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EXTRAOCULAR SENSITIVITY TO POLARIZED LIGHT IN AN ECHINODERM

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Summary

This study tests the hypotheses that the birefringent calcite and stereom structure of the brittlestar (Ophiuroidea, Echinodermata) endoskeleton polarizes light and that certain brittlestars respond to polarized light. The first hypothesis was tested in *Ophioderma brevispinum* by examining ossicles from freshly killed specimens under polarized light. This analysis revealed that the lateral arm shields, oral arm shields, arm spines and aboral disk ossicles are dichroic and thus polarize light. The second hypothesis was tested in two orientation experiments under polarized light. The results from the first orientation experiment showed (1) that, under polarized light, animals oriented significantly and unimodally, (2) that, under polarized light with the ***e***-vector perpendicular to that in 1, animals oriented significantly and unimodally to within 18 ° of the bearing of the animals in 1, and (3) that, under unpolarized light, animals did not orient significantly. The results from the second orientation experiment showed that, under polarized light, animals oriented significantly and unimodally to within 17 ° of an individual preference previously established under polarized light; but under unpolarized light, animals did not orient significantly to an individual preference established under polarized light. Thus, *O. brevispinum* orients under polarized, but not unpolarized, light. The unimodal orientation and lack of consistent alignment with the ***e***-vector suggest that polarized light is not used as a directional cue but instead as a signal to sustain oriented behavior. The dichroism of the ossicles of *O. brevispinum* and the animal's capacity (though eyeless) to discriminate between polarized and unpolarized light suggest that the mechanism of polarization sensitivity may rely on polarizing filters built from the animal's skeleton.

Introduction

Since Karl von Frisch discovered sensitivity to polarized light in honeybees (von Frisch, 1948, 1949), many other arthropods and certain molluscs (cephalopods) and vertebrates (teleosts, amphibians) have been shown to exhibit this sensitivity (reviewed in Menzel, 1974; Waterman, 1975, 1981; Wehner, 1983). In each of these animals, the sensory mechanism mediating this sensitivity is based on dichroism of photopigments in organized membrane structures. Although there is evidence that polarization sensitivity

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in some vertebrates is mediated by specialized midbrain structures, in almost all cases these membrane structures are photoreceptors grouped in retinae or ocelli (reviewed in Waterman, 1981).

This study provides the first evidence that an echinoderm (*Ophioderma brevispinum*: Ophiuroidea) can discriminate between polarized and unpolarized light. In addition, it demonstrates that certain ossicles of *O. brevispinum* polarize light. Because *O. brevispinum*, like all ophiuroids, has no eyes or ocelli (Reese, 1966), it is likely that its mechanism of polarization sensitivity is unrelated to previously described mechanisms. The capacity of the animal's ossicles to polarize light suggests that polarization sensitivity in *O. brevispinum* may be due to differential light transmission through its skeleton.

Materials and methods

Source and maintenance of animals

Two sets of approximately 45 specimens each were collected from a narrow sound near the mouth of the Ochlockonee River by Gulf Specimen Marine Laboratories Inc., Panacea, Florida, USA. Animals were kept in a 120 l holding tank on a 12 h:12 h light:dark cycle. Because preliminary experiments showed a decline in orientation behavior with increasing length of captivity, animals were tested within 1 week of delivery.

Examination of ossicles under linearly polarized light

Ossicles were dissected from freshly killed specimens and viewed under transmitted polarized light. Polarization of the ossicles was detected by rotating the plane of polarization and looking for periodic extinction of light transmission through the ossicles. Only superficial ossicles were examined. These were the oral arm shields, lateral arm shields, aboral arm shields, arm spines and aboral central disk ossicles. Approximately 15–20 ossicles of each type were examined.

Construction of the orientation arena

The arena for the first orientation experiment was constructed of two circular fiberglass tanks (1.2 m diameter, 0.45 m height, light-blue interior) (Fig. 1). A hole (85 cm in diameter) was cut in the bottom of the lower tank and covered with glass (6 mm thick). A plastic cylinder (10 cm diameter, 10 cm long, attached to a monofilament) was used to contain and release the animal in the center of the arena. The arena was levelled and placed on foam-covered blocks to reduce the possibility of orientation to slope and vibration.

Six fluorescent bulbs (20 W, 60 cm long) were mounted in parallel approximately 6 cm apart and 15 cm above the arena. Three of the bulbs emitted full-spectrum light (True-Lite, Interlectric Corp. Warren, PA) and three emitted blue light (Brite-Blue, Interlectric Corp., peak 433 nm, bandwidth 114 nm). It is not known which wavelengths are necessary for polarization sensitivity, but many echinoderms are most sensitive to blue-green light (reviewed in Yoshida, 1979; Yoshida *et al.* 1983). Therefore, a compromise of full-spectrum light with extra intensity in the blue and green regions was made. The degree of polarization of the downwelling light was less than 0.7 %, measured by a light meter (built to the specifications of Oriel Detector Head, model 71920, Oriel Corp.,

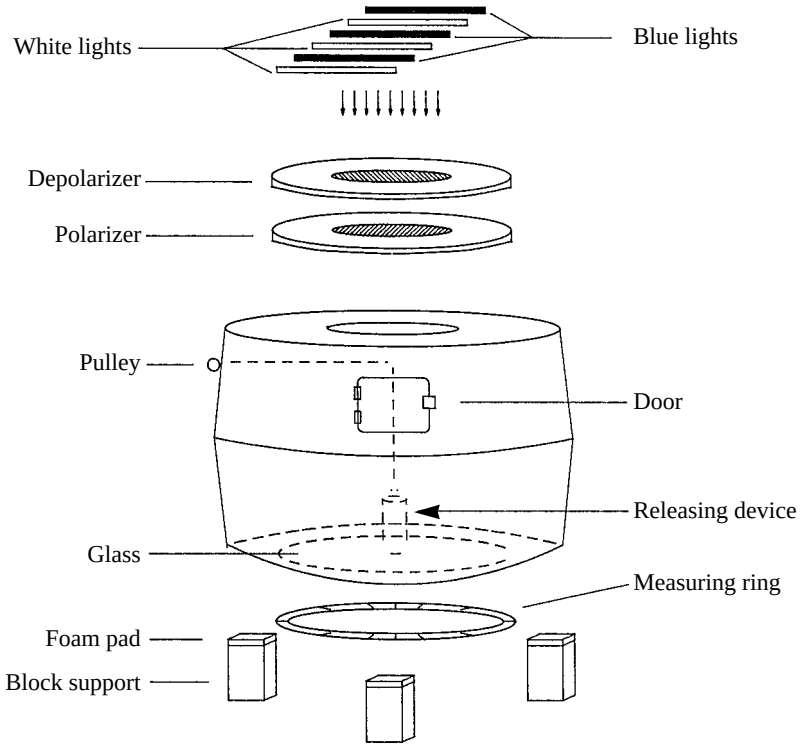

Fig. 1. Vertically 'exploded' schematic diagram of the testing arena used in the first orientation experiment (population preference experiment). Further details are given in the text.

Stratford, CT, USA) covered with a polarizing filter that was oriented first parallel and then perpendicular to the long axis of the bulbs.

Two thin films spanned the 66 cm diameter opening at the top of the arena: (1) a linear polarizer (model HN38s, Polaroid Corp. Norwood, MA), and (2) a diffuser/depolarizer (two sheets of waxed paper). The HN38s polarizer was chosen because of its high light transmission (38 %), neutral density and high extinction coefficient (0.04–0.001). The depolarization efficiency of the two sheets of waxed paper was 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 was designed to ensure that the light in the polarized and unpolarized trials differed only in polarization and not in intensity or spectral distribution (Via and Forward, 1975). Reflected light from the glossy polarizer, however, brightened the arena in polarized light trials by approximately 5 %.

Procedure for the population preference experiment

At the start of the day's trials, the arena was filled to approximately 8 cm with water from the holding tank. Bearings were then taken from each animal using the following procedure. First, an animal was transferred from the holding tank to the releasing device.

The arena was then sealed and the animal was released by lifting the releasing device to the top of the arena. As the animal moved towards the arena wall, its course was observed through the glass floor from underneath the arena. When its central disk first contacted a 66 cm diameter circle centered at the point of release, the animal was removed and its bearing was recorded to within ±2 °. The tank was then vigorously scoured before testing the next animal. Although preliminary experiments showed no evidence for trail following, and although trail following is unknown in ophiuroids, cleansing between trials was carried out as an added precaution against this possibility.

An animal was removed from the arena in the middle of a trial and rejected if it either did not move for more than 90 s or completely circled the arena. Approximately 8 % of the animals were rejected.

The animals were tested under three lighting conditions: (1) linearly polarized light, (2) linearly polarized light with the ***e***-vector perpendicular to the ***e***-vector in condition 1, and (3) unpolarized light.

Six sets of bearings, two for each of the three conditions, were taken each day, with seven animals in each set. The order of the six sets was chosen randomly. Each animal was tested twice, 1–4 days apart, under each condition. It is assumed that bearings taken from the same animal a day or more apart are independent. The experiments were performed during December 1992.

Changes in the testing apparatus before the normalized orientation experiment

The testing arena was modified in several ways before the second orientation experiment was performed. First, the lights were modified. The number of bulbs was increased from six to eight. Of these eight, four were the full-spectrum fluorescent bulbs described above. The other four were ultraviolet bulbs (350 Blacklight, Interlectric Corp., peak 350 nm, bandwidth 41 nm). The ultraviolet bulbs were added because the polarization-sensitive receptors of many animals are sensitive only to ultraviolet wavelengths (Waterman, 1981; Wehner, 1983).

Since most commercially available polarizers transmit very little ultraviolet light, the polarizer used in the first experiment was replaced with an experimental ultraviolet polarizer obtained from Polaroid Corporation. The ultraviolet (300–400 nm) transmissions of the polarizer and the wax paper depolarizer were approximately 25 % and 60 % respectively.

Because the above transmission values were still rather low, irradiance at the arena floor was increased by decreasing the distance between the lights and the glass floor. The inverted top tank of the arena was replaced with a sheet of plywood with a similarly sized opening for the lights. This approximately halved the distance between the lights and the glass floor, thereby quadrupling the light intensity. The underside of the plywood was painted flat black.

A more precisely symmetrical light distribution was obtained by measuring the irradiance at 37 points on the glass floor and shifting the lights to give a symmetrical distribution. The final position of the lights was less than 1 cm from the position arrived at by centering the lights for the population preference experiment.

Finally, the glass floor of the arena was covered with a sheet of white Teflon to reduce

the possibility of food, mucus or other substances becoming attached to the arena floor and influencing orientation behavior.

Procedure for the normalized orientation experiment

The first experimental method could not detect individuals that sustained oriented movement at a bearing that did not coincide with the preference of the population. Therefore, the testing procedure was modified to test an individual's orientation to its own recently determined preference. Certain asteroids show a short-term persistence of directional response, even against adverse stimuli (Cole, 1913). This second experiment was designed to exploit the possible occurrence of this trait under polarized light in *O. brevispinum*.

Each animal was tested in the following way. First, using the methods described for the population preference experiment, three consecutive bearings (to within ±1 °) were taken under polarized light. These bearings are referred to hereafter as sub-bearings. The animal was removed and the arena was cleaned between measurements of each sub-bearing. If the three sub-bearings subtended an arc greater than or equal to 180 °, the animal was rejected and tested at a later time. This was done to exclude animals that were not in a motivational state to respond consistently to any cue. Approximately 10 % of the animals tested in this way were rejected. If the sub-bearings subtended an angle of less than 180 °, a mean angle was calculated. Three more sub-bearings were immediately taken from the same animal and, if those three sub-bearings also subtended an angle of less than 180 °, a second mean angle (calculated from the second set of three sub-bearings only) was calculated. The first mean angle was subtracted from the second and the difference was recorded as the normalized bearing for that animal. If the first three sub-bearings are similar to the last three sub-bearings (indicating little change in orientation behavior), the normalized bearing approximates zero. If the first three sub-bearings are different from the last three sub-bearings (indicating a significant change in orientation behavior), the normalized bearing is far from zero.

The animals were tested under two conditions. In condition 1, the second three sub-bearings were taken under polarized light with the same ***e***-vector orientation as in the first three sub-bearings. In condition 2, the second three sub-bearings were taken under unpolarized light. Each animal was tested once under each condition. The normalized orientation experiment was performed during October 1993.

Results

Examination of ossicles under polarized light

All lateral arm shields, oral arm shields, arm spines and aboral central disk ossicles examined were found to polarize light. The aboral arm shields showed no polarization. Fig. 2 shows an arm spine under transmitted polarized light.

Population preference experiment

The results from the population preference experiment are shown in Fig. 3. The animals under polarized light were significantly oriented with a mean angle of -12 ± 37 ° (mean ±95 % confidence interval of the mean). The animals under turned polarized light

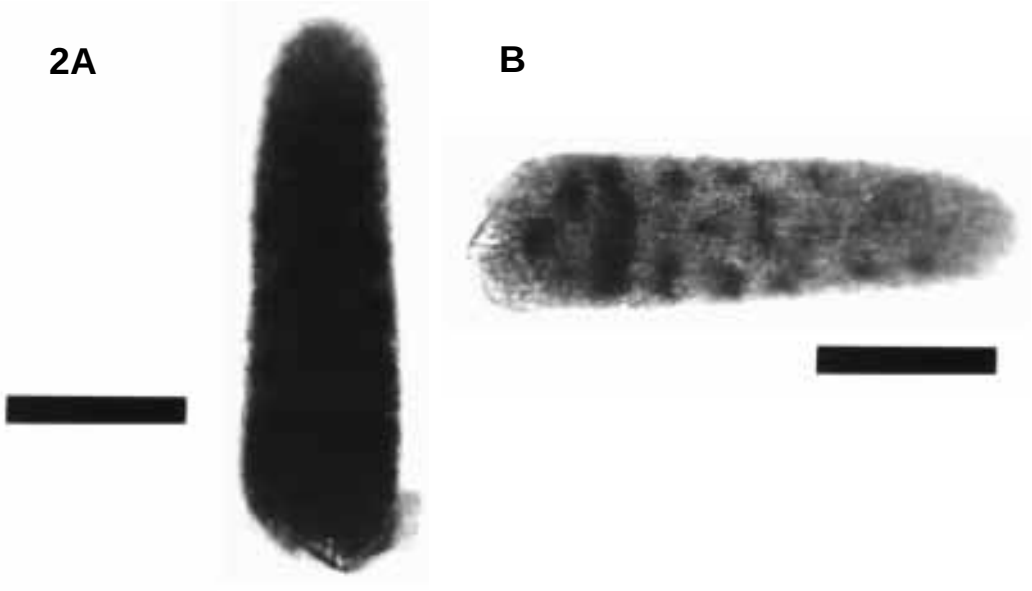

Fig. 2. Photographs of an arm spine of *Ophioderma brevispinum* under transmitted polarized light. (A) Long axis of spine is perpendicular to the ***e***-vector. (B) Long axis of spine is parallel to the ***e***-vector. Photographs were printed such that the contrast between them matched the contrast seen under the microscope by the author and five impartial observers. Scale bars, 0.25 mm.

(***e***-vector perpendicular to the ***e***-vector in the first condition) were also significantly oriented with a mean angle of $-30\pm37^{\circ}$. These two bearings are not significantly different. The animals under unpolarized light were not significantly oriented. The distribution of the animals' bearings under polarized light (combining both ***e***-vector orientations) is not significantly different from the distribution of the animals' bearings under unpolarized light, although the *P*-value is quite low (N_{polar}=165, $N_{unpolar}$=83, U^2=0.183, P<0.06, Watson U^2 two-sample test; Batschelet, 1981).

Normalized orientation experiment

The results from the normalized orientation experiment are shown in Fig. 4. The animals in which the second three sub-bearings were taken under polarized light were significantly oriented in relation to their individual preferences determined in the first three sub-bearings (mean normalized bearing $-17\pm43^{\circ}$). The animals in which the second three sub-bearings were taken under unpolarized light were not significantly oriented in relation to their previously demonstrated preferences. The distributions of normalized bearings in the first condition (six sub-bearings under polarized light) and the second condition (three sub-bearings under polarized light, three sub-bearings under unpolarized light) are significantly different (U^2=0.236, P<0.02, Watson U^2 two-sample test).

Discussion

The results from the population preference experiment show (1) that the population of *O. brevispinum* from Panacea exhibits a directional preference under polarized light, and (2) that this preference vanishes under unpolarized light. The results from the normalized

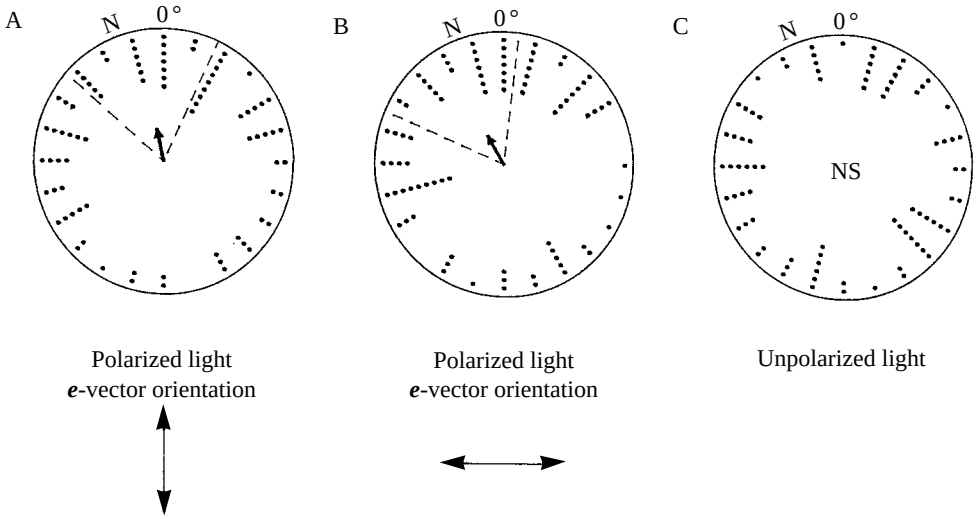

Fig. 3. Data from the population preference experiment. The arrow denotes the mean vector of each distribution. The dashed lines delimit the 95 % confidence interval for the mean angle. N marks the bearing of magnetic north. (A) Bearings taken from animals under polarized light. The animals are significantly oriented (mean angle −12°, r=0.24, N=82, Z=4.86, P<0.01, Rayleigh test). (B) Bearings taken from animals under polarized light with the ***e***-vector perpendicular to the ***e***-vector in A. The animals are significantly oriented (mean angle −30°, r=0.25, N=83, Z=5.18, P<0.01, Rayleigh test). (C) Bearings taken from animals under unpolarized light. The animals are not significantly oriented (mean angle 234°, r=0.01, N=83, Z=0.00298, P>0.9, Rayleigh test). Each dot is the mean bearing for one animal.

orientation experiment show (1) that, under polarized light, *O. brevispinum* orients to an individual preference previously established under polarized light, but (2) that, under unpolarized light, *O. brevispinum* does not orient to a previously established preference. The results from these two experiments show that *O. brevispinum* can discriminate between polarized and unpolarized light. This is the first demonstration of polarization sensitivity in an echinoderm.

The results from the examination of the ossicles show that certain ossicles in *O. brevispinum* can polarize light. This suggests that the mechanism for polarization sensitivity in *O. brevispinum* may be based not on photoreceptor organization but on polarizing filters built from the animal's skeleton.

Orientation is not a simple phototaxis

To demonstrate polarization sensitivity, it is necessary to eliminate the possibility that the orientation behavior is simply phototactic. Owing to differential reflection from the arena walls and differential scattering in the water, downwelling polarized light creates an axial light distribution in which the two quadrants parallel to the ***e***-vector are somewhat darker than the two quadrants perpendicular to the ***e***-vector (Jander and Waterman, 1960). Therefore, a negatively phototactic animal orienting parallel to the ***e***-vector may be mistakenly attributed with polarization sensitivity.

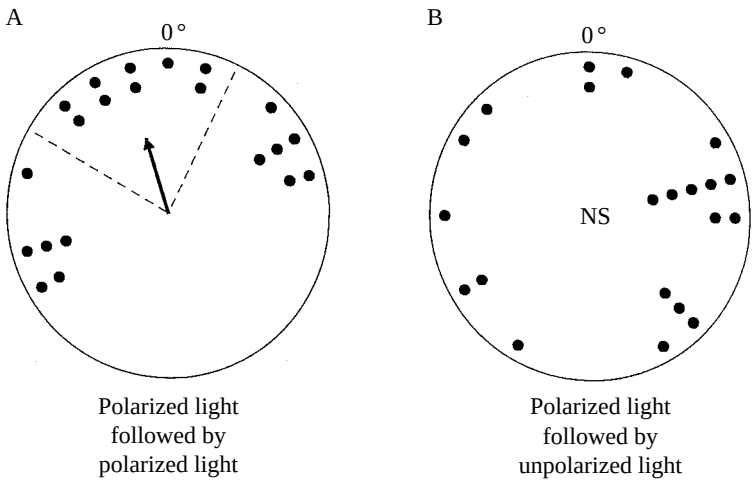

Fig. 4. Data from the normalized orientation experiment. (A) Normalized bearings from animals in which the second three sub-bearings were taken under polarized light. The animals are significantly oriented in relation to their previously established individual preferences (mean angle −17°, predicted mean angle 0°, r=0.46, N=21, P<0.005, V-test). (B) Normalized bearings from animals in which second three sub-bearings were taken under unpolarized light. The animals are not significantly oriented in relation to their previously established individual preferences (mean angle 80°, predicted mean angle 0°, r=0.27, N=21, P>0.5, V-test). Each dot is the normalized bearing for one animal.

This, however, is not the case in *O. brevispinum*. First, as mentioned above, the light distribution pattern is axial and the distributions in both experiments are unimodal. Second, a 90° rotation of the ***e***-vector (and the associated light distribution) in the population preference experiment did not significantly rotate the mean vector of the bearings.

Presence and nature of a second cue

The lack of a consistent relationship between the ***e***-vector and the mean vectors of the bearings in the population preference experiment suggests that, although polarized light was a necessary cue for the animals' orientation behavior, the direction chosen was based on a second cue. Two possible second cues are considered here: (1) the earth's magnetic field, and (2) sound/vibration.

A pilot experiment (an adaptation of the normalized orientation experiment) was performed to test whether *O. brevispinum* oriented to the earth's magnetic field (S. Johnsen, unpublished results). The animals were tested in a Rubens coil (Rubens, 1945) that neutralized and reversed the horizontal component of the earth's magnetic field within the arena. No significant effect on orientation due to earth-strength magnetic fields was found. The small sample sizes in each treatment (N=11–14), however, preclude a definitive answer to this question.

A radio at moderate volume and an aquarium air pump were present during both experiments. Both were approximately 2 m from the center of the arena, the first at a

bearing of 45°, the second at 240°. No orientation towards or away from either source was noted in either experiment.

It must be stressed that the unknown nature of the second cue in no way invalidates the conclusions drawn from the two orientation experiments.

Mechanism of polarization sensitivity may be based on the optical properties of the echinoderm endoskeleton

Raup (1965) argued that echinoderm ossicles may polarize light. Echinoderm ossicles are a sponge-like composite of high-magnesium calcite and an organic matrix of sclerocytes, phagocytes, collagen fibers and extracellular fluids (Pilkington, 1969). Calcite is highly birefringent, while the organic matrix (wherever the collagen fibres are randomly oriented on a large scale) has a single index of refraction. Although calcite alone does not polarize light, calcite in a stereom structure can. If the refractive index of the organic matrix approximates one of the two refractive indices of calcite, then the ossicle will polarize light. This occurs because light of the polarization that has nearly the same refractive index in calcite as it does in the organic matrix is transmitted through the ossicle, while light of the polarization perpendicular to the first has a refractive index in calcite that is quite different from that in the organic matrix and is attenuated as a result of internal reflection at the interfaces between the calcite and the matrix. The ossicle is opaque to the second polarization orientation for the same reason that snow, a sponge-like composite of two transparent materials (air and water) with different refractive indices, is opaque (see Raup, 1965, for more details).

This study shows that certain ossicles from *O. brevispinum* polarize light. Given this, many mechanisms for the detection of polarized light are possible. A simple example involves two neighboring polarizing ossicles oriented perpendicular to each other above a photosensitive substratum. Under unpolarized light, the substratum is evenly illuminated. Under polarized light, the substratum under one ossicle receives more light than the substratum under the other. Therefore, by comparing the light intensity under the two ossicles, it is possible to detect the presence of polarized light. Any mechanism of this sort requires, of course, that at least part of the photoreceptive structure of *O. brevispinum* lies below or inside some of the polarizing ossicles. An immunocytochemical study designed to detect and localize the photopigments in *O. brevispinum* is now in progress. If this study detects photopigments within or below polarizing ossicles, the optical properties of these ossicles (degree of polarization, spectral characteristics, etc.) will be quantified.

The function of polarization sensitivity in Ophioderma brevispinum

At present, the ecological function of polarization sensitivity in *O. brevispinum* is unknown. The data presented here suggest that *O. brevispinum* discriminates between polarized and unpolarized light, but not between polarized light with different ***e***-vector orientations. The observed lack of ***e***-vector discrimination may be due to a limitation of the polarization sensitivity of *O. brevispinum* or to experimental conditions. Jander and Waterman (1960) reported qualitative differences in the orientation responses of certain arthropods depending on the shade of the arena wall and the absolute illumination level.

If polarization sensitivity in *O. brevispinum* is limited to discrimination between polarized and unpolarized light, one possible function is crude depth gauging. The degree of skylight polarization observed under water decreases with water depth (Waterman, 1981; McFarland, 1991). Therefore, it is theoretically possible for an animal that is sensitive to the degree of polarization to gauge its own depth. The rate of decrease of polarization with depth, however, depends on the number, size and refractive index of suspended particles – factors that vary over a wide range in coastal waters. In addition, the original degree of polarization of skylight depends on the number, size and refractive index of suspended particles in the atmosphere – factors that vary from day to day. For these two reasons, an accurate polarization-dependent depth gauge is unlikely.

Nevertheless, a simple gauge is possible. It is possible that the turbidity of the water and the polarization sensitivity of *O. brevispinum* is such that the animals only perceive polarized light when in dangerously shallow water. Although *O. brevispinum* has been found in as little as 15 cm of water (G. Hendler, personal communication, 1993), in certain environments shallow water can be dangerous. Shallow water in an isolated pool can warm to a lethal temperature and, through evaporation, reach a lethally high salinity. In estuarine environments, shallow water can bring a floating lens of brackish water in contact with the sea bottom, exposing benthic animals to possibly lethal low salinities. Finally, further decrease in depth over banks and reefs can expose animals to desiccation and airborne predators. Since all three dangers develop gradually, they may not be noticed until escape for a slow-moving animal is impossible. Downwelling polarized light could be perceived as an early danger sign, prompting the animal to escape. The unimodal orientation and the persistence of direction under polarized light observed in the population preference and normalized orientation experiments could be evidence of such an escape response.

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