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ORIGINAL PAPER

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Wavelength-dependent polarization orientation in *Daphnia*

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Abstract The ability to detect and use the polarization of light for orientation is widespread among invertebrates. Among terrestrial insects, the retinula cells that are responsible for polarization detection contain a single visual pigment, either ultraviolet or short (blue) wavelength sensitive. With the exception of a few aquatic insects, the visual pigments underlying polarization sensitivity in aquatic invertebrates have yet to be determined. Here we report that polarotaxis in *Daphnia pulex*, a freshwater crustacean, is wavelength dependent and most likely mediated by two visual pigments with absorbance maxima in the middle (green) and long wavelength (red) parts of the spectrum. This contrasts with the response of a closely related species, *D. magna*, in which polarotaxis is wavelength independent and based on a single middle wavelength visual pigment. The visual systems in *Daphnia* are the first among crustaceans shown to utilize a middle wavelength pigment for polarization detection and, in the case of *D. pulex*, the first shown to use more than one visual pigment for such a purpose.

Key words Polarotaxis · Crustacean · Visual pigments · Rhabdom · *Daphnia*

Introduction

Many terrestrial and aquatic invertebrates can detect the polarization of light and use it for orientation and navigation (Waterman 1981; Wehner 1983, 1997; Goddard and Forward 1991). This capability arises from the dichroic properties of photoreceptor microvilli, which confine visual pigment chromophores along their axes (Goldsmith and Wehner 1977), to incident illumination on the retina (Nilsson et al. 1987; Labhart and Meyer 1999). In arthropods, polarization sensitivity is mediated by ultraviolet (UV), short (S, blue) or middle (M, green) wavelength sensitive retinula cells (Duelli and Wehner 1973; Herzmann and Labhart 1989; Schwind 1995). In terrestrial insects, these cells have non-overlapping microvilli and are spread in fan shape fashion over the dorsal rim area (DRA) of the compound eye (Wehner 1989; Labhart and Meyer 1999). Among aquatic invertebrates, microvilli may be arranged in a mostly non-overlapping fashion as in insect rhabdoms (e.g. the compound eye of some aquatic beetles, cladocerans and other zooplankton; Röhlich and Törö 1965; Waterman 1981; Schwind 1995), or in stacks with alternating sections of orthogonal microvilli (as in cephalopods and decapod crustaceans, e.g. Moody and Parriss 1961; Waterman and Horch 1966; Waterman 1981). In species with microvillar stacks, the entire retina appears devoted to polarization sensitivity (Waterman 1981). The visual pigment underlying polarization sensitivity is likely a rhodopsin ($\lambda_{max} \approx 490$–$500$ nm) in cephalopods (Hagins 1970; F.I. Hárosi, personal communication), and a potential UV, S or M wavelength sensitive pigment, or a combination of UV/M wavelength sensitive pigments, in decapod crustaceans (Wehner 1983; Cronin and Forward 1988; Marshall et al. 1999). With the exception of some aquatic beetles (e.g. the genus *Haliplidae*), whose polarization systems are most sensitive in the M region of the spectrum (500–540 nm; Schwind 1995), the pigment types and associated retinula cells that underlie polarization sensitivity in aquatic invertebrates have yet to be determined for any species.

Among the cladocerans, various species of *Daphnia* have long been known to spontaneously swim perpendicular to the direction of a broadband, "White", linearly polarized light field (Baylor and Smith 1953;

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Waterman 1960, 1981; Watt and Young 1994; Gocken and McNaught 1995; Schwind 1999). This behaviour is believed to be mediated by two sets of retinula cells with orthogonally-arranged microvilli in the rhabdoms of the compound eye (Waterman 1981; Smith and Macagno 1990). *Daphnia magna* possesses four types of visual pigments with maximum absorbances in the UV, S, M and long (L, red) wavelength regions of the spectrum (Smith and Macagno 1990). Until now, however, the visual pigments underlying polarization in this or other species of *Daphnia* have not been identified. In this study, we carried out orientation experiments to various types of light stimuli that varied in spectral and percent polarization content (the percent polarization of a light source refers to the degree to which the electric fields of the photons that comprise it vibrate in the same plane; all the photons from a source that is 100% linearly polarized vibrate in the same plane – E_{max}). These experiments permitted us to identify the photoreceptor mechanisms and thus, the underlying visual pigments, that contribute to polarization sensitivity in *D. magna* and *D. pulex*, two species of *Daphnia* with identical eye structure (Röhlich and Törö 1965).

Materials and methods

Animals

Daphnia magna and *D. pulex* were obtained from cultures maintained at the University of Bergen, Norway. The *D. magna* originated from wild stocks in Binnensee (Germany) while *D. pulex* came from Lake Myravaan (near Bergen, Norway). Animals were maintained in 24 l aerated aquaria containing lake water, and were fed *Scenedesmus* algae. The photoperiod was maintained on a 12 h L:12 h D cycle. All experiments were performed using water from the holding aquaria and were conducted during the daylight part of the animals' diel cycle.

Imaging system

We used silhouette (shadow) video photography (SVP; Arnold and Nutall-Smith 1974; Edgerton 1977; Browman et al. 1989) to record the orientation responses of *Daphnia* exposed to various types of illumination. Silhouette video photography is superior to standard cinematographic or video imaging techniques in various ways. First, it allows filming of events in a large depth of field (~15 cm) with a relatively large field of view (limited only by the size of the collimating lenses, here 14.5 cm). Second, magnification is independent of distance from the cameras and the resolution is excellent; objects as small as 0.2 mm can be resolved. Third, image quality is unaffected by ambient light levels and the system does not require intense light sources, which would increase temperature in the observation room and/or introduce a confounding stimulus. As a result, *Daphnia* behaviour could be observed under relatively natural conditions.

Our SVP observation and motion analysis system consists of two orthogonally oriented optical rails, with the observation aquarium placed at their intersection (Fig. 1). The imaging optics on each rail consist of a far-red light emitting diode (LED) placed at the focal point of a 14.5 cm diameter biconvex collimating lens whose output passes through the aquarium. The LED's output is below that measurable using a 100 mm diameter submersible integrating sphere sensor (OL-IS-470-WP) attached via a quartz fibre optic cable to a scanning spectroradiometer (OL 754-O-PMT, Optronic Laboratories, Orlando, Fla.; the threshold sensitivity and spectral resolution of this instrument are $\sim 10^{12}$ photons m^{-2} s^{-1} and 1 nm, respectively). The use of a collimated beam prevents perspective distortion; clear, sharp shadows of any organism (even a small virtually transparent one) in the beam's path are projected. Shadow images are collected by a lens (Tamron 70–210 mm zoom) attached to a 0.5 inch CCD sensor video camera (Panasonic WV-BL730) and recorded using a S-VHS video tape recorder (Panasonic AG-6730). The optical components on each rail are aligned using lasers, which also allow the vertical viewing heights and orthogonal orientation of the two rails to be established precisely. The synchronously-recorded orthogonal views allow for exact determination of the three-dimensional positions of particles which appear in both fields of view simultaneously.

The outermost 2.5 cm of the aquarium walls were covered with black plastic (matt surface) contact paper. This restricted the field of view to the central 3.38 l (15 cm × 15 cm × 15 cm) volume of water and ensured that the behaviours observed were not influenced by surface or edge effects; only *Daphnia* freely swimming in the water column were imaged and their displacements analyzed.

Fig. 1 Silhouette video photography (SVP) system used to make behavioural observations of *Daphnia*. Light from a 1 kW xenon arc lamp (not shown) is focused onto the aperture of an ultraviolet (UV)-visible liquid light guide (*O*) and passes through various optical components in a filter holder (*F*) before reaching the test aquarium (*A*) in which the animals are freely swimming in 7 l of lake water. The aquarium (20-cm square) is located at the intersection of two 3 m long optical rails. Each rail supports a far-red light emitting diode (*D*) placed at the focal point of a 14.5 cm diameter biconvex collimating lens (*L*), and a video camera (*C*) to image the shadows projected by the collimated beam that passes through the aquarium. Also shown are the lasers (*Ar*) that are used to align all the optical components on the rails prior to a given set of experiments. On several experiments, a UV-visible light guide from a 150 W arc lamp was positioned parallel to one of the optical rails (the nearest one to the reader in the picture). This light guide (not shown) projected a cone of diffuse light that overlapped approximately the volume illuminated by the downwelling illumination

Illumination systems

The main illumination component consisted of a light intensity-controlled 1 kW Xenon arc lamp (Oriel Instruments) connected to an ultraviolet (UV, 280–400 nm)-visible liquid light guide with a wax paper diffuser at its end. The light guide was coupled to the arc lamp housing using an Oriel 77800 lens assembly. The projecting end of the light guide (placed directly above the observation aquarium) was fitted with a lens assembly (Oriel 77800) and a filter holder. The filter holder always contained a KG-3 type quartz substrate heat filter. Additional optical filters used to modify the spectral and polarization characteristics of the downwelling light were added as appropriate for any given trial (see below). Without any additional filters, polarized light measurements indicated that the field was completely unpolarized (i.e. the same intensity was found irrespective of the rotation of a polarizer placed in front of the OL-754 spectroradiometer sensor, inside the aquarium). The light field exiting the lens assembly-filter holder combination formed an 8° aperture cone that projected a circle (20 cm in diameter) of uniform polarization into the aquarium that did not make direct contact with the walls (see the Appendix). This optical configuration minimized the non-illuminated volume of water and, hence, the chances of edge effects during the experiments. For the *Daphnia* visual system, the illumination was that of a point source subtending an angle of ~2°. This angle is smaller than the acceptance angle of a single ommatidium, which is about 40–60° (Young and Downing 1976), but ensures that the entire cross section of the rhabdom is illuminated (Young and Downing 1976; Ringelberg 1987), allowing for neural interactions between the retinula cells within a rhabdom.

In some experiments, the polarizer was removed from the filter holder and, instead, a 20 cm × 20 cm wax paper diffuser and polarizer (Edmund Scientific) were placed on top of the aquarium (in that order, or reversed to obtain an unpolarized stimulus). The light stimulus under these conditions covered the entire water surface, mimicking the broad downwelling field present in nature under crepuscular skies. Under these conditions, most rhabdoms would be stimulated simultaneously.

Measurements and experiments to demonstrate orientation to the downwelling light

In natural water bodies, where the path length of light is sizeable (>1 m), Rayleigh-type scattering of polarized light by water molecules and dissolved particles will induce intensity gradients and differences in percent polarization with horizontal direction of observation (see Hecht and Zajac 1974; Novales Flamarique and Hawryshyn 1997). To demonstrate that *Daphnia* were orienting to the polarization of the downwelling light source, and not to intensity gradients or differences in percent polarization arising from sidewelling scattered light, we measured the intensity and polarization of the sidewelling scattered light along the two optical rail directions (see Fig. 1). The light measurements showed that, under a white point source stimulus with polarization along either of the optical rails, the difference in percent polarization of sidewelling scattered light between both rail directions was less than 5% (for a given direction, the percent polarization was computed from two intensity measurements corresponding to orientations of the analyzer along the vertical and horizontal directions; percent polarizations ranged from 8% to 12.6%). Furthermore, the integrated intensity of the scattered light in any direction was similar and small (~10^{13} photons m^{-2} s^{-1}), about 10^4 times lower than the downwelling intensity. The low percent polarizations (lower than the minimum levels required by each *Daphnia* species to detect E_{max}; see Results), the small difference in these values between the two perpendicular rail directions, and the large difference between downwelling and sidewelling intensities indicates that, under these conditions, the *Daphnia* were orienting to the downwelling polarized light cue.

To further show behaviourally that the *Daphnia* were not orienting to differences in horizontal intensity gradients, we carried out orientation experiments with two light sources positioned perpendicular to each other. In these experiments, an additional 150 W source containing a KG-3 filter was used to project light onto one of the sides of the aquarium through a liquid light guide equipped with a diffuser at its end. This point source illuminated the central ~3 l volume of water, overlapping to a large extent the volume illuminated by the downwelling light field. The intensity of this light relative to the downwelling field was in the ratio ~6:1. In some experiments, a 20 cm × 20 cm wax paper diffuser and polarizer combination was used to cover the side of the aquarium illuminated by the 150 W source. The diffuser and polarizer (Edmund Scientific) were the same as those used for the downwelling light field in the same experiments.

Single downwelling point source experiments

With the downwelling illumination in the point source configuration, we observed the swimming behaviour of *Daphnia* under unpolarized (diffuse) white light and under two series of polarization stimuli. The first series consisted of 100% linearly polarized light that varied in spectral content [see long pass (LP) stimuli, Fig. 2A]. The spectral compositions and intensities of the LP stimuli were chosen to induce different photon catches by the various visual pigments present in *Daphnia* rhabdoms (Fig. 2B, Table 1). These experiments were designed to determine the visual pigments and associated photoreceptors involved in polarization detection of both species. The second series of stimuli consisted of a broadband (white) light field (Fig. 2a) that varied in percent polarization; the aim here was to test the accuracy of the polarization detection system of each species (i.e. the minimum percent polarization of the source required to detect the maximum plane of polarization, E_{max}).

Linearly polarized stimuli (100%) were created by placing neutral density filters, the appropriate LP filter (none for the white background), and a linear polarizer (HNP'B, Polaroid), in that order, into the filter holder. For the unpolarized white light stimulus, the linear polarizer was removed and the photon flux adjusted with neutral density filters to match that of the corresponding white polarization stimulus. The intensity and spectral distribution of the white light stimulus was chosen to mimic spectra obtained in lakes inhabited by *Daphnia* (Novales Flamarique et al. 1992).

White light polarization stimuli of different elliptical (percent) polarizations were created with a mica quarter wave plate (Melles Griot) placed into the filter holder in series with the polarizer. Elliptical polarization is functionally equivalent to partially linearly polarized light for microvillar-based photoreceptors similar to those of *Daphnia* (see Labhart 1996). Percent polarizations for various rotations of the quarter wave plate were calculated from intensity measurements at the maximum (E_{max}) and minimum (E_{min}) planes of polarization (determined visually, ± 5°, at the level of the aquarium with an Oriel E-vector finder) using the OL-754 scanning spectroradiometer. Percent polarizations were computed using the formula: $\text{Polarization}(\%) = 100(I_{E_{max}} - I_{E_{min}})/(I_{E_{max}} + I_{E_{min}})$, where $I_{E_{max}}$ and $I_{E_{min}}$ are the intensities in the E_{max} and E_{min} polarization planes, respectively. These stimuli ranged from ~8% to 92% in percent polarization (see Results).

Single downwelling large field source experiments

With the 20 cm × 20 cm diffuser and polarizer combination directly over the aquarium, we observed the orientation behaviour of *Daphnia* to a 100% linearly polarized white light field (>380 nm) and to an unpolarized light field of the same intensity and spectral quality.

Experiments with two light sources

These experiments used both the 1 kW downwelling source and the 150 W sidewelling source simultaneously. The orientation behaviour of *Daphnia* was observed for various combinations of white

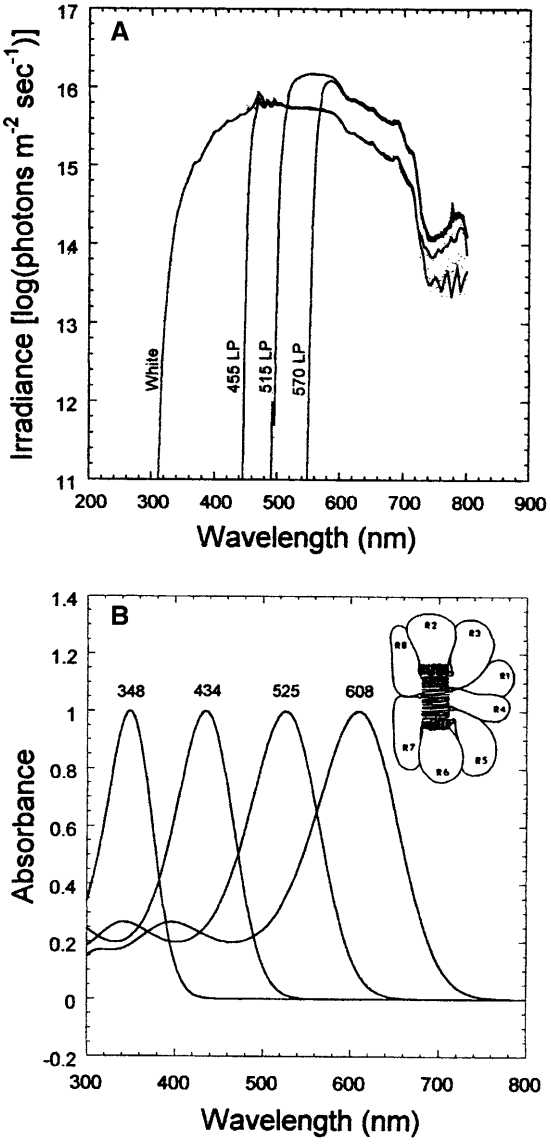

Fig. 2 **A** Spectral fields used during *Daphnia* orientation experiments. *White* white light, *LP* long pass. **B** *Daphnia* visual pigment absorbance spectra determined from an eighth-order polynomial template (Bernard 1987) using absorbance maxima measured electrophysiologically (Smith and Macagno 1990). The spectral types of the known retinula cells (depicted in the rhabdom shown) are as follows: R6 and R8 (blue), R2, R3 and R5 (green), R1 (red). Table 1 shows the relative photon catches of the various pigments given the light stimuli presented in this figure

Table 1 Relative photon catch results for the four visual pigments using the light stimuli in Fig. 2A and accounting for the smaller size of the red rhabdom (R1, ca. one-half length of rhabdom used here). Relative photon catches were obtained by integrating the product of absorbance and spectral irradiance for each pigment (for a given light stimulus) and dividing these by the highest value

Pigment type	White	455 LP	515 LP	570 LP
UV	0.042	0	0	0
Short	0.56	0.20	0.073	0
Middle	1	1	1	0.42
Long	0.44	0.45	0.81	1

light stimuli that were either polarized or unpolarized. Experiments were carried out with the sources in the point-like or large field configurations.

Procedure and analysis for individual experiments with multiple *Daphnia*

For each experiment, 25 *Daphnia* were placed in a 20 cm × 20 cm × 20 cm glass aquarium filled with 7 l of lake water and allowed to acclimate for 10 min under the stimulus light. Responses were then videotaped for the next 10 min. There were five replicate trials (each consisting of 25 new *Daphnia* tested in every condition) per light stimulus per species.

Videotaped observations of *Daphnia* responses were analyzed frame by frame using motion tracking software (TRAKFISH, Racca Scientific Consulting, Victoria, B.C., Canada) that extracted the three-dimensional path coordinates of every *Daphnia* simultaneously present on both camera views during the first 3 min of the experiment. The large number of paths obtained during these 3 min (>50) were representative of the behaviour of *Daphnia* under a given light stimulus. This was shown to be the case by analyzing the orientation behaviour of *Daphnia* under both white polarized and unpolarized light stimuli for the remaining 7 min of the experiment (see Results). The path coordinates generated by TRAKFISH were further examined using path analysis software (ANAPATHS, Racca Scientific Consulting) which allowed path length to be determined. The four longest paths from each trial were chosen for further analysis; every one of these paths was at least 10 cm long as most *Daphnia* crossed the viewing field at least once (in the case of unpolarized light conditions, the paths were much less direct but path length remained >10 cm). Each path was then plotted in the horizontal (x, y) plane and the swim angle made by the *Daphnia* displacement vector (i.e. the vector linking the start and end points of the path, or the average vector of all sweeps made by the *Daphnia* across the field of view) with respect to the direction of the plane E_{max} determined (Fig. 3).

The 20 angles obtained per light background (5 trials × 4 *Daphnia* per trial) were pooled and analyzed as follows. First, the data were tested for circular uniformity using Rayleigh's test. Second, a V-test was performed on data that was not uniformly distributed to determine whether there was a preferred distribution in the direction perpendicular to E_{max} (Batschelet 1981).

The choice of four longest paths in our analysis was representative of the behaviour observed because all animals oriented similarly under a given light stimulus. This was demonstrated by analyzing the behaviour of a variable number of *Daphnia*, for each species, present at the same interval of time on both camera views during the 1st minute of experimentation.

Single *Daphnia* experiments

To show that the orientation behaviours were independent of *Daphnia* numbers in the aquarium (within the densities used), we performed experiments on each of 20 individual *Daphnia* per species under the 100% linearly polarized white stimulus. Experiments were carried out both under the point source and under a wide angle downwelling field. The same software and statistical techniques were used in these experiments with the exception that the statistics were performed on the ensemble of pooled individual paths.

Procedure for experiments with two light sources

In addition to documenting the orientation behaviour, we carried out a count of *Daphnia* present within 2.5 cm of the aquarium side closest to the sidewelling light source after 3 min of observation, under the wide angle source configuration. This was prompted by differences in *Daphnia* behaviour under the wide angle versus the point source configurations of the two sources.

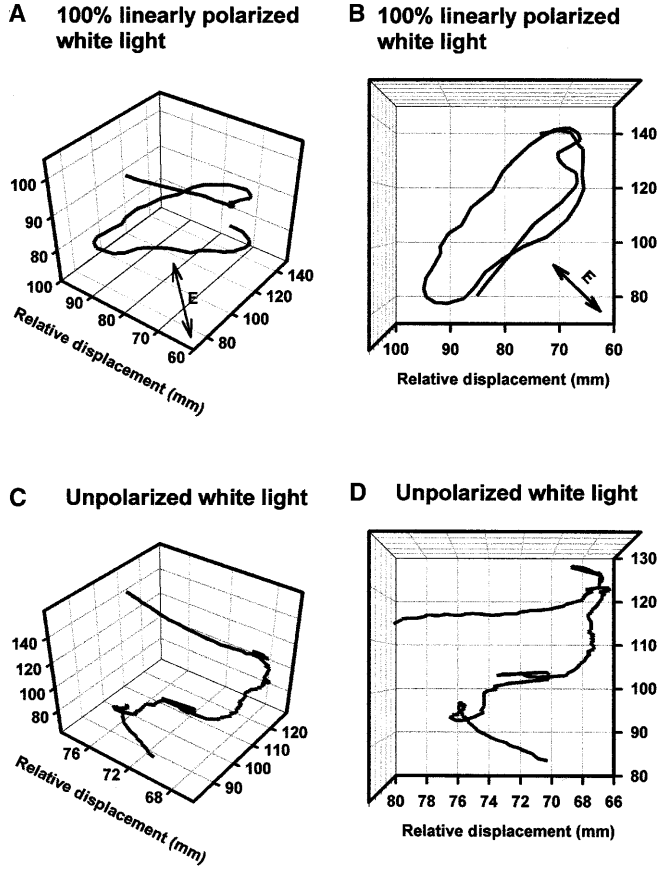

Fig. 3 Representative paths of individual *Daphnia* swimming under 100% linearly polarized white light (**A**, **B**), and diffuse white light (**C**, **D**). Panels **B** and **D** are two dimensional top-down projections of the paths in **A** and **C**. *Daphnia* swim perpendicular to E_{max} under 100% polarization and randomly under diffuse white light. Note that the path in **A** comprises more than one sweep across the field of view, i.e. upon reaching the periphery of the illuminating cone of light, the *Daphnia* turns around and swims back, always perpendicular to E_{max}. For statistical analyses, paths that comprised multiple sweeps were divided into individual sweeps and the average vector direction from all sweeps taken as the orientation angle for that particular *Daphnia*. Path length was therefore set constant in the statistical analyses. The orientation of the path was arbitrarily chosen in the direction of the first sweep

Results

Orientation to point sources

Under a diffuse white light stimulus (Fig. 2A), both species of *Daphnia* swam in random orientation (Figs. 4A, D). When the light field was linearly polarized, however, *Daphnia* oriented predominantly perpendicular to E_{max} (Figs. 4B, C, E, F). This behaviour was similar regardless of the time chosen for analysis (Figs. 5, 6), the individuals (paths) selected (Fig. 7), or on the number of *Daphnia* in the aquarium (Fig. 8). The statistics corresponding to these results and those that follow are presented in Table 2.

When, in addition to the polarized downwelling light field, an unpolarized light source is projected from the side of the aquarium along the E_{max} direction, *Daphnia* continue swimming perpendicular to E_{max} (and to the unpolarized light source, Fig. 9), despite the sidewelling intensity being approximately six times higher. The same orientation occurs if a polarizer is added in front of the sidewelling source, with polarization either perpendicular or horizontal. If, however, only the sidewelling source is turned on (unpolarized), *Daphnia* disappear from the observation volume as they rush to either of the two aquarium's sides along the path of the beam (in five trials conducted, over 80% of the *Daphnia* were found next to the glass wall near the light source, while fewer than 20% were on the opposite side). Once at the glass walls, the *Daphnia* maintained their depth with their usual 'hop and sink' movements without further horizontal displacement. This phototactic behaviour, to or away from a light source, has been documented previously (see Ringelberg 1987).

If the downwelling light is unpolarized but the sidewelling light is 100% linearly polarized, the orientation behaviour of *Daphnia* depends on the species. In the case of *D. magna*, neither a vertical nor horizontal sidewelling polarization perturbs their random swim within the downwelling cone of light near the centre of the aquarium (orientation is similar to that under a downwelling unpolarized light field alone). Nonetheless, most of the animals (61–78%, $n=3$ trials) are found on the side of the aquarium nearest to the sidewelling light source. In the case of *D. pulex*, a horizontal polarization induces a more pronounced shift toward the sidewelling light source, with many animals swimming perpendicular to the horizontal E_{max} (see results from wide angle experiments below). A vertical polarization, on the other hand, does not alter their random swim near the centre of the aquarium.

Orientation to wide angle stimuli

When the diffuser-polarizer combination is placed directly on top of the aquarium, creating a wide field stimulus that is either unpolarized or 100% linearly polarized, the orientation behaviour of each *Daphnia* species is the same as when subjected to the corresponding point source stimuli. The swim is random under unpolarized light and perpendicular to E_{max} under 100% polarization (see Figs. 4, 5, 6, 7, 8).

If the sidewelling source is also present with the same combination of diffuser and polarizer on the side of the aquarium, the following behaviours take place. In the case of a polarized downwelling light field of higher intensity than the sidewelling one, *D. magna* swims perpendicular to the downwelling E_{max} irrespective of the polarization state of the sidewelling light source (i.e. whether unpolarized or polarized vertically or horizontally). Most animals are found swimming on the half of the aquarium closest to the 150 W source, but near the centre of the aquarium. If the sidewelling light is unpolarized or vertically polarized, the *Daphnia* are found

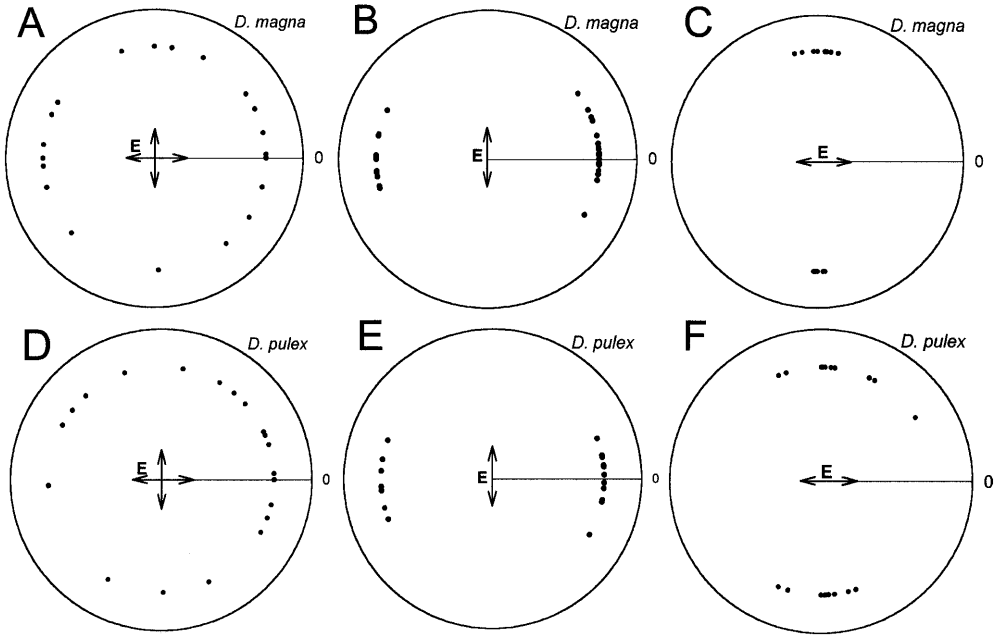

Fig. 4A–F Angular orientation of *Daphnia* to diffuse and 100% linearly polarized white light stimuli. Each *dot* represents the orientation angle of an individual *Daphnia* with respect to the 0° (radius) line. Angles increase anti-clockwise from the 0° line. The orientation of E_{max} is indicated in each panel. Orthogonal *E*-vectors indicate diffuse, unpolarized, light. Panels show the following (*from left to right*): swimming orientation to diffuse white light, to 100% linearly polarized white light, and to 100% linearly polarized white light with E_{max} perpendicular to the previous direction, for *D. magna* (**A–C**), and *D. pulex* (**D–F**)

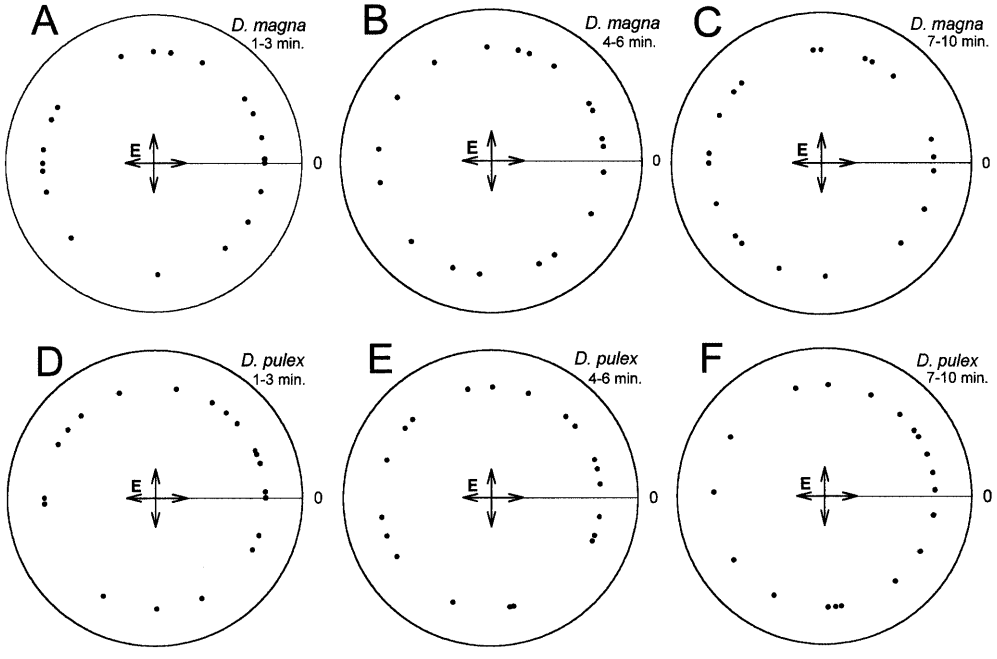

Fig. 5A–F Angular orientation of *Daphnia* to diffuse white light as a function of time. Panels represent angular data from 20 animals (five replicates × four longest paths) during time intervals 1–3 mins (**A**, **D**), 4–6 mins (**B**, **E**) and 7–10 min (**C**, **F**) for *D. magna* (**A–C**) and *D. pulex* (**D–F**). Presentation of data as in Fig. 4

swimming within a restricted vertical band (about 3 cm wide). If the polarization is horizontal, there is a small horizontal dispersal (~1 cm) toward the sidewelling light source and, in one trial out of three, 5 animals out of 25 swam to the aquarium wall near the sidewelling light source. Similar distributions are observed if the downwelling light is unpolarized, except that in this case the animals swim randomly.

If, however, the two sources are equally intense, about 28–46% of the animals ($n=3$) will swim toward the sidewelling light source and stay close to the wall. This result is similar whether the sidewelling light is diffuse, vertically or horizontally polarized. The rest of the animals are observed to be more scattered in the aquarium than if they swam under point sources of comparable intensities and polarizations. Those that swim near the centre of the aquarium swim primarily perpendicular to the downwelling E_{max} but their bodies are inclined toward the sidewelling light source. Some (~8–24%, $n=3$) remain near the wall of the aquarium that is furthest from the sidewelling light source.

For similar light stimuli combinations, the behaviour of *D. pulex* is markedly different from that of *D. magna*. Whether the downwelling light field is polarized or not and whether it is more intense or not than the sidewelling light field, a horizontal polarization from the side

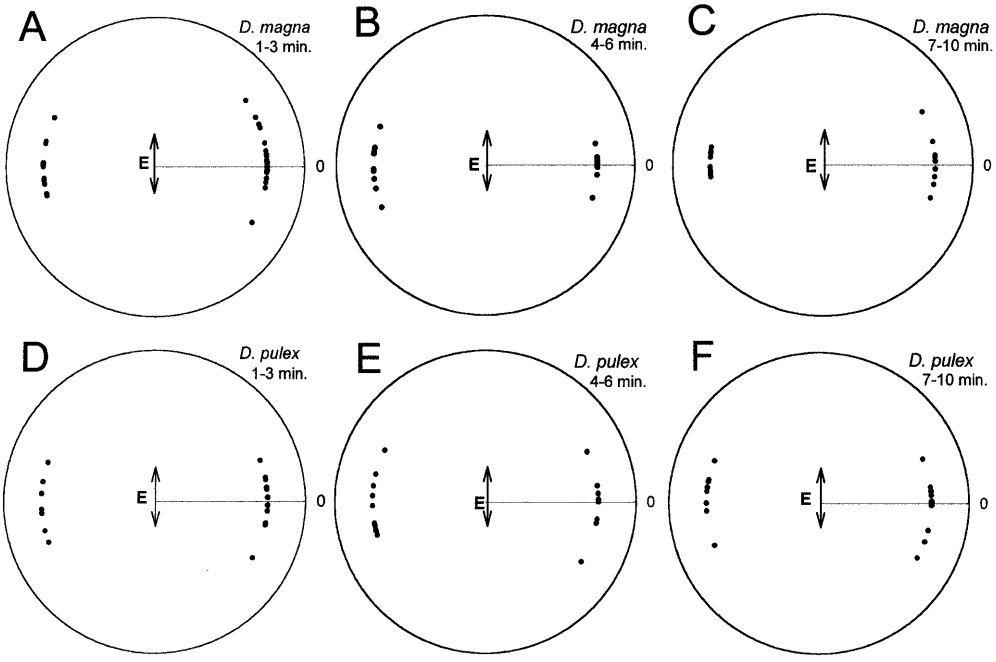

Fig. 6A–F Angular orientation of *Daphnia* to 100% linearly polarized white light as a function of time. Treatment and presentation of data as in Figs. 4 and 5

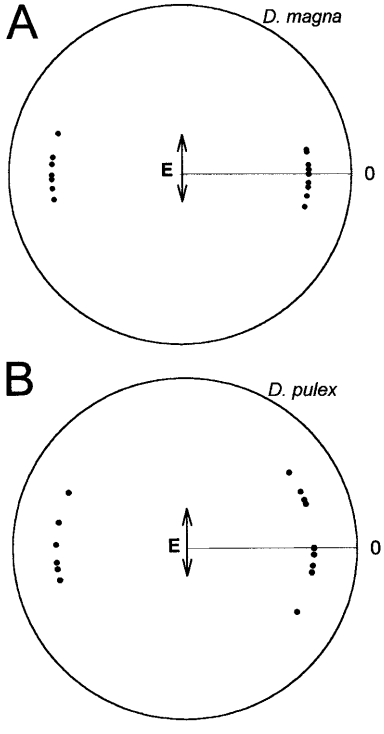

Fig. 7A, B Angular orientation of *Daphnia*, present at the same time interval in both camera views during the 1st minute of recording, to 100% linearly polarized white light. **A** *D. magna*, $n=16$; **B** *D. pulex*, $n=15$. Presentation of data as in Fig. 4

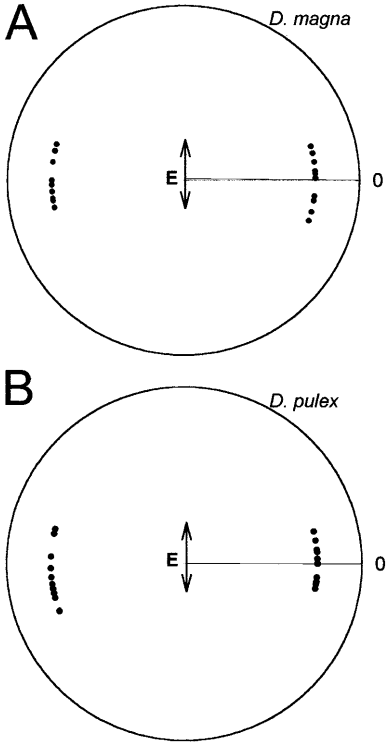

Fig. 8A, B Angular orientation of *Daphnia*, from 20 experiments using a single *Daphnia* per experiment, to 100% linearly polarized white light. Presentation of data as in Fig. 7

induces a swim toward the sidewelling light source in the majority of animals (Fig. 10). Even at intensity ratios of ~600:1 in favour of the downwelling light field, a major proportion of *Daphnia* swim toward the sidewelling light source, and stay within 2.5 cm of the aquarium wall (Fig. 10). If the sidewelling polarization is switched to vertical, the animals swim away from the aquarium wall and toward the centre where they orient, primarily, perpendicular to the downwelling E_{max}. A similar distribution is observed if the downwelling light field is polarized and the sidewelling one unpolarized, provided the downwelling field is more intense. Under these

Table 2 Statistical results of Rayleigh's test applied to *Daphnia*'s doubled swimming angles. Symbols are as follows: ā = average double angle, *SD* standard deviation, *a* theoretical double angle, *R* Rayleigh's R statistic, *z* statistic for circular uniformity, *u* statistic for distribution along a mean direction (see Batschelet 1981). In the following, the data represented by the mean double angle are uniformly distributed around the trigonometric circle if $z \leq z_{0.05,20} = 2.958$ (in the case of 16 and 15 observations – Fig. 7 – the corresponding *z* numbers are: $z_{0.05,16} = 2.948$, and $z_{0.05,15} = 2.945$). The data are distributed along a specific mean direction (the angle *a*) if $u \geq u_{0.05,20} = 1.646$ (in the case of $n = 16$ or 15, $u_{0.05,15 \text{ or } 16} = 1.647$). The Figure no. corresponding to each statistic is indicated in the table

Species	Figure no.	ā	SD	a	R	z	u
magna	4a	141	123	–	6.30	1.98	–
magna	4b	7.4	21.8	0	18.3	16.7	5.73
magna	4c	179	10.4	180	19.7	19.4	6.23
pulex	4d	153	118	–	5.14	1.32	–
pulex	4e	0.7	26.6	0	18.1	16.3	5.71
pulex	4f	173	37.2	180	16.5	13.7	5.19
magna	5a	141	123	–	6.30	1.98	–
magna	5b	147	114	–	4.26	0.91	–
magna	5c	156	121	–	3.38	0.57	–
pulex	5d	153	118	–	5.14	1.32	–
pulex	5e	145	124	–	7.04	2.48	–
pulex	5f	172	109	–	1.68	0.14	–
magna	6a	7.4	21.8	0	18.3	16.7	5.73
magna	6b	0.8	19.1	0	19.0	18.0	6.00
magna	6c	0.7	19.0	0	19.0	18.0	6.00
pulex	6d	0.7	26.6	0	18.1	16.3	5.71
pulex	6e	1	28.9	0	17.7	15.7	5.60
pulex	6f	354	26.3	0	18.1	16.4	4.25
magna	7a	357	17.2	0	19.1	18.2	6.03
pulex	7b	358	38.0	0	16.2	13.1	5.12
magna	8a	0.3	20.3	0	18.8	17.7	5.92
pulex	8b	4.9	20.6	0	18.8	13.1	5.12
magna	9a	2.2	30.0	0	17.5	15.4	5.54
pulex	9b	359	29.0	0	17.7	15.7	5.95
magna	11a	4	13.8	0	19.4	18.9	6.14
magna	11b	6	20.6	0	18.8	17.7	5.92
magna	11c	0.1	15.0	0	19.0	18.7	6.12
pulex	11d	8.1	13.9	0	19.5	18.9	6.09
pulex	11e	170	101	–	2.73	0.37	–
pulex	11f	176	32.5	180	17.1	14.7	5.41
magna	12a	2	13.4	6	19.5	19.0	6.15
magna	12b	335	13.4	332	19.5	19.0	6.15
magna	12c	305	28.6	274	17.7	15.7	4.83
magna	12d	286	20.2	234	18.6	17.3	3.62
magna	12e	184	122	0	4.00	0.80	–
magna	12f	143	61.4	196	11.1	6.21	2.11
magna	12g	70.1	20.3	110	18.9	17.8	4.57
magna	12h	43.7	18.4	76	19.1	18.1	5.09
magna	12i	11.8	20.7	20	18.8	17.7	5.88
pulex	13a	2.7	12.9	6	19.5	19.1	6.16
pulex	13b	344	23.1	332	18.5	17.1	5.35
pulex	13c	332	43.8	274	14.7	10.8	2.46
pulex	13d	241	109	234	9.57	4.58	3.01
pulex	13e	161	120	0	4.56	1.04	–
pulex	13f	167	109	196	1.42	0.10	–
pulex	13g	107	51.6	110	10.6	5.62	3.35
pulex	13h	59.8	22.2	76	18.6	17.3	5.65
pulex	13i	19.4	20.1	20	18.9	17.8	5.96

conditions, if both downwelling and sidewelling fields are unpolarized, *D. pulex* swims randomly, as was the case for *D. magna*, and for both species under point source illuminations.

Behaviour under polarization backgrounds of different spectral and percentpolarization content

Under 455 LP illumination, both *D. magna* and *D. pulex* oriented perpendicular to E_{max} (Fig. 11A, D). When tested under 515 LP and 570 LP stimuli, *D. magna* continued to swim perpendicular to E_{max} (Fig. 11B, C). *D. pulex*, however, swam randomly under 515 LP illumination (Fig. 11E) and parallel to E_{max} under the 570 LP stimulus (Fig. 11F). Orientation to E_{max} in *D. magna* is observed to be independent of the spectral content of the stimulus, while *D. pulex*'s orientation behaviour is wavelength dependent.

Results with white light stimuli that varied in percent polarization showed that the two *Daphnia* species differed in their resolution of E_{max}. *Daphnia magna*

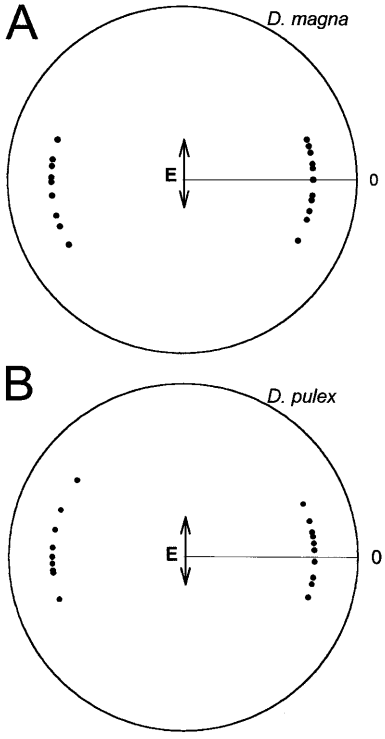

Fig. 9A, B Angular orientation of *Daphnia* when exposed to both a downwelling 100% linearly polarized white light field, and a diffuse white light field of ~6 times the intensity from the side. The E_{max} vector direction is also the direction of light propagation from the sidewelling unpolarized source. Note that the animals continue swimming perpendicular to E_{max} (and to the unpolarized light source) in these conditions. The movement is inversed, i.e. along the unpolarized light source and onto the tank walls, when only the sidewelling light is on. Presentation of data as in Fig. 7

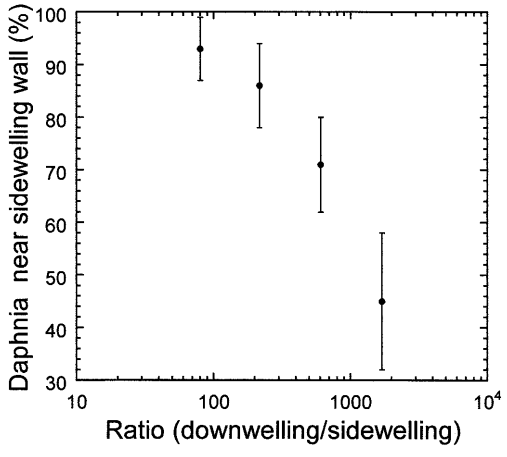

Fig. 10 Daphnia numbers (as a percentage of the total) found within 2.5 cm of the aquarium wall closest to the 150 W sidewelling source 3 min after initial illumination. Each trial used 25 *Daphnia* ($n = 5$)

exhibited oriented swimming behaviour primarily perpendicular to E_{max} down to 16% polarization (Fig. 12), while *D. pulex* required at least 31% polarization (Fig. 13) for similar orientation behaviour. Under most polarization stimuli the variability in orientation responses exhibited by *D. pulex* was greater than that for *D. magna* (see standard deviations in Table 2).

Discussion

Daphnia orient perpendicular to the E_{max} of downwelling polarized light

Under 100% linearly polarized white light, both species of *Daphnia* oriented perpendicular to E_{max} (Figs. 4, 6, 7, 8; recall that we did not measure any significant differences in intensity or percent polarization with horizontal direction of view in these experiments). The orientation was bimodal and independent of intensity gradients (Fig. 9) demonstrating that the animals were orienting to the polarization of the downwelling light field, as previously observed for *D. magna* and other species (Baylor and Smith 1953).

The similarity in results obtained irrespective of whether the downwelling source was point-like or wide angle attests to the validity of point source studies in the determination of photoreceptor mechanisms responsible for polarization orientation in *Daphnia*. Although such stimuli may not occur frequently in nature (Schwind 1999, except for potential polarization emissions from other animals), the practical advantages of point sources makes them a valid and indispensable tool in many studies (e.g. in the percent polarization experiments presented here). Our results suggest that the findings from previous studies, the majority of which used downwelling point sources, should concur with the orientation behaviour of the species tested under a wide angle downwelling polarized light field as well.

The similarity in orientation behaviours irrespective of the angular dimensions of the downwelling light field suggests various possibilities for the detection of polarization information in *Daphnia*. Since the downwelling point source stimulus in our study could only have directly activated a single ommatidium at a time (and since it is this cue that was responsible for the behaviour), it is conceivable that polarization discrimination only requires stimulation of a single polarization rhabdom. Alternatively, *Daphnia* may be comparing information from various polarization rhabdoms using rotatory eye movements (Frost 1974; Ringelberg 1987), or by quick body movements away from the vertical. Either system may require some form of short-term memory.

The preference for a downwelling or sidewelling E_{max} is species specific

Under wide field stimuli, *D. pulex* swam toward a sidewelling horizontally polarized light field irrespective of the downwelling polarized light field (within a wide range of intensity ratios between the two sources). In contrast, the animals swam away from a vertically

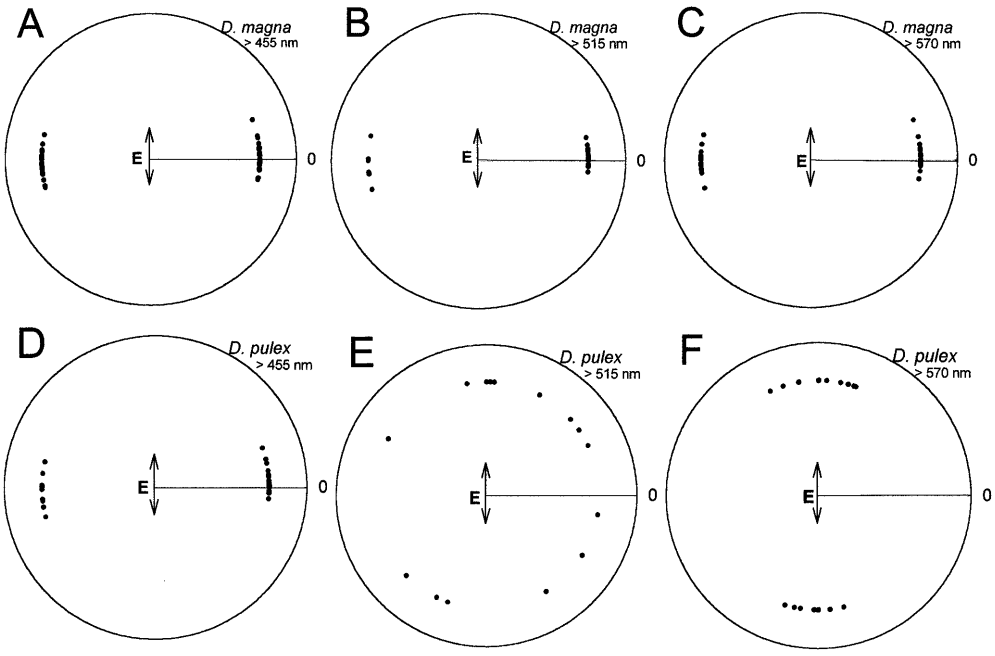

Fig. 11 Angular orientation to 455 LP, 515 LP, and 570 LP 100% linearly polarized stimuli, in this order, for *D. magna* (**A–C**) and *D. pulex* (**D–F**). Data presented as in Fig. 4

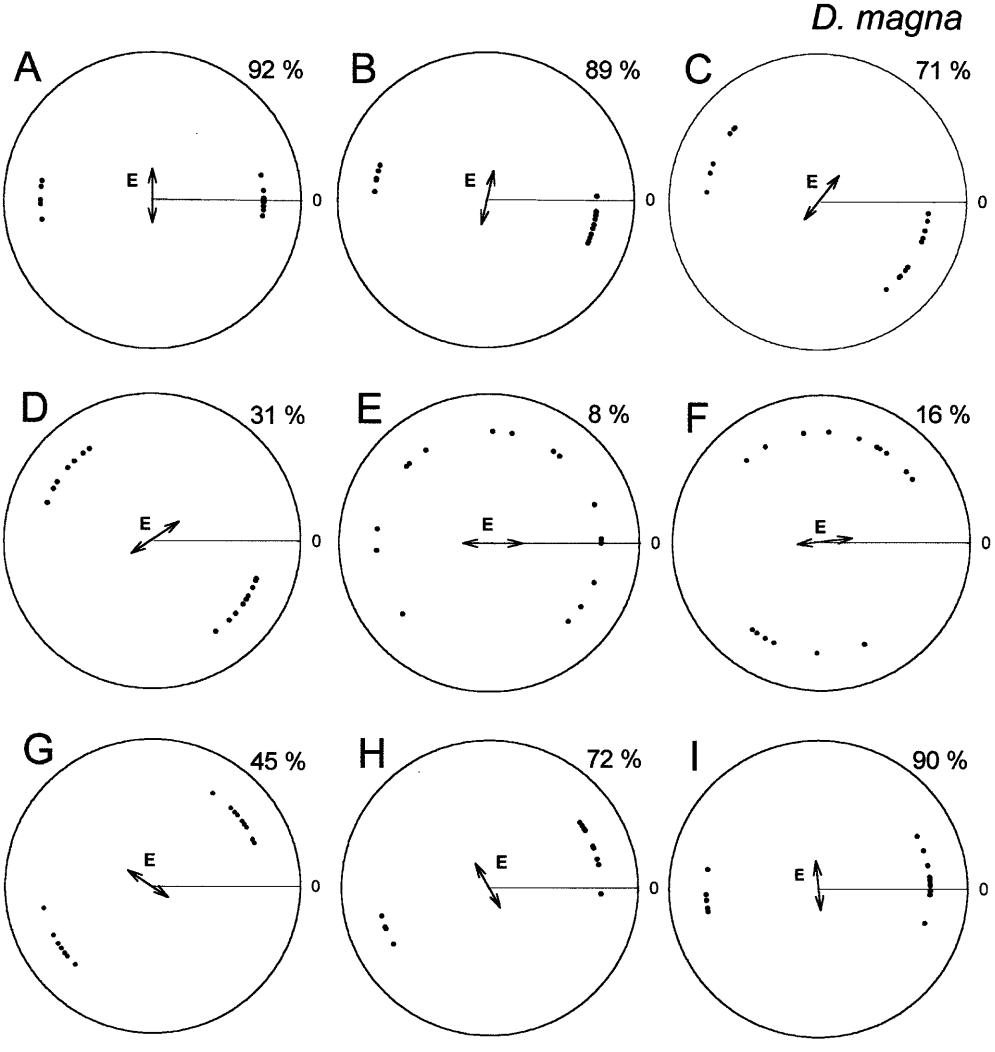

Fig. 12 Angular orientation of *D. magna* to white light stimuli of different percent polarization (diagrammatic representation as in Fig. 4). The measured E_{max} bearing and percent polarization are indicated on each panel. The presentation of percent polarizations follows from progressive ~10° rotations of the quarter wave plate with respect to the linear polarizer, after initial alignment of one of the optical axes with the transmission axis

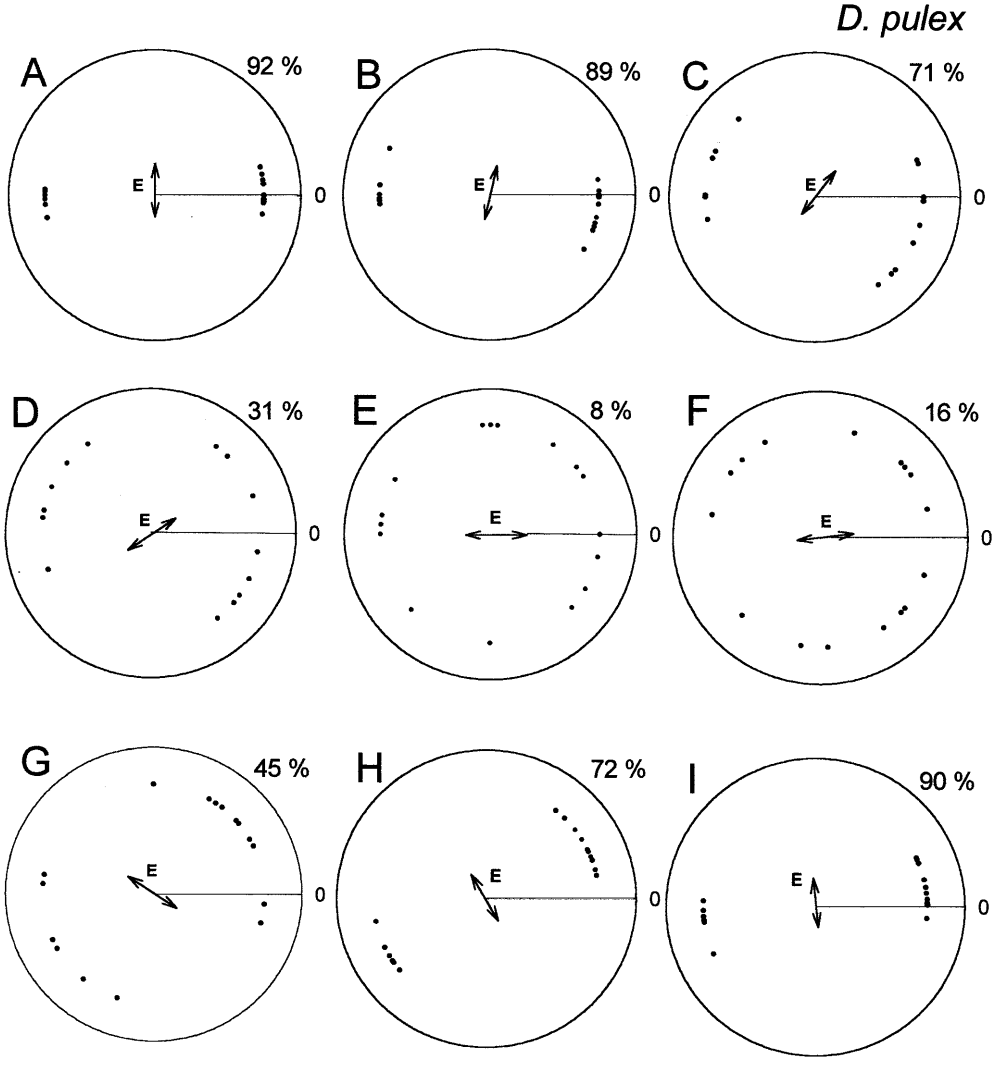

Fig. 13 Angular orientation of *D. pulex* to white light stimuli of different percent polarization (data presented as in Fig. 12)

oriented sidewelling polarized light source. These results agree with those reported by Schwind (1999) and point to the potential importance of sidewelling polarized light in the orientation behaviour of *D. pulex* in nature.

The behaviour of *D. magna*, which swims perpendicular to the E_{max} of a downwelling light field irrespective of the polarization of a less intense sidewelling light field, suggests that it is the downwelling light field that is most important for this species in nature. The illumination used in some of our experiments approaches that found during clear crepuscular periods, when the polarized light field is in the downwelling direction and percent polarization is at its maximum (see Novales Flamarique and Hawryshyn 1997). Polarization orientation in *D. magna* may thus be an adaptation for displacements during crepuscular periods, when the sun is no longer visible. The mechanistic reasons for the differences in orientation behaviour between species are unknown to us, but they may arise from differences in the location of polarization receptors and visual pigment types in the retinas of each species.

Spectral characteristics of the polarization detection systems

The spectral dependence of polarotaxis and the photoreceptor mechanisms underlying polarization discrimination were different in the two species (Fig. 11). Previous observations on the structure and photopigment content of *Daphnia* rhabdoms, in conjunction with our observations, provide a basis for interpreting these results.

The rhabdoms of *D. magna* and *D. pulex* are composed of eight retinula cells comprising two orthogonal microvillar orientations (Fig. 2B; Röhlich and Törö 1965; Smith and Macagno 1990). In *D. magna* (Smith and Macagno 1990) and presumably in *D. pulex* as well (Röhlich and Törö 1965), the only retinula cells that exhibit two orientations of microvilli are those that contain the S or M visual pigments (Fig. 2B). To date, only one retinula cell has been found to contain the L visual pigment, and this cell (R1, Fig. 2B) only contributes microvilli to the proximal end of the rhabdom

(Smith and Macagno 1990). Results from experiments with the 515 LP and 570 LP stimuli indicate that neither retinula cells containing the UV nor the S visual pigments are necessary for polarization discrimination. This is because polarization orientation persisted under these stimuli which had spectral content beyond the absorption range of the UV pigment (515 LP and 570 LP) and that of the S pigment (570 LP, Fig. 2B). In the case of *D. magna*, considering the higher relative photon catch of the L visual pigment when illuminated with the 570 LP stimulus (Table 1), and the assumption that polarization discrimination in crustaceans is a function of the relative photon catch between cell types with orthogonal microvilli (Waterman and Horch 1966), the persistent orientation of *D. magna* perpendicular to E_{max} under this illumination suggests that only the M retinula cells are involved in polarization discrimination in this species. This is because the only polarization channel with orthogonal microvilli that is stimulated under the 570 LP stimulus is the M channel. In contrast, the 90° shift in the orientation of *D. pulex* relative to E_{max} under 570 LP illumination, and the random orientation under a background (515 LP) that produces similar total photon catch for the M and L visual pigments (Table 1), implies a two-pigment polarization visual system. This system is most likely based on the action of the M and L retinula cells, although the S retinula cells cannot be ruled out completely without detailed information on the absorbance of visual pigments in this species. Under the present assumption of similar visual pigments to *D. magna*, the S retinula cells of *D. pulex* always have a photon catch smaller than those containing the M or the L visual pigments under the 455 LP, 515 LP and 570 LP illuminations. Thus, if the system were based on an S/M or S/L interaction, the orientation behaviour (were it antagonistically linear) would lead to the same swim, that dictated by the retinula cells containing the M or L visual pigments respectively. We can only conclude, under the present assumptions, that the potential contribution of the S retinula cells to the polarization channel would be of the same polarity as that of the M channel.

To our knowledge, the two pigment polarization detection system of *D. pulex* is the first of its kind reported for any invertebrate. We propose that the detection systems of both *Daphnia* species, comprising one or two visual pigments, function in a similar way to that suggested for arthropods (Wehner 1983; Labhart and Petzold 1993). In other words, second order processing by interneurons equivalent to those found in the cricket visual system (Labhart 1988) would sum antagonistic inputs from the two structural classes of polarization receptors with orthogonal microvilli. In the case of *D. magna*, polarization discrimination leads to an oriented swim perpendicular to E_{max}; in *D. pulex* the relative photon catch of M versus L receptors is most likely determined in order to swim perpendicular or parallel to E_{max}. The majority of light backgrounds that *Daphnia* encounters in nature would result in higher photon catch by the M receptors (Fig. 2, Table 1) – this would produce a swim oriented perpendicular to E_{max} for both species, which is what has been observed previously (Baylor and Smith 1953; Waterman 1981).

A polarization system based on two visual pigments must account for spectral changes in intensity that could confound the polarization signal (Bernard and Wehner 1977). In *D. pulex*, this could be accomplished by the UV (or the S) retinula cells as they probably sample fields of view similar to those of polarization-discrimination receptors. Alternatively, because of the large receptive fields of *Daphnia* ommatidia (~40–60°, Young and Downing 1976), spectrally-induced changes in intensity between the fields of view sampled by the limited number of dorsal ommatidia involved in downwelling light capture may not be sufficiently large to disrupt the polarization detection system (see Smith and Macagno 1990 for a drawing of the compound eye with the distribution of ommatidia). The fact that the downwelling spectrum in surface waters of lakes inhabited by these animals is more homogeneous in the middle to long wavelengths (Novales Flamarique et al. 1992; Novales Flamarique and Hawryshyn 1997) would reduce the probability of spectrally induced errors.

Responses to white light backgrounds of varying percent polarization

The percent polarization thresholds required to detect E_{max} for both species of *Daphnia* are close to the value of 20% reported for another cladoceran, *D. schødleri* (Waterman 1981). The higher threshold percentage associated with *D. pulex*'s orientation behaviour may reflect a lower resolution of polarization discrimination systems based on multiple visual pigments, versus those based on one. This may be due to the added neurological complexity (and intrinsic tolerance of the components) required to compensate for changes in spectral intensity that could confound the polarization signal, or the errors involved in comparing average polarization signals between rhabdoms, should there be little spectral compensation. It is noteworthy that our percent polarization results for *D. pulex* agree with observations reported by Schwind (1999). In that study, *D. pulex* were subjected to different percent polarizations from either side of an aquarium. The animals swam to the side with higher percent polarization; animals preferred the half of the aquarium nearest the 37% polarized light source versus that which was diffuse (0%), but their distribution was statistically the same when the sources were 22% and 0% polarized, respectively. Further experiments similar to the ones reported in this study should be conducted with other *Daphnia* species to determine the resolution of single versus multiple pigment-based polarization detection systems.

From the polarization thresholds observed, we conclude that *Daphnia* can detect and use the partially polarized light field present in the water column

throughout the day, which may vary in percent polarization between 20% and 65% under cloudless skies and near the water surface (Novales Flamarique and Hawryshyn 1997). The polarization detection systems of cladocerans are significantly more sensitive than those of fishes (Hawryshyn and Bolger 1990; Novales Flamarique and Hawryshyn 1997) but slightly less than those of arthropods for which polarization percentages in the range 5–10% are sufficient for discrimination and orientation to E_{max} (Waterman 1981; Labhart 1996). These differences are likely based on two facts. First, the arthropod microvillar polarization system utilizes a single pigment and involves more receptors (an indication of higher spatial resolution) than the corresponding system in *Daphnia* (Waterman 1981; Wehner 1983, 1989; Labhart and Petzold 1993). Second, except for anchovies (whose percent polarization thresholds are unknown, Novales Flamarique and Hawryshyn 1998), the polarization detection system of fishes uses multiple visual pigments and lacks dichroic structures (Novales Flamarique and Hawryshyn 1997; Novales Flamarique et al. 1998).

Ecological implications of polarization orientation in *Daphnia*

The functional significance of polarization orientation in *Daphnia* is unknown. Aggregation into lines perpendicular to E_{max} has been proposed as a mechanism to avoid mutual interference while feeding in food rich surface waters (Hazen and Baylor 1962). Under these conditions, polarization-induced swarms may minimize predation risk because: (1) predators sometimes hesitate to attack aggregated prey (Neill and Cullen 1974), (2) on average, the individual is better protected (the selfish herd effect, Hamilton 1971), and (3) optical mechanisms (such as thin layer interference by cuticle layers when oriented perpendicular to the wave front) may operate to reduce spectral contrast to predators in the short wavelengths (Giguère and Dunbrack 1990). In addition, Rayleigh scattering by microscopic algae could produce horizontally polarized light (see Hecht and Zajac 1974 for scattering patterns by Rayleight-type particles); swimming perpendicular to this polarization would lead *Daphnia* to the algal patches.

Recently, it has been suggested that *D. pulex*'s response to broadband horizontally polarized light explains the phenomenon of "shore flight" (Schwind 1999). In lakes, *Daphnia* will see higher polarizations coming in the direction of the open water, rather than the shore, leading to the reported "shore flight" to open water (Schwind 1999). Although this is an interesting hypothesis, our results indicate that it does not fully explain the orientation behaviour of *D. magna*. Furthermore, on many cloudy or misty days, the polarization cue could not be used given the low percent polarizations found in natural water bodies (see Novales Flamarique and Hawryshyn 1997). If polarization orientation promotes "shore flight", then this displacement should occur primarily during clear sunny days, a hypothesis that requires testing. In conclusion, because of the variations in percent polarization and E_{max} orientation (from horizontal) in natural water bodies (Novales Flamarique and Hawryshyn 1997), the reliability of a mechanism to get away from shore predators based on polarization cues alone appears limited.

The parallel swim with respect to E_{max} exhibited by *D. pulex* under long wavelength illumination is intrigu-

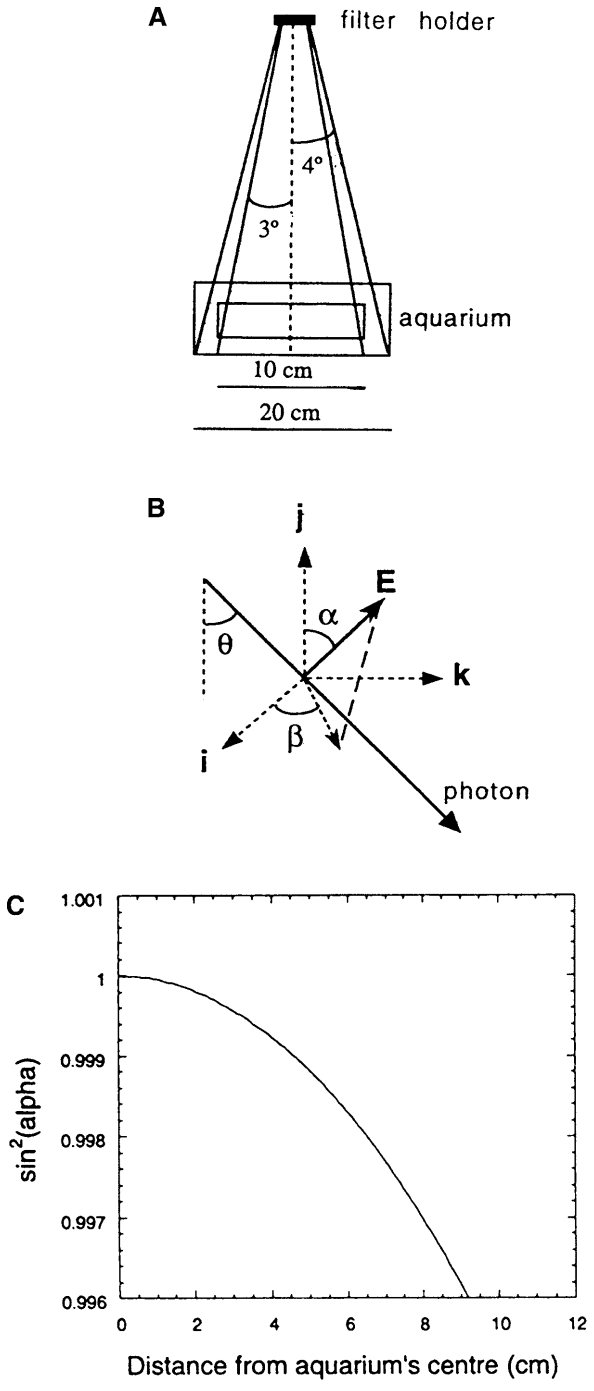

Fig. 14A–C

ing. Irradiance profiles that contain only long wavelengths are characteristic of sub-surface layers of dystrophic lakes (e.g. McDonald and Hawryshyn 1995). These environments lack the abundance of food algae present in non-dystrophic systems such that the advantages of foraging perpendicular to the polarization, and the camouflage created by enhanced reflectivity of short wavelengths under broadband illumination (Giguère and Dunbrack 1990), would be absent. Further research is needed to understand the advantages of swimming parallel to E_{max}, if any, under these conditions.

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Appendix

The illumination field consisted of a light cone of 4° half aperture as depicted in Fig. 14A. The viewing field extended 7.5 cm on either side of the normal to the centre of the aquarium. It is important to know how the electric field of linearly polarized light changes with aperture angle, as this will determine the intensity across the field of observation.

Figure 14B shows a photon with electric field (E) travelling with angle θ with respect to the vertical (z axis). The electric field makes an angle α with the z axis and an angle β with the x axis. The components of E are given by $E = Esin(\alpha)cos(\beta)i + Esin(\alpha)sin(\beta)j - Ecos(90° - \alpha)k$, or, $E = Esin(\alpha)cos(\beta)i + Esin(\alpha)sin(\beta)j - Esin(\alpha)k$, where i, j, and k are unit vectors in the x, y, and z directions, and $\alpha = 90° - \theta$.

Since intensity (I) is proportional to E^2 (Hecht and Zajac 1974), this quantity will vary as $\sin^2(\alpha)$ in any direction away from the centre of the aquarium (Fig. 14C). For the most peripheral ray entirely within the field of view in this study (most paths were in the lower half of the aquarium and within this restricted cone), $\theta = tan^{-1}(7.5/145) \approx 3°$, so that $\alpha = 87°$, and the magnitude square of the field in any direction will be, theoretically, 99.7% of the corresponding value for the axial ray ($\theta = 0°$). The percent polarization will not change.

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