water. The training of fish indoors followed by subsequent testing outdoors precluded the use of landmark orientation. We used an e-vector axis finder (Edmund Scientific, Barrington, New Jersey, U.S.A.) to locate both the region of the sky with maximum polarized light and the orientation of plane-polarized light in that region. A radiometer (Photodyne, Optikon) with a radiance collector (10° acceptance angle) was used to measure integrated solar energy from 200 to 1200 nm. We used these values to calculate the percentage of polarized light in the region with maximal polarization. Testing was conducted with the sun on the horizon, 24 May and 27 May 1994, to provide the fish with a natural polarized light cue in approximately the same position, zenith, as in the laboratory. Responses of fish were recorded on videotape for analysis with the F-chase program. Unlike the laboratory experiments, the polarized light field had the potential to change in both position and relative percentage between replicates and over the course of this experiment. Therefore, we plotted each fish's response as the difference between the fish's position and the orientation of plane-polarized light at zenith.

## RESULTS

### Orientation of Naïve Juvenile Rainbow Trout Under Laboratory Conditions

The movements of experimentally naïve fish from the release area towards the periphery of the tank varied between fish from undirected to direct (0.18–0.92; Fig. 5).



$$\bar{\phi}_g = 1.96 \pm 1.17 \text{ radians}, r_v = 0.08$$

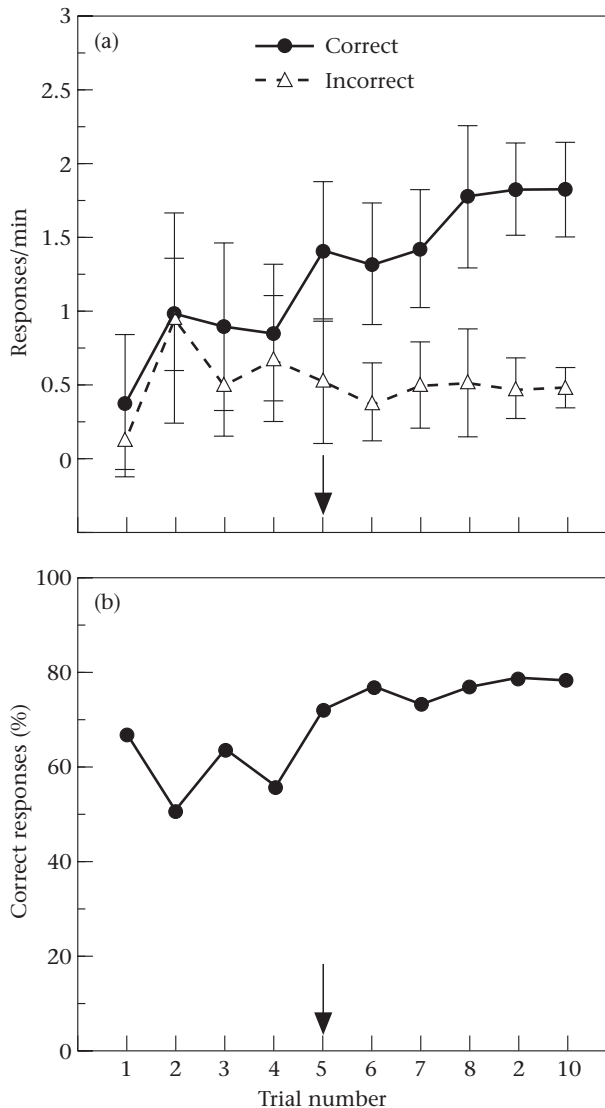
Rayleigh test:  $Z = 0.340$ ,  $N = 50$ ,  $P > 0.05$

**Figure 5.** Orientation responses of individual, experimentally naïve rainbow trout (●) to a plane-polarized stimulus. Individual points represent  $\bar{\phi}_s$ , the mean angular orientation of individual fish. The dashed line represents  $\bar{\phi}_g$ , the grand mean of all the trout. The radial length of this dashed line from the centre of the plot represents  $\bar{r}_v$ , the mean vector of directionality (axis scale 0–1). The arc between the dotted lines represents the circular standard deviation from the mean,  $s_g$ .

The mean  $\pm$  SD angle of orientation ( $\bar{\phi}_g$ ) of untrained fish was  $1.96 \pm 1.17$  radians ( $112.3 \pm 67.0^\circ$ ;  $N=50$ ), and  $\bar{r}_v$  was 0.08 (Fig. 5). The distribution of the angular responses of naïve fish did not differ significantly from a uniform distribution (Rayleigh test: NS; Fig. 5). This result indicated that, as a group, these juvenile rainbow trout had no common, innate orientation response. Thirteen fish did not move from the release area in the centre of the tank during the trials and thus were excluded from the analysis.

### Acquisition of an Orientation Response to a Plane-polarized Cue

During the training protocol, the total number of responses of rainbow trout doubled from the first training session to the second (Fig. 6a). During the first and second sessions, however, the fish did not associate the orientation of the plane of polarized light with the location of food rewards, as evidenced by an equal percentage of correct and incorrect responses (Fig. 6a). Following the second training session, the mean number of both correct and incorrect responses decreased slightly, although the rates of correct and incorrect responses began to diverge (Fig. 6a). After we began an intermittent reinforcement schedule at the fifth training session, the mean number of correct responses increased and reached a constant level by session eight (Fig. 6a). The percentage of correct responses also remained relatively constant from the fifth session on (range 70–80%; Fig.



**Figure 6.** Responses of juvenile rainbow trout ( $N=12$ ) during entrainment to a linearly polarized light field, as a function of number of trials: (a) Mean  $\pm$  SE number of responses/min. (b) Percentage of correct responses. Arrow indicates start of variable-ratio reinforcement schedule.

6b). However, the mean number of incorrect responses declined slightly after the intermittent reinforcement schedule was begun, then remained relatively constant for the remainder of the training sessions (Fig. 6b).

### Testing of Fish Trained Under a Linearly Polarized Light Field

In contrast to the naïve fish ( $r_v=0.08$ ), trained rainbow trout typically showed more direct movements towards the edge of the testing tank during testing, for both parallel-trained fish (Fig. 7a) and perpendicular-trained fish (Fig. 7b). The vector  $r_v$  of individual fish varied from 0.48 (relatively undirected) to 0.98 (very directed; Fig. 7a, b). Rainbow trout trained to orient either parallel or

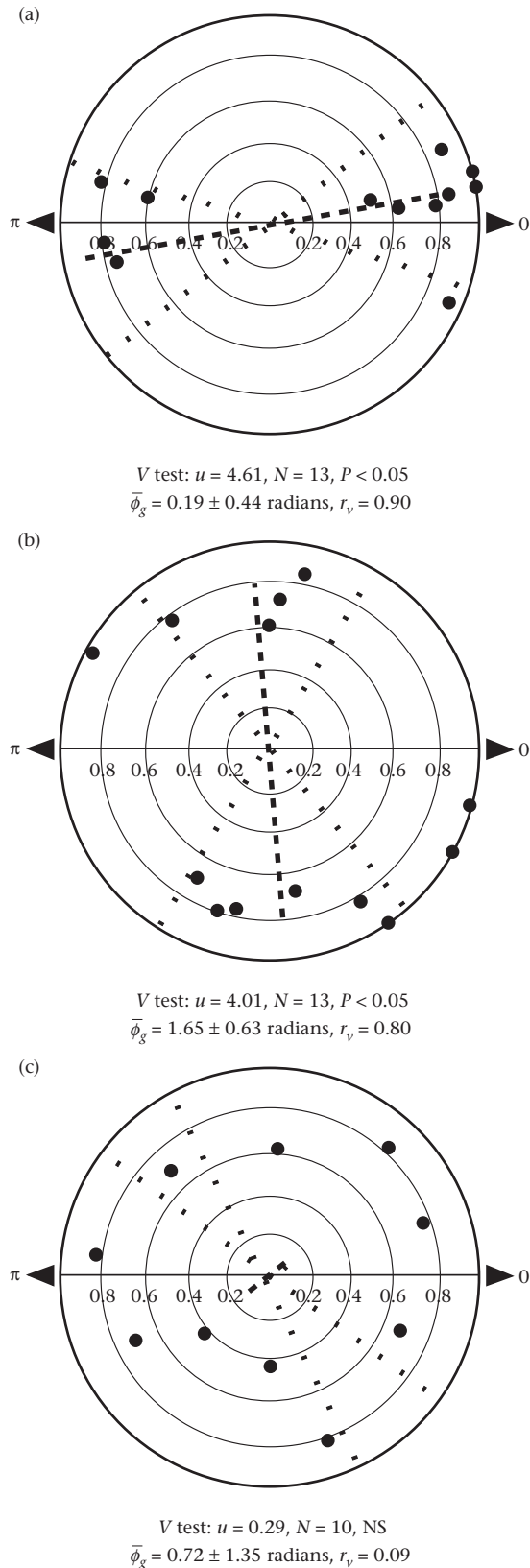
perpendicular to the plane of polarized light also responded nonrandomly. Responses were clumped either parallel ( $V$  test:  $u=4.61$ ,  $P<0.05$ ; Fig. 7a) or perpendicular ( $V$  test:  $u=4.01$ ,  $P<0.05$ ; Fig. 7b) to the axis of the polarizer. The axial distributions of the orientation responses of the fish under parallel or perpendicular training were not significantly different from each other (Watson's  $U^2$  test:  $U=0.155$ ,  $N_1=13$ ,  $N_2=13$ , NS), indicating that they oriented equally well. In contrast, when a UV-transmitting diffuser (Table 1) was used to diffuse light from the light source, juvenile rainbow trout trained to orient parallel to the plane of polarized light responded with an overall uniform distribution (Rayleigh test: NS; Fig. 7c).

Like rainbow trout, steelhead and brook char also showed bimodal orientation responses, which in these cases, were aligned parallel to the plane of polarized light (Fig. 8a, b, respectively). Individual values of  $r_v$  varied between 0.42 and 0.99, with most values greater than 0.70. Thus, these fish responded in a directed manner upon release. The responses of both steelhead and brook char were not distributed uniformly and were significantly clustered around the angle corresponding to the position of the polarizer ( $V$  tests both  $P<0.05$ ; Fig. 8a, b). No differences were detected among the orientation responses of rainbow trout, steelhead and brook char trained to orient parallel to the plane of polarized light (Wheeler & Watson test:  $\chi^2_{0.05,4}=9.488$ ,  $W=6.067$ , NS).

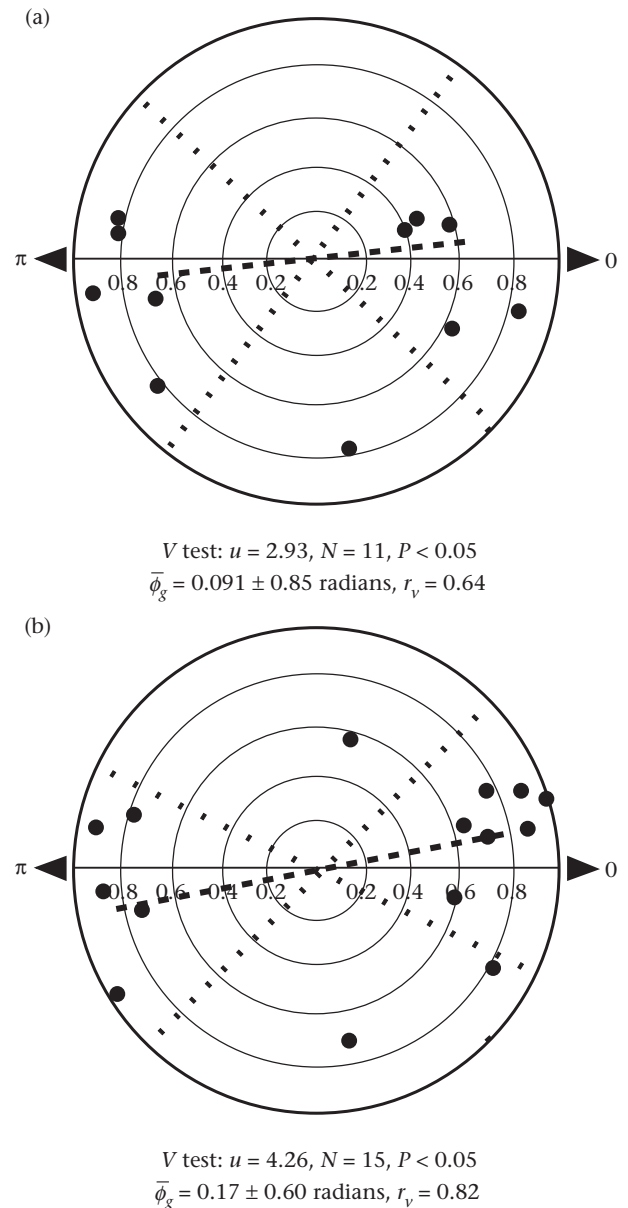
### Outdoor Tests of Laboratory-trained Fish

Rainbow trout trained in the laboratory to orient parallel to the plane of linearly polarized light were tested under seminatural conditions in a tank under natural lighting at sunset ( $\sim 2045$  hours Pacific Daylight Time, 24–27 May 1994; Fig. 9). The plane of maximally polarized light was near zenith along a compass bearing of approximately  $131^\circ/311^\circ$  (corrected for magnetic declination). The maximum percentage of polarized light (HNP'B polarizer) in the sky during twilight at the onset of testing on 24 May was 59% and was 67% on 27 May when the tests were completed. The responses of these laboratory-trained fish were consistent with the responses of fish trained to orient parallel of the plane of maximally polarized light ( $V$  test:  $P<0.05$ ; cf. Fig. 9, Fig. 6a). All 10 fish responded within  $47^\circ$  of the axis of the ambient plane of maximally polarized light. Individual values for  $r_v$  varied between 0.43 and 0.90, with nine of 10 fish having an  $r_v$  greater than 0.5, indicating that the fish maintained a relatively constant bearing following release.

In summary, experimentally naïve rainbow trout did not orient spontaneously in a unified direction, but conditioned rainbow trout, steelhead and brook char oriented in a directed manner to plane-polarized light under laboratory conditions. When the trained rainbow trout were also tested outside under natural celestial light patterns, they oriented relative to the ambient polarized light field, despite a lack both of training and previous exposure to the natural pattern of polarized light.



**Figure 7.** Orientation responses of individual rainbow trout (●) trained to orient (a) parallel to the plane of polarized light, (b) perpendicular to the plane of polarized light and (c) parallel to plane of polarized light with a diffuser present. See Fig. 5 for a description of plot attributes.

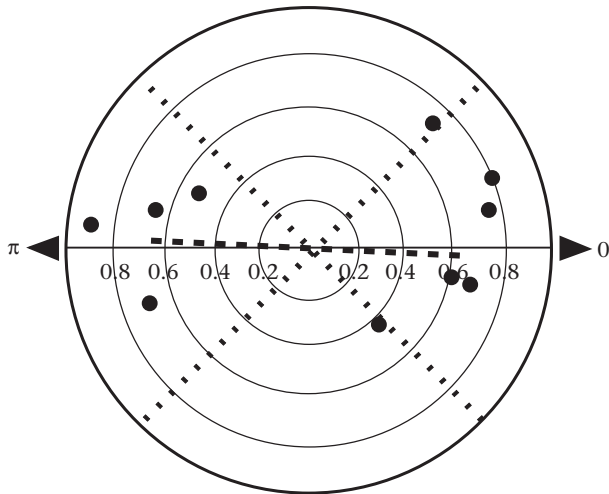


**Figure 8.** Orientation responses of steelhead (a) and brook char (b) trained to orient parallel to the plane of polarized light. See Fig. 5 for a description of plot attributes.

## DISCUSSION

### Orientation of Naïve Juvenile Rainbow Trout Under Laboratory Conditions

Although trained fish demonstrated the ability to learn to orient relative to the angular position of a linearly polarized light stimulus, the lack of a common directional response in untrained juvenile rainbow trout suggests that these fish did not have an innate orientation response to polarized light during testing. Several interpretations are possible. First, orientation to polarized light may be a learned response, and therefore these juvenile fish did not have the opportunity to acquire a response to polarized light. As an alternative, naïve fish



$V$  test:  $u = 2.74$ ,  $N = 10$ ,  $P < 0.05$   
 $\bar{\phi}_g = 6.24 \pm 0.82$  radians,  $r_v = 0.66$

**Figure 9.** Orientation responses of rainbow trout entrained in the laboratory to orient parallel to the plane of polarization, following exposure to a clear celestial hemisphere with the sun on the horizon. See Fig. 5 for a description of plot attributes.

may not have responded because of handling stress. All fish were acclimated before testing to minimize effects of handling, but repeated handling of trained fish may have been a confounding factor, because naïve fish were handled only once. Another explanation for the lack of a common or unified orientation response in naïve fish is that individual fish may have an innate directional preference, or relevant releasers for polarized light may have been absent. Potential releasers might include photoperiod, water temperature and size or stage of development of fish. Age and physiological condition have long been known to influence the timing and type of behavioural responses of salmonids to their environment (Hoar 1953, 1954). Therefore, at the parr or stream-dwelling, non-migratory phase of development, an innate directional preference, even if present, may not have been expressed because of the behavioural or physiological state of the fish. If a critical period does exist for the acquisition of the ability to orient to polarized light, this would necessitate exposure of young fish to natural polarized-light patterns, which could be relevant to stocking programmes intent on establishing self-sustaining populations. Further investigation of laboratory-reared (naïve) fish at different stages of their lives is needed to address this issue.

### Acquisition of Orientation to Polarized Light in Laboratory Studies

Rainbow trout demonstrated their ability to learn to orient relative to the angular position of the polarized light cue by selecting the correct channel in the L-shaped training tank. The learning curve for rainbow trout for acquisition of the correct behavioural response was similar to that observed by Adron et al. (1973) for two-choice

learning of colour patches using food rewards. However, compared with Adron et al.'s results, the mean number of correct responses that we observed was lower ( $\leq 79\%$ ). This result could be attributable to a number of factors, including duration of training, motivation of the fish and complexity of the task. The neurobiological and ethological foundations of memory-based orientation in fish are poorly understood and fields of inquiry are in need of development.

### Indoor Tests of Laboratory-trained Fish

In contrast to the naïve fish, fish that associated the polarized light cue with a relevant resource (food), oriented relative to the plane of polarized light under laboratory conditions, corroborating previous observations with rainbow trout (Kawamura et al. 1981; Hawryshyn & Bolger 1990; Hawryshyn et al. 1990; Parkyn 1998; Degner & Hawryshyn 2000). The similarity in the accuracy of orientation of juvenile rainbow trout, regardless of whether they were trained to orient perpendicular or parallel, indicated that trained fish could orient relative to the angular orientation of polarized light. The lack of a unified directional response in trained rainbow trout exposed to a depolarized light source also further supports the idea that the fish oriented relative to the plane of polarized light, rather than to brightness cues from the light source. Based on the results of our laboratory experiments, anadromous steelhead and potamodromous brook char also perform compass orientation behaviour relative to a plane-polarized light. These results support those of earlier behavioural experiments involving sockeye salmon (*O. nerka*: Groot 1965; Dill 1971), as well as more recent findings that steelhead, brook char and sockeye salmon physiologically discriminate between different angular orientations of a plane-polarized stimulus (Coughlin & Hawryshyn 1995; Parkyn & Hawryshyn 2000).

### Outdoor Tests of Laboratory-trained Fish

Fish trained to orient parallel to a single plane of polarized light in the laboratory oriented parallel relative to the band of maximally polarized light at zenith, despite inexperience with the complexity of brightness and spectral cues in natural outdoor conditions. When the sun is lower on the horizon, the polarized-light field is least complicated for an observer sensitive to polarized light (e.g. see Horváth & Varjú 1995). Thus, at this time of day, the position of the sun can be determined unambiguously by the distribution of polarized light. Under the simple design that we used, it is difficult to eliminate the potential influence of these brightness and chromatic cues, although, as we mentioned, the height of the test tank walls may have reduced or prevented this artefact. More complete designs have experimentally addressed these issues (Groot 1965; Parkyn 1998). Furthermore, in the present study, the fish were reared

in an indoor hatchery without exposure to patterns of natural sunlight. Thus, it is doubtful that the bidirectional response of this group of tested fish was due to orientation to brightness or chromatic cues. Finally, the training of fish indoors followed by a single test outdoors for each fish precluded the use of landmarks.

The biological roles of polarization sensitivity have only recently begun to be explored. Although fish have been shown to respond to celestial patterns of polarization (Groot 1965; Forward et al. 1972; Waterman & Forward 1972; Forward & Waterman 1973; Parkyn 1998; this study), it is not known whether wild fish orienting in nature make use of this information. This is a challenging question to address. Polarized light orientation has potential to be useful for compass orientation in large bodies of water (Groot 1965) but for stream-dwelling (lotic) fish where rheotactic and landmark cues are present, it seems less plausible that it provides useful information. It is therefore curious that both anadromous and lotic salmonids have equally reliable polarized-light orientation abilities. However, for many species of adult salmon that undergo extensive migrations, the utility of polarized light as a compass mechanism has great appeal. Although it is now known that juvenile salmonids of several species can orient to the plane of polarized light in the laboratory, few studies have examined older and larger reproductive fish (e.g. Parkyn 1998). The possibility of adult reproductive fish being able to orient using polarized light is not precluded by the apparent ontogenetic loss of UV cones and UV sensitivity (Bowmaker & Kunz 1987; Hawryshyn et al. 1989; Browman & Hawryshyn 1992; Parkyn & Hawryshyn 1993; Deutschlander et al. 2001), because some species of salmon returning to their natal streams (i.e. reproductive fish) have UV cones in their retina (Beaudet et al. 1997).

Another issue that remains to be addressed is the role of the underwater polarized-light field for orientation of organisms. The underwater polarized-light field is not always distributed symmetrically in three dimensions around a fish (McFarland 1991; Hawryshyn 1992; Novales Flamarique & Hawryshyn 1997). Consequently, aquatic organisms monitoring the distribution of aquatic polarized-light distribution outside of Snell's window (Horváth & Varjú 1995) might be expected to show unidirectional orientation responses instead of bidirectional responses. Therefore, the relative importance of the underwater polarized-light field versus the celestial polarized-light field as viewed through Snell's window is still unknown. We know that other aquatic organisms besides fish also appear to orient using the celestial pattern of polarized light (Goddard & Forward 1991). In the present study, the shallow depth of the tanks (15 cm) in both testing situations resulted in a short path length for light. As a consequence, the proportion of plane-polarized light resulting from Rayleigh and Mie scattering in the water would have been less than would be observed with a deeper water column (Born & Wolf 1999). This effect, coupled with the observation of bimodal directional responses in both the laboratory and the field, suggests that, at least in this study, the fish oriented to celestial plane-polarized light.

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