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DAPHNIA PULEX SWIMS TOWARDS THE MOST STRONGLY POLARIZED LIGHT – A RESPONSE THAT LEADS TO 'SHORE FLIGHT'

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Summary

When *Daphnia pulex* are presented on one side of their visual field with diffuse, large-area linearly polarized light with a horizontal e-vector and on the other side of their visual field with large-area polarized light with a lower degree of polarization, they swim towards the place with the higher degree of polarization. The response is intensity-invariant: *Daphnia pulex* swim towards the place of maximal polarization regardless of which side of their visual field has the higher intensity of light. As a result of Rayleigh scattering in a pond, the light surrounding the *Daphnia* is polarized and has a horizontal e-vector. Near the shore, polarization is not homogeneous. The light seen in the direction of the open water has a higher degree of polarization than that seen in the direction towards the shore. Therefore, in a pond, swimming towards the place with the highest degree of polarization leads the *Daphnia* away from the shore. For *Daphnia*, this response explains a mechanism that underlies the well-known phenomenon of 'shore flight', the active departure of small pelagic crustaceans from shore zones.

Key words: polarization vision, *Daphnia pulex*, shore flight, eye movement, vision.

Introduction

The visual receptor cells of many arthropods are polarization-sensitive; that is, their response to linearly polarized light depends on its direction of oscillation. Some arthropods are able to exploit this property of their receptor cells to orient themselves in space with reference to the naturally occurring linear polarization of the light. For example, bees and other Hymenoptera navigate by the polarization pattern of sky light produced by Rayleigh scattering (von Frisch, 1949; Wehner and Rossel, 1985). Aquatic insects flying in search of new bodies of water to colonize and other insects with modes of life closely associated with water, such as dragonflies and mayflies, identify water surfaces by the polarization of the reflected light (Schwind, 1985, 1995; Kriska et al., 1998; Wildermuth, 1998).

In these examples, it has been clearly demonstrated that the polarization sensitivity of the visual systems is biologically significant. However, in the case of other arthropods known to respond to the direction of linear polarization under particular experimental conditions, it remained an open question whether the ability to discriminate polarization had any adaptive value for the animals in their natural habitat. An example of this group is the 'water flea' *Daphnia*.

Baylor and Smith (1953) had shown that when *Daphnia* and other small crustaceans are in a vertical beam of linearly polarized light, they preferentially swim perpendicular to the direction of the e-vector of the light while travelling horizontally. In later experiments on *Daphnia*, the stimulus was usually a beam of linearly polarized light produced by a projection apparatus (e.g. Waterman, 1960; Jander and Waterman, 1960; see also Waterman, 1981, p. 322). One purpose of such experiments was to determine whether *Daphnia*, like bees, set their course with reference to the polarization pattern of sky light. This pattern is visible even under water, although it is distorted by Snell's window (the circular transparent sector of the water surface seen above an observer in the water). The results of the tests confirmed the polarization sensitivity of the *Daphnia* visual system, but Waterman (1981, p. 331) concluded that 'there is no information on the adaptive significance of their polarization sensitivity nor any evidence for its menotactic application to possible course steering'.

Beams of linearly polarized light are not stimuli that *Daphnia* normally encounter: the sunlight that directly penetrates the water is not polarized. The polarized light that *Daphnia* can see is diffuse, covering a large area; it is either the part of the polarized skylight visible through the 97° Snell's window or the light horizontally polarized in the water by Rayleigh scattering. The direction of oscillation of the latter light, which an animal under water sees at its sides, is perpendicular to the direction of its gaze and predominantly horizontal, although when the sun is low there can be considerable departures from horizontality of the e-vector for certain directions of gaze (see Waterman, 1981, p. 300). The degree of polarization is approximately 40 %. The intensity of this background light is low in comparison with that of the light incident from above. This paper describes the reaction of *Daphnia pulex* to diffuse, large-area, partially polarized light of this kind that has adaptive significance to the animals in their natural habitat.

Materials and methods

Adult specimens of *Daphnia pulex* were used that had been collected from a pond in the autumn of 1998 and kept over the winter in aquaria. The enclosure used for the tests was a water-filled acrylic tank with a base measuring 50 cm×30 cm and a height of 20 cm (Fig. 1). To assist observation, the animals were placed into a compartment occupying only 10 cm of the tank's 30 cm width, separated from the rest of the tank by an acrylic plate. The tank was set up in a room with its long side parallel to a window that had been completely covered with depolarizing, translucent paper. Glued to the back of the tank (the long side towards the window), from the bottom of the tank to the meniscus of the water, was a sheet of black film. The two small sides were covered with black material above the water level. The light entering through the window was diffusely reflected by screens set at an angle to the small sides of the tank. Because of the shielding on the upper parts of the small sides of the tank, this reflected light could be seen only below the water surface. To demonstrate the intensity invariance, a white and a nearly black reflector were used, and at one small side of the tank the light was polarized by a polarizing film (Käsemann, type RW 84) that covered the entire surface of that side.

The light entering the sides of the tank is reflected by the totally reflecting water surface and by parts of the side walls of the tank. These reflections are symmetrical in the right and the left sides of the tank. Furthermore, the degree of polarization and the horizontal alignment of the e-vector are retained. Therefore, the crucial variables (the test lights, the degree of polarization and the direction of the e-vector) are not changed by the reflections.

In the experiments with variable degrees of polarization, polarizing films were set into cuvettes filled with water that had been made turbid by adding milk (5 ml of condensed milk in 14 l of water), which were placed next to the small sides of the experimental tank. The cuvettes had an opaque black covering on the back, front, top and bottom surfaces, while the side wall facing the small side of the tank was transparent. Depolarizing translucent paper was attached to the outer surface of the wall facing the reflector. The degree of polarization could be continuously adjusted, without changing the intensity, by moving the films to different distances from the small sides of the tank. To obtain a degree of polarization of zero, the polarizing film was placed outside the cuvette, behind the depolarizer. The ratio of the intensity of the light entering the test tank from above to that entering at the two small sides (when white reflectors were used) was 10:1. The light entering the tank from the front side, through which the *Daphnia* were observed, was greatly reduced by appropriate shielding. The degree of polarization induced by the films or the water was measured by means of an ultraviolet Glan-Thompson prism equipped at its input with a quartz objective; aperture 10°. The intensities of the ordinary and the extraordinary rays were measured using silicon photodiodes. The prism was rotated around the measuring axis until the difference between the two intensities was maximal. The degree of polarization (p, %) was then calculated as:

$$p = 100(I_{max} - I_{min})/(I_{max} + I_{min}),$$

where I_{max} and I_{min} are maximal and minimal intensity measured simultaneously by the two photodiodes.

The experiments were carried out in the morning to midday hours, in overcast weather conditions. For each trial, 8–12 *Daphnia pulex* were placed into the front compartment of the tank, the desired lighting conditions were applied, and the number of animals in the right and left halves of the tank was counted after 5 min. The stimulus arrangement was then reversed, and right/left counts were again obtained after 5 min. For most of the values given in Table 1, three different populations were tested; in principle, all showed the same reactions. The probability (P) values in Table 1 were calculated using a nonparametric binomial test (SPSS software).

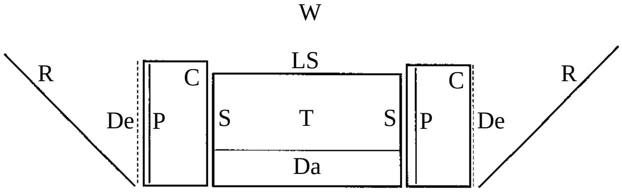

Fig. 1. Diagram of the experimental apparatus. LS, long side of the tank facing the window, black from the bottom to the meniscus of the water; C, cuvette filled with turbid water; Da, compartment of the tank containing *Daphnia pulex*; De, depolarizer; S, small side of the tank, black above water level; P, polarization film; R, reflecting surface; T, acrylic tank; W, window of room, covered with depolarizing, translucent paper.

Results

Daphnia (in this case *Daphnia pulex*) under certain conditions give a clear response to large-area, diffuse polarized light that they view in a lateral direction. When one entire side of an aquarium containing the *Daphnia* is covered from the bottom to the meniscus of the water surface with polarizing film that allows horizontally polarized light to pass, most of the animals swim towards the polarizing film. It is crucial for the e-vector to be approximately horizontal; when the film is rotated so that the e-vector is vertical, the *Daphnia* move away from the film.

The response of swimming towards a film with a horizontal e-vector is intensity-invariant. In one example, most of the animals, always more than 80 % (out of a total of 140 counted), were found in the half of the tank nearer the film-covered small

Table 1. *Distribution of animals in the two halves of the tank under different conditions*

Degree of polarization at the two small sides of the tank	22 % *versus* 0 %	37 % *versus* 0 %	49 % *versus* 0 %	68 % *versus* 0 %	37 % *versus* 22 %	49 % *versus* 37 %
Percentage of animals in the half with the greater polarization	55	61	64	70	56	56
N	210	660	510	510	535	600
P	–	<1 %	<1 %	<1 %	<1 %	<1 %

side, irrespective of whether the intensity of the polarized light was higher (intensity ratio 1.7:1) or lower (ratio 0.1:1) than that of the unpolarized light entering the other small side of the tank. The animals are not, therefore, exhibiting a positive or negative phototactic response but are indeed responding specifically to the polarization of the background light visible in a lateral direction. This swimming towards a polarizing film at the small side of the tank was not prevented by placing another polarizing film horizontally above the water surface, regardless of the e-vector direction of the light allowed to pass by that film and of whether that film covered the whole water surface or, for example, only the half of the tank opposite to the side with the polarizing film. In all cases, approximately the same large percentage of animals stayed in the half of the tank with the horizontally polarizing wall. An intense vertical beam of polarized light also failed to distract the animals from their positive polarotactic course towards the small side covered with polarizing film.

In the experiments described above, the degree of polarization produced by the film was 77 %. In another series of experiments, the responses of *Daphnia* to lower degrees of polarization were tested: 22, 37, 49 and 68 % polarization with a horizontal e-vector at one small side of the tank and, in each case, a polarization of 0 % at the other side. The brightness and the spectral composition of the light were the same at both small sides. The response proved to be stronger, the higher the degree of polarization; the proportion of the animals showing polarotaxis was lower for 37 % polarization than for the higher degrees, but there was still a clear, significant response (Table 1). A degree of polarization of 37 % corresponds to that recorded with the same instrument for light incident horizontally within a natural pond, when the instrument was aimed away from the shore, towards the open water (Fig. 2).

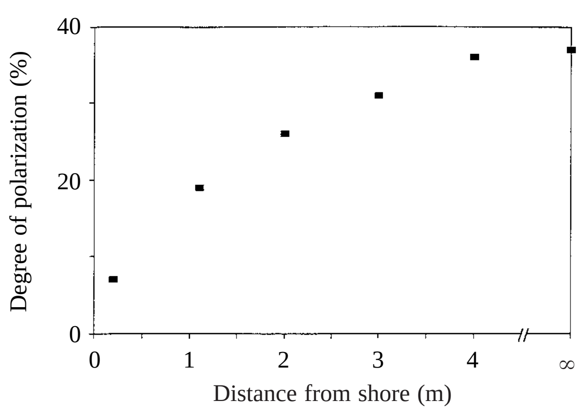

Fig. 2. Degrees of polarization (ordinate) measured under water in a pond with an instrument aimed towards the shore, parallel to the water surface, as a function of the distance from shore (abscissa). The e-vector was horizontal. The data point on the far right was obtained by aiming the instrument away from the shore.

When the *Daphnia* see polarized light with a horizontal e-vector at both small sides of the tank, they prefer the side with the higher degree of polarization (Table 1): 49 % polarization at one side is significantly more attractive than 37 % at the other side of the tank, and 37 % is significantly more attractive than 22 %.

Discussion

In the present paper, a response to inhomogeneously polarized, diffuse side light is described for *Daphnia pulex*: if the degree of polarization differs in different horizontal directions, the animals swim on a horizontal course towards the place with the highest degree of polarization if the e-vector of the side light is horizontal.

This finding provides an explanation of the response of *Daphnia* to intense linearly polarized light entering a container from above. As documented by Baylor and Smith (1953), Waterman (1960, 1981) and Jander and Waterman (1960), the animals swim within the vertical beam of light on a horizontal course in a direction perpendicular to the e-vector of the incident polarized light. This can be explained by the response of the *Daphnia* to stray light. A strong vertical beam of polarized light produces scattered light that is differently polarized when viewed in different horizontal directions. My own investigations of the stray light emerging from such a beam in unfiltered ion-exchanger water showed that, when measured from the side, perpendicular to the e-vector direction of the light incident from above, the degree of polarization of such a beam was 77 % and the e-vector direction was horizontal. When the meter was moved so that it was aiming in a direction parallel to the direction of oscillation of the vertical light beam, the e-vector of the scattered light was found to be vertical and the degree of polarization was 30 %. Because *Daphnia* swim towards polarized light from the side whenever the e-vector of the light is horizontal, regardless of its intensity, but avoid polarized light with a vertical e-vector, they will swim on a horizontal course at 90 ° to the direction of polarization of the vertical light beam. Furthermore, the *Daphnia* show a similar reaction if they do not see the incident light of the vertical beam but only the stray light. When a vertical beam of polarized light was projected into an aquarium at a position distant from a group of *Daphnia*, the animals swam into the beam if they faced one of its sides where the stray light had a horizontal e-vector. If they faced one of the sides where the stray light had a vertical e-vector, they did not swim into the beam of light.

In experiments by Jander and Waterman (1960), the *Daphnia* gave a clear response by swimming on a horizontal course perpendicular to the e-vector of the vertical polarized light beam only if the walls of the aquarium were black. When the side walls were light, the response became indistinct and other swimming directions were also observed. This, too, indicates the crucial significance of polarized scattered light: the response became indistinct because the degree of polarization of the scattered light was reduced by the unpolarized light reflected diffusely from the light side walls.

In the vertical beam of polarized light, the response of *Daphnia* was more pronounced in turbid than in filtered water (Waterman, 1960). Again, this finding implicates scattered light. When the water is made more turbid, for instance by adding yeast cells, the scattered light becomes more strongly polarized, especially for the direction from which the vertical direction of oscillation can be seen. That is, the difference among the degrees of polarization observed from the various lateral viewing directions is increased.

Unlike a vertical beam of polarized light, inhomogeneously polarized, diffuse light from the side is a stimulus that occurs in the natural habitat of *Daphnia*. In water near the shore, different degrees of polarization can be seen in different horizontal directions. For example, Fig. 2 shows measurements made in overcast weather conditions in a pond with a range of sight of only a few metres. In the direction towards the open water, the degree of polarization was 37 %. Light diffusely reflected under water from the shore is not polarized, so the degree of polarization measured in the shoreward direction next to the shore is zero. However, in the viewing direction towards the shore at greater distances, the degree of polarization gradually increases, reaching a maximum several metres away. In bodies of clear water, a higher maximal degree of polarization would be expected, and it would be reached at a point even farther away from the shore (Ivanoff, 1974). Regardless of the light intensity, *Daphnia* swim in the direction of higher polarization, and polarized light above the animals (i.e. polarized sky light) does not interfere with this response. Therefore, in their natural habitat, *Daphnia* will swim away from the shore in a great variety of lighting conditions. Once they have travelled a certain distance into the open water, they will also see suprathreshold polarization in the direction towards the shore. In the example of Fig. 2, a *Daphnia* 1.5 m away from the shore sees 22 % polarization in the shore direction and 37 % towards the open water. Even under these conditions, the *Daphnia* in the test tank preferred the side with the higher degree of polarization. In the open water of a pond, where the fields of view with polarized background light are more extensive than in the tank and there are no walls to interfere with locomotion, a swarm of *Daphnia* will travel farther away from the shore.

It has long been known that the shore zones of lakes are almost devoid of small pelagic crustaceans. These animals actively move away from the shore. The biological significance of this response is probably that it removes them from the vicinity of shoreline-inhabiting predators. Siebeck (1968) has shown that this directed movement can be elicited by raising the shore horizon visible to the animals through Snell's window, and he proposed such an elevation as a mechanism underlying 'shore flight'. However, this mechanism cannot account for the response in cases where the shore is not appreciably elevated. Furthermore, the amount of elevation visible to aquatic animals in Snell's window is reduced by the refraction of light as it enters the water: a shore height that subtends a visual angle of 10 ° above the water will appear to subtend only 1 ° in Snell's window. As shown here, at least the *Daphnia* (in this case *Daphnia pulex*) among the small crustaceans, because they are able to orient using the polarization gradients near shore, possess a mechanism that enables them to avoid the shore region regardless of the height of the shore.

The analyzers in the eyes of *Daphnia* are the rhabdomeres of the receptor cells, which are so arranged that the microvilli in different cells are orthogonal to one another (Waterman, 1981); hence, there are two orthogonally oriented analyzer directions. To determine the degree of polarization of partially linearly polarized light using such a system, the analyzer direction of one of the two analyzers must be brought into alignment with the direction of oscillation of the light. Because an animal floating in the water has no fixed orientation relative to the direction of light polarization, the analyzer system will usually have to be rotated to measure the degree of polarization. It may be that this is one function of the marked rotatory movements regularly carried out by the eyes of *Daphnia* (Frost, 1974).

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