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# TURBIDITY AND THE POLARIZED LIGHT ORIENTATION OF THE CRUSTACEAN *MYSIDIUM**

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## INTRODUCTION

While behavioural responses to polarized light have been demonstrated in many arthropods, relatively little is known about the mechanism of polarized light analysis or its possible application to the navigation of aquatic animals (Waterman, 1958). We have found that the marine mysid, *Mysidium gracile* (Dana), exposed to a vertical beam of linearly polarized light shows preferential orientation by swimming predominantly in directions at right angles to the plane of polarization (Bainbridge & Waterman, 1957). While statistical analysis of the data indicated that this orientation to the *e* vector was significant overall, these crustaceans showed random horizontal orientation 40% of the time on an average. Orientation within 15° of the normal to the polarization plane occupied less than 40% of their time.

This lack of precision in the observed orientation pattern may depend on sensory inaccuracy, behavioural inattentiveness or some unsuspected factor. However, the possibility that the turbidity of the medium might also somehow be involved occurred to us because of the earlier work on *Daphnia*. The quantitative results of Eckert (1953) are not suggestive in this respect, but the observations of Baylor & Smith (1953) are. These authors usually conducted their experiments in water made turbid by the addition of yeast.

Under this condition *Daphnia* and a variety of other cladocerans were reported to swim with a high degree of reliability perpendicular to the *e* vector of a vertical beam of linearly polarized light. A repetition of our previous work on *Mysidium* seemed desirable to test whether or not such high turbidity might affect its polarized light behaviour. Consequently, a series of experiments was designed to compare the mysid's behaviour in carefully clarified sea water and in water deliberately made turbid with yeast.

## PROCEDURE

The general experimental method and organism, adult *M. gracile* have been described previously (Bainbridge & Waterman, 1957). However, accuracy and objectivity were increased in the present experiments by photographing the mysid's position every

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5 sec. instead of having an observer manually record the orientation of its antero-posterior axis. A Grass kymograph camera was placed beneath the experimental vessel so that the animal's horizontal orientation was photographed with light provided by the vertical polarized beam itself. Exposures of 0·01 sec. at *f*/8 were appropriate with the Kodak Plus-X film used. Twenty-five pictures were made in each run for a given polarization plane; ten choices from six planes 30° apart and within 180° were tested in random order to comprise a set of runs. Each of such complete groups consisted therefore of 250 orientation measurements and the total data here reported of nearly 4000. In analysing the results the orientation of the animal's longitudinal axis was measured on the photographs relative to the polarization plane; these measurements have been tabulated in nine groups comprising 10° intervals from this plane. 'Clear' and 'turbid' sets of runs were carried out in counterbalanced order. As in previous work a plane cover for the circular experimental vessel eliminated the water-air meniscus. Proper shielding prevented other optical artifacts from originating in reflexions from the vessel's walls.

Clear sea water was prepared at first by careful paper filtration; in later sets it was filtered through a porcelain candle. For comparison, turbidity was produced by adding specific numbers of drops of a standard yeast suspension of about 10 mg./ml. of dried yeast powder. While a suspension of such irregular particles, large relative to the wavelength of the light, is not ideal for causing optically simple and reproducible light scattering (Stacey, 1956), it does have the obvious advantages of being nontoxic, convenient, and of being similar to the suspension used for *Daphnia* by Baylor & Smith (1953).

## RESULTS

In all, five paired comparisons were made of clear water *v.* turbid water produced with three to four drops of yeast suspension. In addition two three-way tests were carried out with clear water compared with a moderately turbid medium containing four drops of the standard yeast suspension and with more turbid water containing eight drops of yeast. Each set was done with a different individual *Mysidium*. The resulting distribution of the mysid's horizontal orientation within the nine 10° intervals is shown in Table 1. These figures demonstrate that in clear water the animal's orientation is ordinarily more random than in turbid water. In the latter medium there is usually a distinct tendency to swim perpendicular to the *e* vector, as reported earlier. These generalities become more obvious when all these results are summed as shown in Fig. 1.

The total data show that in clear water the mysids spent on the average 10·9% of their time swimming within 10° of the polarization plane and 11·8% swimming within a similar 10° range at 90° to the *e* vector. The intermediate orientations also show a small, but persistent trend favouring perpendicular orientation. However, the statistical significance is low according to a $\chi^2$-test. By contrast in the turbid media an average of 8·3% of the time was spent within 10° of the plane and 15·1% within 10° of its perpendicular. Furthermore, the overall tendency to orientate at right angles to the *e* vector is here significant at better than the 1% level.

Table 1. *Summary of* Mysidium *orientation data*

| Date | Turbidity | Angular groups | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–10° | 11–20° | 21–30° | 31–40° | 41–50° | 51–60° | 61–70° | 71–80° | 81–90° |
| 31 July 1957 | Clear | 25 | 25 | 25 | 24 | 24 | 30 | 18 | 17 | 36 |
| | Turbid (low) | 24 | 16 | 25 | 22 | 24 | 34 | 35 | 30 | 39 |
| 2 Aug. 1957 | Clear | 31 | 35 | 25 | 26 | 23 | 28 | 29 | 31 | 22 |
| | Turbid (low) | 20 | 28 | 36 | 18 | 29 | 27 | 34 | 25 | 30 |
| 5 Aug. 1957 | Clear | 25 | 23 | 24 | 33 | 24 | 25 | 25 | 37 | 32 |
| | Turbid (low) | 19 | 14 | 17 | 25 | 20 | 26 | 31 | 39 | 57 |
| 8 Aug. 1957 | Clear | 34 | 21 | 34 | 22 | 27 | 29 | 29 | 26 | 22 |
| | Turbid (low) | 29 | 22 | 30 | 28 | 22 | 17 | 36 | 29 | 33 |
| 21 Aug. 1957 | Clear | 25 | 25 | 20 | 30 | 29 | 31 | 24 | 28 | 27 |
| | Turbid (low) | 25 | 27 | 25 | 19 | 19 | 20 | 36 | 36 | 39 |
| 22 Aug. 1957 | Clear | 26 | 17 | 21 | 26 | 26 | 27 | 38 | 26 | 35 |
| | Turbid (low) | 20 | 19 | 29 | 26 | 25 | 27 | 32 | 35 | 34 |
| | Turbid (high) | 15 | 22 | 22 | 20 | 24 | 34 | 32 | 44 | 33 |
| 30 Aug. 1957 | Clear | 16 | 23 | 24 | 26 | 30 | 21 | 24 | 33 | 22 |
| | Turbid (low) | 18 | 25 | 22 | 30 | 38 | 37 | 43 | 35 | 39 |
| | Turbid (high) | 21 | 21 | 21 | 26 | 23 | 41 | 50 | 43 | 45 |
| Totals | Clear (No.) | 182 | 169 | 173 | 187 | 183 | 191 | 187 | 198 | 196 |
| | (%) | *10·9* | *10·1* | *10·4* | *11·2* | *11·0* | *11·5* | *11·2* | *11·9* | *11·8* |
| | Turbid (No.) | 191 | 194 | 227 | 214 | 224 | 263 | 329 | 316 | 349 |
| | (%) | *8·3* | *8·4* | *9·8* | *9·3* | *9·7* | *11·4* | *14·3* | *13·7* | *15·1* |

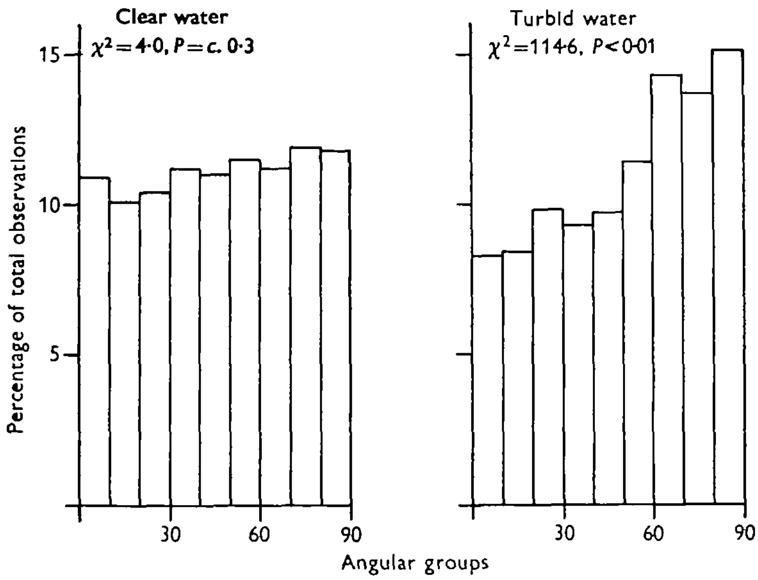


Fig. 1. Comparison of the swimming orientation of *Mysidium* to polarized light during nearly 4000 measurements in filtered water and in water made turbid with suspended yeast. These results show a significant overall tendency to orientation at right angles to the *e* vector in turbid water, but the similar trend in clear water is not statistically significant. This figure displays all the numerical data in Table 1.

If the two sets performed at three turbidity levels (data of 22 and 30 August) are examined separately, an increasing trend towards perpendicular orientation is evident in the measurements for clear, moderately turbid and more turbid media (Fig. 2). But, as in the overall data, the statistical significance is low in the case of clear water and high in the two cases with yeast added. The comparison between the three conditions is not significant when tested for the 10° intervals in a contingency table, probably because *n* is too small, but is significant if 30° intervals are considered.

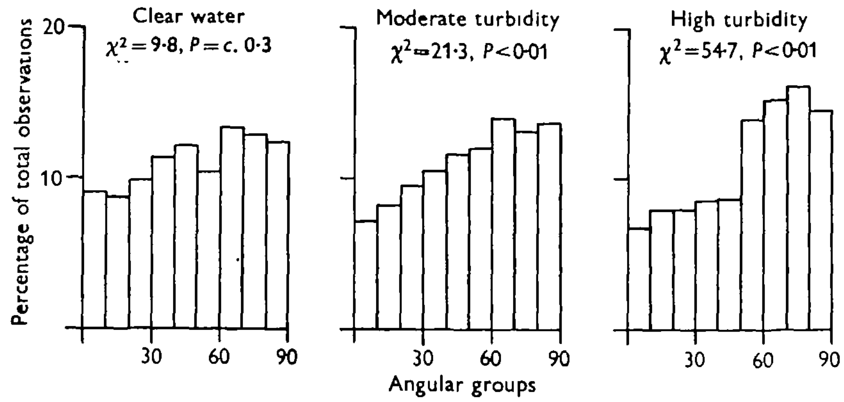


Fig. 2. Orientation of *Mysidium* to polarized light during nearly 1500 measurements in filtered water, in water of moderate turbidity (four drops of yeast suspension), and in water of high turbidity (eight drops of yeast). An increasing trend towards perpendicular orientation with increasing turbidity is apparent. The slight tendency towards such orientation in the clear water is not statistically significant. This figure displays the numerical data for 22 and 30 August in Table 1.

## CONCLUSIONS

These experiments prove that the turbidity of the medium has a marked effect on the responses of *Mysidium* in a vertical beam of polarized light. On a statistical basis no significant orientation occurs if the water has been carefully clarified. Consequently, under the conditions of these experiments some suspended material in the medium is necessary to obtain a strong response. In our previous work the inconsistent nature of the response was undoubtedly largely due to slight, but uncontrolled changes in the turbidity of the medium in which the mysid was swimming. However, it is also apparent that a response to polarized light cannot be invoked invariably by making the water turbid. Two of the seven animals tested showed a random orientation in both clear and turbid water (data of 2 and 8 August).

The positive correlation between turbidity and orientation raises some important points relative to the sensory mechanism of this crustacean's response to polarized light. In general, turbidity reduces the polarization by multiple scattering but, more important, the primary scattering of the incident polarized light varies strongly in intensity with the line of sight. More specifically, the intensity of the light originating by Rayleigh scattering is proportional to $\sin^2 \psi$, where $\psi$ is the angle between the *e* vector and the line of sight. In the experimental set-up this means that the horizontally scattered light, visible to the animal against a dark background, will (for primary scattering only) vary from zero when the animal is looking in a direction parallel to the place of polarization, to a maximum when it is looking in a direction at right angles to this. Consequently, whenever there is appreciable turbidity the mysid's horizontal visual field must consist of two dark and two more or less light sectors related to the polarization plane.

If these sectors are detectable by the test organism and if it is phototactic, preferential orientation could arise from intensity discrimination without any visual analysis of the polarized light *per se*. Orientation perpendicular to the plane would be expected of a positively phototactic form provided that this mechanism were operative. Similarly, one should obtain comparable orientation patterns in a vertical beam of unpolarized light if this were reflected horizontally by two diametrical light quadrants separated by intervening dark quadrants. Such a pattern of illumination can be readily obtained by inserting sectors of white paper in the normally completely dark screen surrounding the experimental vessel

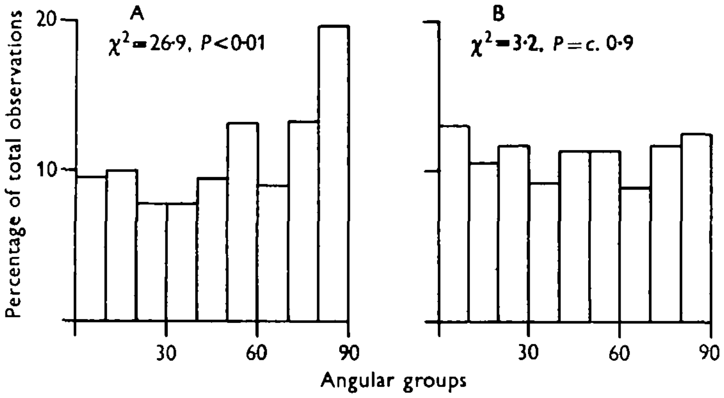


Fig. 3. Orientation of two different *Mysidum* during 250 measurements each in unpolarized light reflected horizontally by alternate light and dark quadrants. One run (B) shows random orientation, the other (A) shows significant preference for swimming in the line of the light quadrants, thus simulating the polarized light response in turbid water. These data are additional to those in Table 1.

This kind of control experiment was run for two sets of data. In one, completely random orientation was recorded; in the other, swimming was strongly directed towards the light sectors as would be expected of a positively phototactic form (Fig. 3). Thus, there is a definite possibility that the intensity of horizontally scattered light is the critical factor in the situation involving a vertical beam of polarized light. But more data are obviously required to make certain of this.

Although our results suggest that *Mysidium*'s response to polarized light under experimental conditions may rest on an external mechanism rather than a specific internal physiological one, the evidence is not entirely conclusive. In this connexion Baylor & Smith (1953) found that black and white sectors in unpolarized light produced orientation in *Daphnia* which simulated their responses to vertical beams of polarized light. Yet they postulated that an analytical mechanism involving reflexion and refraction within this animal's cyclopean compound eye was responsible in the case of polarized light, rather than the external scattering phenomenon which would seem most readily to account for our data.

If the observed orientation of *Mysidium* to polarized light depends on the perception of an intensity pattern rather than of the polarization as such, several important problems are raised. First, it must be determined whether the polarized light responses of other aquatic animals are critically dependent on turbidity. A new series of experiments is currently being done by one of us (T. H. W.) to test how much of the well-marked response of *Daphnia* to polarized light (Baylor & Smith, 1953) remains when it is swimming in very clear water with a minimum of scattering particles. Similar studies need to be made of the various other aquatic forms which respond to polarized light.

The case of terrestrial animals should also be reviewed. While atmospheric turbidity does affect the polarization of the sky (Sekera *et al.* 1955) its influence would probably be less likely to have phototactic significance than in the case of directional illumination underwater. On the other hand, the amount of surface reflexion of polarized light is strongly influenced by the angle between the polarization plane and the plane of incidence. Consequently, any reflected polarized light seen by an animal can include intensity gradients quite comparable to those resulting from the scattering of polarized light. Phototactic forms could use such intensity differences for orientation quite apart from the polarization as such.* Clearly the nearly inevitable occurrence of both scattering and reflexion artifacts in any experimental situation involving linearly polarized light calls for great care in assessing the relative significance of the various potential stimuli available to the organism. The determination of their relative importance is a considerable experimental challenge.

The ambiguities described above are critical for understanding the physiological or ethological mechanism of response to polarized light. But they do not restrict the possibility that animals utilize natural polarized light in the sky or sea. As long as the polarization patterns give rise to any adequate sensory cue detectable by the animal, appropriate orienting or navigational responses could ensue.

## SUMMARY

1. The influence of the turbidity of the medium on the previously reported directional orientation of the littoral mysid, *Mysidium gracile*, swimming in a vertical beam of linearly polarized light, has been studied.

2. In carefully clarified sea water the slight preference shown for orientation perpendicular to the polarization plane was not statistically significant.

* An interesting example of this sort has been described for the phototropic response of the mould, *Phycomyces* (Castle, 1934). Here the growing sporangiophore distinguishes different polarization planes of horizontal light beams by the various intensities of refracted light which penetrate its cylindrical stalk. These vary differentially as a result of changes in the ratio of surface reflexion to refraction.

3. In water made turbid with known amounts of suspended yeast a statistically significant preference for swimming perpendicular to the plane of polarization appeared.

4. This response to the pattern of polarized light illumination appears stronger in highly turbid water than it is in water of moderate turbidity.

5. The mechanism of the observed response seems largely dependent upon discrimination of intensity differences in the light scattered horizontally.

6. These results emphasize the need for careful consideration of the scattering and reflexion artifacts almost invariably present with linearly polarized light.

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