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Shade-seeking behaviour under polarized light by the brittlestar *Ophioderma brevispinum* (Echinodermata: Ophiuroidea)

Sönke Johnsen* and William M. Kier

Department of Biology, University of North Carolina, Chapel Hill, NC 27599–3280, USA. *E-mail: sonkej@hboi.edu

The effect of polarized light on the shade-seeking behaviour of the ophiuroid *Ophioderma brevispinum* was investigated at the Keys Marine Laboratory, Long Key, Florida, USA. Animals were collected and placed in a partially shaded arena. When the arena was illuminated with unpolarized light, the number of animals settling under the shaded portion of the arena was not significantly different from random ($N=30$, $P>0.3$). When the arena was illuminated with linearly polarized light, the number of animals settling in the shaded portion of the arena was approximately double what would be expected in a random distribution ($N=30$, $P<0.001$). The results are further evidence that *O. brevispinum* is sensitive to polarized light and are consistent with the hypothesis that polarized light may be used by the animals as an indicator of harmful levels of solar ultraviolet radiation.

Previous work has shown that the ophiuroid *Ophioderma brevispinum* is sensitive to polarized light (Johnsen, 1994). Many of the ossicles of the animal's endoskeleton polarize light and it is hypothesized that these ossicles are used as polarizing filters for the detection of polarized light (Johnsen, 1994, 1997). However, while the previous experiments showed that the animals could discriminate between polarized and unpolarized light, the experimental design precluded any hypothesis of the ecological function of this ability. This study further investigates this unusual ability by examining the animals' behaviour under polarized and unpolarized light in a partially shaded arena. This approach was chosen because shade-seeking behaviour is common in ophiuroids (reviewed in Hendler et al., 1995). Shade-seeking behaviour is particularly interesting in *O. brevispinum* because this species is diurnal and moves between various shelters during the day (personal observations). Also, since *O. brevispinum* is easily damaged by environmental levels of solar ultraviolet radiation (Johnsen & Kier, 1998), shade-seeking behaviour under polarized sky light (which is more apparent in shallow, clear water where ultraviolet light levels are high (Jerlov, 1976; Waterman, 1981)) may be advantageous.

The apparatus used is similar to the one described by Johnsen (1994). An arena was constructed of a large, covered fiberglass tank (Figure 1). Holes were cut in the cover and the bottom of the tank. The bottom hole was covered with glass and Teflon™. The Teflon reduced the possibility of food, mucus or other substances becoming attached to the arena floor and influencing orientation behaviour. A plastic cylinder (0.15 m diameter, 0.095 m high) was used to contain the animal in the centre of the arena. A black, one-quarter pie-shaped light shield was attached to the top of this cylinder. Since the shield was above the arena floor, the shaded region on the arena floor was larger than the size of the light shield (about one-third of the total floor area within the cylinder). The arena was levelled and placed on foam-covered concrete blocks to reduce the possibility of orientation to slope or vibration.

Eight fluorescent bulbs (0.6 m long) were mounted in parallel approximately 0.06 m apart and 0.6 m above the Teflon floor of the arena. A parallel arrangement was used because it creates a highly uniform light field (Feynman et al., 1964). The bulbs were full spectrum (400–700 nm) (True-Lite, Interlectric Corp. Warren, Pennsylvania, USA). The visible irradiance at the arena floor was approximately $6\ W\ m^{-2}$ (approximately equal to sunrise or sunset). A light meter covered with a polarizing filter that was orientated first parallel and then perpendicular to the long axis of the bulbs measured the degree of polarization of the downwelling light at less than 0.7%. The light meter was also used to centre the lights in order to create a symmetrical light distribution.

Two thin films spanned the opening in the plywood: (1) a linear polarizer (model HN38s, Polaroid Corp. Norwood, Massachusetts, USA); and (2) a diffuser/depolarizer (two sheets of wax paper). The HN38s polarizer was chosen because of its high light transmission (38%), neutral density and high extinction coefficient (0.04–0.001). The wax paper was used to both diffuse and, in unpolarized light trials, depolarize the downwelling light. Using methods similar to those used for measuring the polarization of the lights, the depolarization efficiency of two sheets of wax paper was measured to be approximately 97%. For the polarized light trials, the polarizer followed the depolarizer in the light path. For the unpolarized light trials, the depolarizer followed the polarizer. This method ensures that the light in the polarized and unpolarized trials differs only in polarization and not in intensity or spectra (Via & Forward, 1975). However, the glossiness of the polarizer brightened the arena slightly ($\sim$10%) under polarized light. In general, the relationship between light intensity and visual response in animals has been found to be logarithmic (reviewed in Dowling, 1987). Therefore, small differences in general illumination are difficult to detect. So, while it is possible that a 10% difference in intensity influenced the results, it is unlikely.

Animals were collected from Old Dan Bank, Long Key, Florida, USA (24°50′N 80°50′W) at a depth of 0.4–1.0 m and maintained in water from the collection site. Within five hours of collection, an animal (average arm span $\sim$0.08 m) was transferred to the central cylinder of the arena. The arena was then sealed and the animal's silhouette was observed from below through the glass and Teflon floor. Typically, the animal moved about quickly initially, circling the central cylinder several times, and then settled against a portion of the wall. Once the animal's central disk remained motionless for 10 s, its position

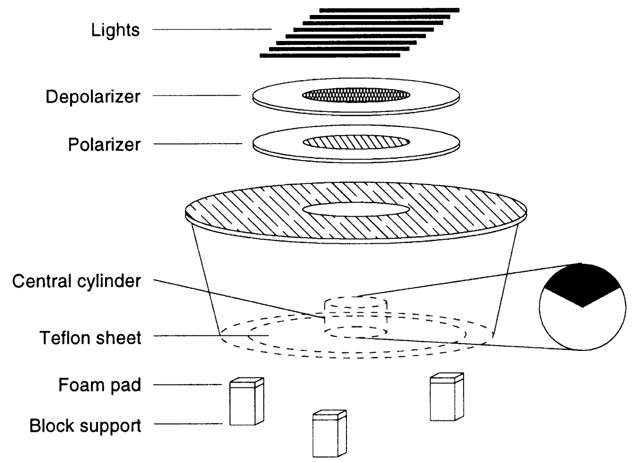

Figure 1. Vertically exploded diagram of the partially shaded arena. The circle to the right shows the portion of the central cylinder that is shaded.

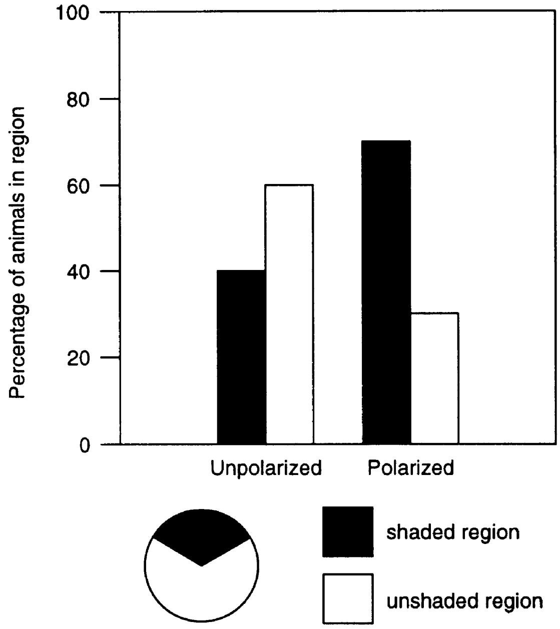

Figure 2. Graph showing the percentage of animals settling in the shaded and unshaded portions of the central cylinder under polarized and unpolarized light. The distribution under unpolarized light is not significantly different from random (N=30, $P>0.3$). The distribution under polarized light is different from random (N=30, $P<0.001$) and from the distribution under unpolarized light ($P<0.02$).

relative to the shaded region was recorded. A time of 10 s was chosen because once an animal remained motionless for 10 s, it generally remained motionless indefinitely. The tank was then vigorously scoured before testing the next animal. Although preliminary experiments showed no evidence for trail following, and though trail following is unknown in ophiuroids, cleansing between trials was done as an added precaution against this possibility. Sixty animals were tested, 30 under polarized light (*e*-vector at 60° to the centre of the shaded region) and 30 under unpolarized light. Trials under polarized light were alternated with trials under unpolarized light. It is important to note that while *O. brevispinum* usually seeks shade in bright sunlight, it does not generally seek shade under lesser illumination. The illumination used in this experiment, while quite bright by indoor standards, is considerably dimmer than full sunlight. The experiment was performed in August 1995.

The data are shown in Figure 2. In a random distribution, approximately 20 animals would be expected to settle in the unshaded region and ten would settle in the shaded region. Under unpolarized light, 18 of the 30 animals settled in the unshaded region of the arena and 12 settled in the shaded region. This distribution is not significantly different from random ($P>0.3$, χ^2-test). Under polarized light, nine animals settled in the unshaded region of the arena and 21 settled in the shaded region, a distribution different from random ($P<0.001$) and from the distribution under unpolarized light ($P<0.02$).

To demonstrate polarization sensitivity, it is necessary to eliminate the possibility that the observed behaviour is simply phototactic. Due to differential reflection, downwelling polarized light creates an axial light distribution in which the cylinder walls in the two quadrants parallel to the *e*-vector are darker than the walls in the two quadrants perpendicular to the *e*-vector (Waterman, 1981). Therefore, a negatively phototactic animal settling at a bearing parallel to the *e*-vector may be mistakenly attributed with polarization sensitivity. This potential artefact was eliminated in the following way. The overhead shield in the central cylinder was removed and a vertical black stripe subtending 15° of the cylinder wall was added. The experiment was repeated with 11 animals released under polarized light with the *e*-vector parallel to the bearing of the stripe, and ten animals released under unpolarized light. The two distributions were not significantly different (Watson U^2-test, $N_1=11$, $N_2=10$, $U^2=0.071$, $P>0.3$; Batschelet, 1981) and so were combined. Seven animals settled in the quadrant centered on the stripe, 14 settled in the other three quadrants. This distribution is not significantly different from random (N=21, $P>0.25$, χ^2-test), suggesting that the settling is not influenced by the relative brightnesses of the various portions of the walls of the central cylinder.

These results provide another line of evidence that *O. brevispinum* is sensitive to polarized light (the first is described in Johnsen (1994)). Since this sensitivity is the only sensitivity to polarized light known in echinoderms and in any eyeless animal, a second line of evidence is particularly important.

These results also suggest a possible ecological function for polarization sensitivity in these animals. As mentioned above, *O. brevispinum* is damaged by environmental levels of ultraviolet radiation. Since (as mentioned above), the polarization of skylight is more apparent in shallow clear water where ultraviolet light levels are high, it is possible that shade-seeking under polarized light in *O. brevispinum* is a behavioural defense against dangerous levels of ultraviolet light. This hypothesis is attractive for several reasons. First, ultraviolet light levels and degree of sky light polarization are similarly affected by clouds and atmospheric haze. Both are reduced by water particles in the atmosphere; ultraviolet light is reduced by absorption and scattering, and degree of polarization is reduced by multiple scattering. Although clouds and haze also attenuate visible light, their effect on degree of polarization and ultraviolet light levels are orders of magnitude greater (Jerlov, 1976). Second, ultraviolet light levels and degree of polarization of overhead light are similarly affected by water turbidity; ultraviolet light is absorbed and scattered and degree of polarization reduced by multiple scattering from suspended particulate matter. Again, though underwater particulate matter also attenuates visible light, its effect on degree of polarization and ultraviolet light are far greater (Jerlov, 1976). Finally, in certain conditions, degree of polarization of downwelling light is a more reliable indicator of ultraviolet light intensity than is visible light intensity. Visible light intensity is strongly affected by any object (cloud, sea grass, sponge, etc.) that comes between the animal and the sun. Polarized sky light, however, comes entirely from the portion of the sky not occupied by the sun. Therefore, the perceived degree of polarization is little affected by the position of the animal relative to shadows. Exposure to ultraviolet light is also little affected by the position of the animal relative to shadows. Because light scattering is inversely proportional to the fourth power of wavelength, a large percentage of solar ultraviolet light is scattered into the sky. From 50% (at noon) to 93% (at dawn and dusk) of the near ultraviolet radiation (397 nm) is contributed by the sky (Jerlov, 1976). The sky's contribution to the lower, more damaging wavelengths is even higher. Extrapolating Jerlov's data shows that, at noon, 70% of 300 nm light (the central wavelength of UVB) is contributed by the sky. Since Old Dan Bank has an extremely uneven terrain formed by the algae, grasses and sessile invertebrates and casts many complex shadows, an ultraviolet light indicator that is sensitive to the whole sky may be more useful than one mediated by visible radiation emanating directly from the sun.

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