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Behavioral responses of a sand-beach amphipod to light and pressure

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Abstract: Light-oriented responses of the sand-beach amphipod *Synchelidium micropleon* (Barnard) in an optical arrangement that simulated the natural underwater angular light distribution were compared with previous measurements upon stimulation with a highly directional light source. Since positive and negative phototaxis occurred in both situations, phototaxis is not a laboratory artifact and will occur in nature. During rising tide amphipods move up the beach at the leading edge of the wave uprush zone. Maintenance of this position during the day results from an interaction of behavioral responses to light and hydrostatic pressure. The sequence of events is that a wave passing over the buried amphipods causes an increase in pressure, which evokes a negative geotaxis, and the animals exit from the sand. The increase in light intensity upon leaving the sand evokes an initial ascent into the wave due to positive phototaxis. This response reverses to negative within a few seconds, and the animals reenter the sand before they are swept back down the beach. The magnitudes of the increases in light intensity and pressure regulate the time until the reversal. This phototactic sequence can reoccur if upon entering the sand the animals are exposed to light levels below the lower threshold for phototaxis for at least 1 s. Reburrowing in the sand to a depth of about 7 mm will reset responsiveness. In this state, the animals are ready to ride the next wave up the beach and remain at the leading edge of the wave uprush.

Key words: Amphipod; Migration; Phototaxis; Geotaxis; Pressure

Introduction

Zooplankton frequently show directional movements toward or away from a directional light source (phototaxis). In most studies phototaxis is measured upon stimulation with a highly directional light source. Although this optical arrangement is ideal for inducing phototaxis, it does not simulate the angular light distribution (ALD) of underwater daylight or moonlight. Thus interpreting behavioral responses under unnatural lighting situations raises many problems (e.g. Schallek, 1942, 1943). In his review, Verheijen (1958) correctly concluded that normal photic orientation is only likely when experimental lighting conditions approximate a normal ALD.

Recent studies have compared phototactic responses of zooplankton in a highly directional light with those in an optical situation which simulates the underwater ALD (Stearns & Forward, 1984a,b; Forward, 1985, 1986). These studies have shown that while both positive and negative responses can occur in a directional light field, negative but not positive phototaxis occurs in conditions simulating the natural ALD. Since

responses varied among species, additional species need to be tested to evaluate the general applicability of these results.

One group of zooplankton which should be considered is the demersal species which live in or directly on the benthic substratum and which migrate short distances up into the water column. The small sand-beach amphipod *Synchelidium micropleon* lives in the wave uprush zone and is considered a demersal zooplankter due to its tidal vertical migration pattern. At low tide the animals occur in the sand as a well defined band, at and slightly above the area covered and uncovered by the uprush of the waves (Enright, 1961a). On rising tide, they enter a wave on the uprush and move back to the sand as the wave recedes. This behavioral pattern moves them up the beach as a distinct band near the upper edge of the wave uprush. Shortly before high tide and during the falling tide, they cease to reenter the sand as the waves recede and hence are distributed throughout the wave uprush zone and probably in the water column close to shore. Shortly before the time of low tide the population reaggregates into the distinct band found at low tide.

Upon stimulation with a highly directional light, *S. micropleon* shows pronounced phototactic responses. Because phototaxis varies rhythmically with the tide, it may be important for normal up- and down-beach migration (Forward, 1980). The present study was initially undertaken to measure phototaxis in a situation which simulates the underwater ALD. Since phototaxis clearly occurs in this situation, the study was expanded to investigate behavioral responses involved in migration up the beach during daytime rising tide. In addition to light, this species is also very responsive to changes in hydrostatic pressure (Enright, 1961b, 1962). The present study demonstrates that responses to light and pressure interact to produce the observed migration on rising tides.

MATERIALS AND METHODS

The oedicerotid amphipod *Synchelidium micropleon* (Barnard, 1977) was collected from the wave uprush area of the beach in front of the Scripps Institution of Oceanography, La Jolla, California. Only females are reported to occur in the area of collection and to attain the large size (cephalothorax length of 1–2 mm) of the test animals (Enright, 1961a). Animals were collected only from one beach area at variable heights above mean low water on rising tides. After sorting in the laboratory, animals were maintained in large white pans containing water from the beach and very little sand. In most cases, they were light adapted under the room fluorescent lamps at an illumination intensity of $0.34\ W \cdot m^{-2}$ as measured with a radiometer (YSI Model 65). The minimum light adaptation time was 0.5 h. Water temperature in the uprush area during the study (May to July) was about 18 °C. The room in which the animals were maintained was usually within 2 °C of this temperature while the environmental room in which experiments were conducted was held at 17–18 °C. Small variations in temperature do not appear to affect photoresponsiveness (Forward, 1980).

Animals were tested only on the day of collection. Test times were rigorously controlled according to tide times, which were based on U.S. Coast and Geodetic Survey predictions for the area of collection (Scripps Institution Pier). Tests during "rising tide" were done 1.5 to 3.5 h after low tide while those during "falling tide" were done during the same time interval after high tide. No consideration was made for variation in tidal height (spring or neap tides) on different days.

Photoresponsiveness was measured in two ways. First, phototaxis was tested using traditional techniques (Forward, 1980). Light stimulation occurred in a horizontal plane. The stimulus source was a slide projector with a 300 W incandescent bulb. The light was filtered by two hot mirrors (Baird Atomic) and a Corning No. 1–75 IR absorbing filter to remove heat and was then interference filtered to 540 nm (Ditric Optics, Inc.; half band pass = 8.7 nm). This wavelength is at the center of the spectral sensitivity maximum for *S. micropleon* (Forward, 1980). Light intensity was regulated by neutral density filters and stimulus duration controlled by an electromagnetic shutter (Uniblitz).

The test chamber consisted of a rectangular Lucite cuvette (15 × 3 × 3 cm), which was divided into five equal sections along the longitudinal axis. The sections were separated by thin partitions constructed so that all could be moved vertically in unison. To determine phototactic responsiveness, the cuvette was filled with sea water and 10 animals were placed in the center section. Irradiation during placement came from a dim microscope lamp filtered to 700 nm (half band pass = 12 nm). Immediately after introduction into the cuvette, the partitions separating the sections were raised. The animals were stimulated with a collimated horizontal light beam for 30 s and then the partitions were lowered. The distribution of animals among the five sections was then recorded. This completed the test, after which all animals were removed, new water was added to the chamber and another group of animals was tested. Thus each animal was tested only once and never used again. Control groups were tested following the same procedure, except the animals remained in darkness for the entire trial.

Only animals located in the section nearest the light source were considered to display positive phototaxis, while those in the furthest section were considered negative. The percent positive and negative response was calculated for each replicate. The combined percentage of positive and negative phototaxis rarely equaled 100%, because some animals remained in the center three sections.

In the second method of measuring photoresponsiveness, animals were tested in an apparatus designed to produce an ALD similar to that occurring underwater during the day (see Forward *et al.*, 1984, for a detailed description). Amphipods were placed in a Lucite chamber (4.7 × 4.7 × 19 cm) which was filled with sea water from the collection beach and positioned at the center of a much larger water bath which had its walls and bottom painted flat black. The bath contained deionized water. The test chamber extended from the bottom of the bath to several mm above the level of the water, which prevented deionized water from entering it. The walls of the bath were outside the critical angle (zenith ± 48.6°) as viewed from the bottom of the test chamber.

The stimulus source was a 750-W incandescent lamp, the light of which was filtered through IR absorbing filters to remove heat and a Corning No. 4–97 filter. The output from this filter matched the spectral sensitivity of the amphipod (approximately uniform sensitivity from 460 to 600 nm with possible peak at 540 nm; Forward, 1980). Broad-band pass filtering was used because high intensities were needed for the experiments. Light intensity was controlled by fixed neutral density filters and measured with a radiometer (EG and G, Model 550). Stimulus duration was again controlled by an electromagnetic shutter.

The light stimulus was aligned to enter the top of the bath. The beam was expanded to a size larger than the bath and then reflected down by a mirror. Before entering the bath it passed through a translucent Lucite plate which was overlaid with wax paper to promote uniform light intensity throughout the bath. The maximum intensity difference between the center and edge of the bath was 25%. The ALD in the bath was similar to that present in shallow water with the sun in the zenith (Forward *et al.*, 1984).

Thin unpainted slits existed in opposite walls of the bath. Amphipods were illuminated through one slit with far-red light (maximum transmission 775 nm) and observed via a closed circuit television system through the other. Amphipods are not responsive to light at this wavelength (Forward, 1980). All experiments were conducted in a light-tight environmental room. Light from the stimulus source was shielded to minimize the amount of stray light which reached the bath.

The equipment for measuring responses to an increase in hydrostatic pressure was patterned after that used by Enright (1962). The animals were enclosed in a Lucite chamber (inside dimensions 4.7 × 4.7 × 19 cm) connected to a water-filled 50 cm^3 syringe by Tygon tubing. The total height of the water column for the chamber and syringe was about 30 cm. Pressure was increased by adding calibrated weights to the piston of the syringe with the units of increase being millibars (mb). For all experiments the chamber was positioned in the middle of the water bath and behavioral responses viewed with the closed circuit television system.

The general experimental procedure was to stimulate a group of amphipods with different combinations of light and/or pressure changes and record the behavioral responses on video tape. In most cases animals were tested only once. For those few situations where animals were reused, at least an hour elapsed between trials and the amphipods received different stimuli for each trial. Direction of movement under different conditions was measured at 10° intervals (e.g. 90° ± 5°) with 0° being directly up. In experiments designed to measure phototaxis, a positive response was defined as movement toward the light within 15° (i.e. 0° ± 15°), while a negative response was movement directly away ± 15°. For geotaxis, a positive response was movement directly down (± 15°) and a negative response in the opposite direction.

For analysis of directional movement during each replicate, the movement of about 25 animals was measured and a percent response for phototaxis/geotaxis calculated. Means and standard error for responses under the different stimulus conditions were calculated from arc-sine square root transformed data and are plotted in the figures.

Statistical comparisons varied with each type of experiment. If separate samples were used to determine the experimental and corresponding control responses then Student's *t*-test was used to test for differences between mean responses. If paired observations were made before (control) and upon stimulation (experimental) of a group of animals then a *t*-test for paired comparisons was used to test for differences. If the control response upon one condition (e.g. darkness) was compared to responses to many stimulus conditions (e.g. a range of light intensities) then the Dunnett's *t*-test for multiple comparisons with a control was used to test for differences (Dunnett, 1964).

The initial set of experiments was designed to determine whether phototaxis occurs during rising and falling tides in a simulated natural light field. Since phototaxis did occur, studies then concentrated on the time of daytime rising tide to determine the behavioral responses to light and pressure that are involved in migration up the beach in the swash zone. It was first necessary to establish the phototactic patterns upon light adaptation to different intensity levels and the rates of light and dark adaptation. Second, the animals show a reversal in phototactic sign over time upon light stimulation. The cues that initiate and restore this response were determined. Finally, behavioral responses to an increase in pressure alone and the interaction between light and pressure change were measured. The detailed experimental procedures for each of these determinations are described in the Results section.

Additional measurements were made of light transmission through sand from the collection beach. A variable amount of sand was added to a Lucite chamber containing a relatively constant depth of water above the sand. The chamber was positioned over the radiometer sensor and light filtered with the Corning No. 4–97 was passed through the sand. Measurements of light levels with sand at different depths were used to calculate the attenuation coefficient.

RESULTS

PHOTOTAXIS IN A NATURAL LIGHT FIELD

To determine whether phototaxis occurred in the simulated natural light field, animals were placed in darkness for 30 s and then stimulated for 4 s. Directional movement was measured 2 s after the beginning of stimulation because measurements of phototaxis over time indicated the greatest response occurred at this time. Control movements were measured 2 s before stimulation.

Under control conditions in darkness, the animals swim up at a variety of angles and then sink down. At any instant about half of the observed animals are ascending or descending. Since sinking results in movement within 15° of the vertical, the control level of movement in the downward direction is around 50% (Fig. 1A,B). During both rising and falling tides positive phototaxis occurred upon stimulation with low intensity light (Fig. 1C, D), and negative to high (Fig. 1A,B), as indicated by the significant increases in upward and downward movements respectively. The same lower threshold

for positive phototaxis (1.25×10^{-5} W · m^{-2}) is observed at both tides. However, the animals are negatively phototactic over a wider range of intensities at the time of falling tide.

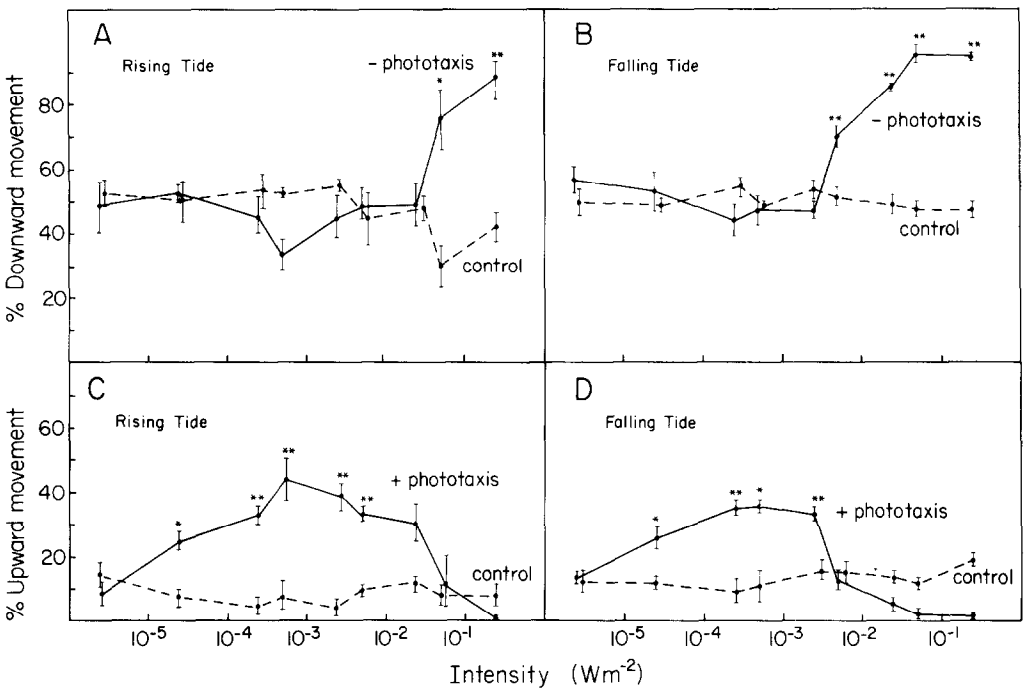

Fig. 1. Percent downward (A and B) and upward (C and D) movement (ordinate) in darkness (dashed line) and upon stimulation with a range (abscissa) of light intensities (solid line) at the time of rising and falling tide: the mean and standard error of six determinations for each condition are shown; asterisk indicates that the mean response level upon stimulation with light is significantly (t-test for paired comparisons) greater than the corresponding control level at the $P < 0.05$ level; double asterisk indicates significant difference at $P < 0.01$ level.

RISING TIDE – LIGHT AND DARK ADAPTATION

When light adapted during rising tide the amphipods are positively phototactic to low intensities and negative to high (Fig. 1A,C). However when dark adapted, they are negatively phototactic to all intensities (Forward, 1980). The light intensity necessary for inducing the phototactic pattern observed upon light adaptation was determined by light adapting animals to various intensities for at least 30 min and then determining phototactic responsiveness in the horizontal chamber using traditional techniques. It will be shown that 30 min is adequate time for light adaptation to occur (Fig. 3). The pattern of phototactic responsiveness to different intensities was used as an indicator of light and dark adaptation. Upon dark adaptation no stimulus intensity induces a positive phototactic response.

The intensity necessary for inducing the light-adapted phototactic pattern (positive to low intensities and negative to high) is below 2.0×10^{-3} W · m^{-2} and above 2.0×10^{-4} W · m^{-2} (Fig. 2). Thus total darkness is not needed for the dark-adapted

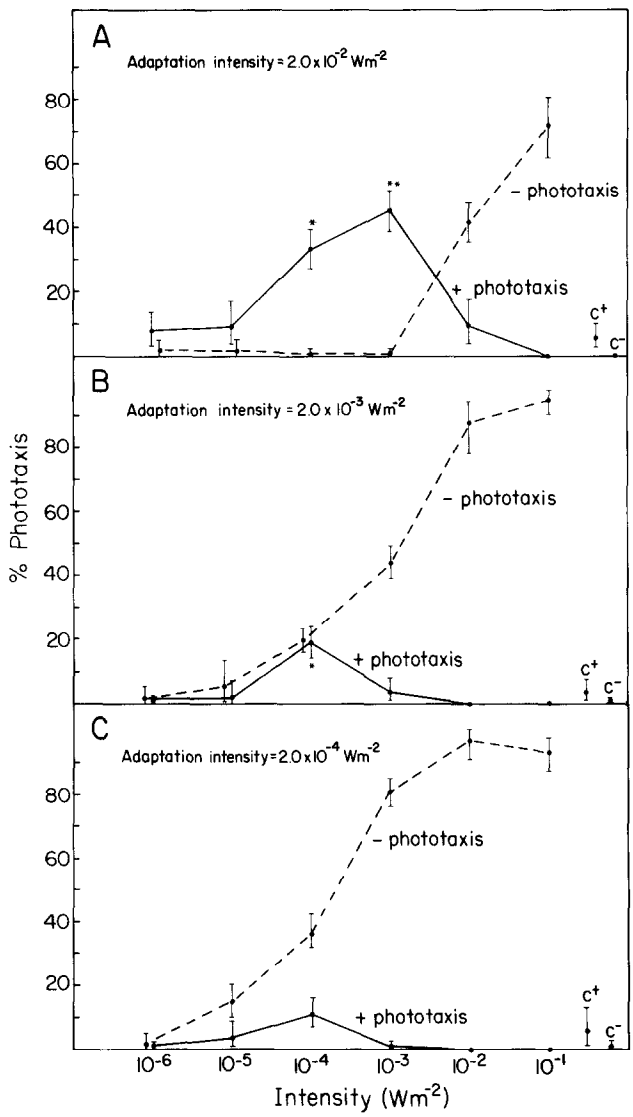

Fig. 2. The percentage of positive (solid line) and negative (dashed line) phototaxis (ordinate) after light adaptation to three intensity levels and upon stimulation with a range of intensities (abscissa): (A) 2.0×10^{-2} W · m^{-2}; (B) 2.0×10^{-3} W · m^{-2}; (C) 2.0×10^{-4} W · m^{-2}; the means and standard errors of four (A, C) or five (B) determinations for each condition are shown. Values indicated as C are the control movements in darkness in the direction of the stimulus light (+) and away (−); asterisk indicates that the mean positive phototaxis level is significantly (Dunnett's *t*-test) greater than the control level at the $P < 0.05$ level; double asterisk indicates significant differences at the $P < 0.01$ level.

pattern because the animals respond phototactically to an intensity of 2.0×10^{-4} W · m^{-2} (Figs. 1 and 2). Also it is important to note that after dark adaptation the intensity which will cause the light-adapted phototaxis pattern (2×10^{-3} W · m^{-2}) evokes a negative phototaxis (Fig. 2C).

The change in the phototactic pattern upon light or dark adaptation was used as a behavioral assay for establishing the rates of light and dark adaptation. The rate of light adaptation was determined by exposing animals that had been dark adapted for at least 1 h, to a light intensity (1.6×10^{-3} W · m^{-2}) near the lowest level which had produced the light-adapted phototactic pattern (Fig. 2). Since this adaptation intensity is near the lowest to evoke this pattern, the rate of light adaptation should be slower than rates upon exposure to higher light levels. At selected times after the beginning of exposure, samples of animals were removed and phototactic responsiveness to an intensity of 1.0×10^{-4} W · m^{-2} was tested in the horizontal chamber. This stimulus intensity was used because it was the only one to evoke a positive response upon adaptation to the above light level (Fig. 2B). Therefore the rate of light adaptation is indicated by the time between placement in light and the onset of a positive response which is significantly greater than that during dark adaptation. A significant decrease in negative phototaxis could similarly be used. The animals light adapted rapidly as there is a significant increase in positive phototaxis within 30 s exposure to the adaptation light (Fig. 3).

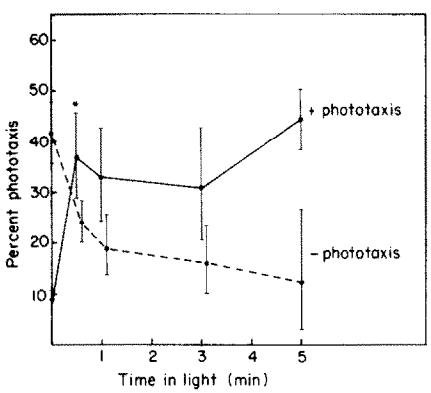

Fig. 3. The percent positive (solid line) and negative (dashed line) phototaxis (ordinate) after different times (abscissa) of light adaptation to an intensity of 1.6×10^{-3} W · m^{-2} and stimulation with 1.0×10^{-4} W · m^{-2}: the mean and standard error are plotted at each time; there were seven determinations in darkness while three were made at each time upon light adaptation; asterisk indicates the first time that the positive response is significantly (Dunnett's t-test) different ($P < 0.05$) from the initial level of dark-adapted animals.

The rate of dark adaptation was monitored using a similar procedure in that animals were light adapted at 1.6×10^{-3} W · m^{-2} for 30 min and then put into darkness. At specific times after placement in darkness groups of animals were removed and responsiveness to 10^{-4} W · m^{-2} tested. Since this adaptation intensity is near the

lowest to induce the light-adapted phototaxis pattern (Fig. 2), the rate of dark adaptation is expected to proceed at a faster rate upon placement in darkness as compared to rates after adaptation to higher intensities. The times for the positive response to be significantly reduced below the initial level in light and for the onset of

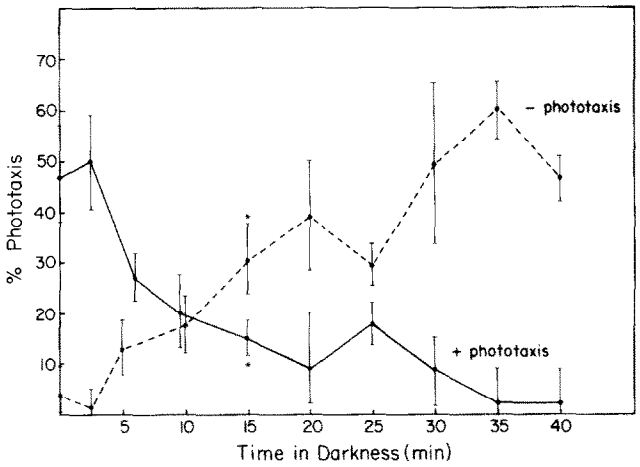

Fig. 4. The percent positive (solid line) and negative (dashed line) phototaxis (ordinate) of animals light adapted to $1.6 \times 10^{-3}\ W \cdot m^{-2}$ and then placed in darkness and stimulated over time (abscissa) with an intensity of $1.0 \times 10^{-4}\ W \cdot m^{-2}$; the mean and standard error for four determinations at each time are plotted; asterisk indicates the first time that the positive and negative responses are significantly (Dunnett's t-test) different ($P < 0.05$) from the initial levels of light-adapted animals.

a negative response significantly greater than the initial level were used to indicate the rate of dark adaptation. The rate was relatively slow as significant differences in both positive and negative phototaxis occurred after only 15 min in darkness (Fig. 4). Thus the animals light adapt very much faster than they dark adapt.

RISING TIDE – REVERSAL IN PHOTOTACTIC SIGN

Forward (1980) showed that if stimulated in a horizontal chamber using standard techniques during rising tide, the amphipods show an initial positive phototaxis, which reverses to negative after a few seconds. Will this reversal also occur when the animals are tested in the simulated natural light field? To learn the answer, light-adapted animals were placed in the test chamber in the water bath. Room lights were extinguished and stimulation continuously occurred at an intensity of $5.0 \times 10^{-3}\ W \cdot m^{-2}$, which is an optimum intensity for the reversal (Forward, 1980). Positive and negative phototaxis were measured over time. The control was to leave the animals in darkness and measure movement in the upward ($\pm 15°$) and downward ($\pm 15°$) directions over time. The expectation from Fig. 1 is that in darkness there will be a low level of upward movement but downward movement should reach about 50%.

There was a significant increase in upward movement during the first 4 s of light stimulation (Fig. 5A) which indicated positive phototaxis was occurring. Similarly, downward movement in light was significantly greater than in darkness at all times except 15 s, indicating the occurrence of negative phototaxis (Fig. 5B). The negative

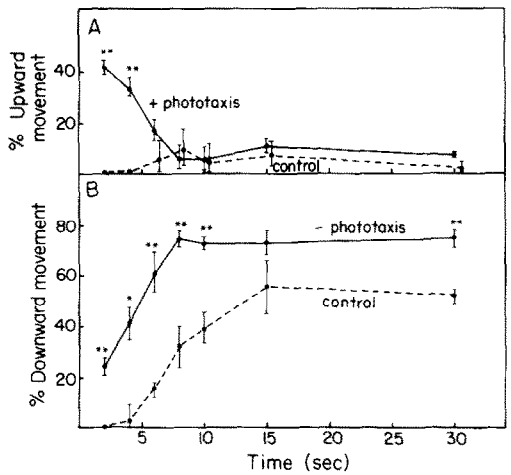

Fig. 5. The percent positive (solid line-A) and negative (solid line-B) phototaxis (ordinate) over time (abscissa) upon continuous stimulation with 5.0×10^{-3} W · m^{-2}: the dashed lines show the level of upward (A) and downward (B) movement over time in darkness; the mean and standard error of four determinations for each condition are shown; asterisks indicate the level of phototaxis is significantly (Student's t-test) ($P < 0.05$) greater than control movements in darkness; double asterisk indicates differences at the $P < 0.01$ level.

response generally leveled off at about 75% at 8 s. Thus the pattern of an initial pronounced positive phototaxis followed by an increase in negative response (phototactic reversal) is observed upon light stimulation in the simulated natural light field.

Since the initial positive response rapidly disappears, a further question is what cue evokes this initial attraction to light. Two possible cues are the onset of light and turbulence. The normal experimental sequence involves pipetting the animals into the test chamber, which subjects them to turbulence, and then suddenly stimulating them with light. To differentiate between these cues, animals were placed in the test chamber in the water bath and subjected to either of two procedures. First, to test the onset of light, animals were either stimulated initially at an intensity of 5.0×10^{-3} W · m^{-2} or kept in darkness for 70 s and then stimulated. In both cases positive phototaxis was measured 2 s after the beginning of stimulation. As controls, upward swimming was measured 2 s before initial stimulation (see Fig. 1), and 2 s before stimulation after 70 s in darkness. Second, amphipods were stimulated continuously in the water bath with light at the above intensity for 60 s and then subjected to 10 s of vigorous agitation by repeatedly filling and emptying a pipette in the test chamber (light remained on). Positive phototaxis was measured 2 s after the end of agitation.

The level of positive phototaxis upon initial stimulation (transformed percentages, mean = 35.1; SD = 3.5; $n = 6$) is not significantly different (Student's t-test) from the level upon stimulation after 70 s in darkness (transformed percentages, mean = 36.4, SD = 3.2; $n = 4$). Positive phototaxis is occurring in both cases because upward movement in darkness 2 s before initial stimulation (transformed percentages, mean = 17.8; SD = 4.8; $n = 5$) and after 68 s in darkness (transformed percentages, mean = 21.5; SD = 2.6; $n = 4$) are significantly less ($P < 0.001$, t-test for paired comparisons) than the values 2 s after stimulation. In contrast, positive phototaxis does not occur after the induced turbulence (transformed percentages, mean = 9.9; SD = 7.0; $n = 5$). Thus the onset of light, not turbulence, is the cue for positive phototaxis.

Since positive phototaxis disappears upon continuous light stimulation, a further consideration is whether the response can be reactivated by turning the light off and back on. This does occur, so experiments were conducted to determine the length of time light must be off before positive phototaxis returns. The procedure was to stimulate for 10 s with an intensity of $5.0 \times 10^{-3}\ W \cdot m^{-2}$. The reversal in phototactic sign occurred by 10 s and the animals were negatively phototactic (Fig. 5). The light was then extinguished for variable lengths of time as regulated by the control unit for the electromagnetic shutter. Positive phototaxis was measured 2 s after the light was restored. For controls, the level of upward movement was measured 2 s before the return of light when the light was off for 5 or 9 s. Since the control results were not significantly different they were combined. A second control determined whether the length of the initial 10 s stimulus affected the results. Amphipods were stimulated continuously for 60 s before lights-off. The lights were off for 5 or 15 s and the level of positive phototaxis was measured 2 s after the return of light.

There is a significant increase in positive phototaxis after only 1 s in darkness (Fig. 6). Longer lengths of darkness cause only small increases in the response level. The initial length of stimulation does not cause the results since response levels after 10 and 60 s of light stimulation are not significantly different (Student's t-test) (Fig. 6; points A and B).

Thus the restoration of phototaxis requires only a short duration decrease in light intensity. The next question is how much intensity reduction is needed for reactivation. The procedure for answering this question was to stimulate at an intensity of $5.0 \times 10^{-3}\ W \cdot m^{-2}$ for 10 s, by which time the reversal in phototactic sign had occurred. The light intensity was then decreased for 10 s by placing neutral density filters in the light path and positive phototaxis was measured 2 s after removal of the filters. The control was determined with different animals and consisted of measuring the level of upward swimming after the initial 10 s in light. A significant increase in upward movement above the control values indicates positive phototaxis was restored.

If the light intensity after reduction remains above the lower phototactic threshold ($1.25 \times 10^{-5}\ W \cdot m^{-2}$; Fig. 1C), the positive response is not restored (Fig. 7). Exposure to light levels below this threshold, however, induces positive responses which are

significantly greater than the control level but not different from the level after exposure to total darkness (Fig. 7). Thus the minimum conditions for restoration of the reversal

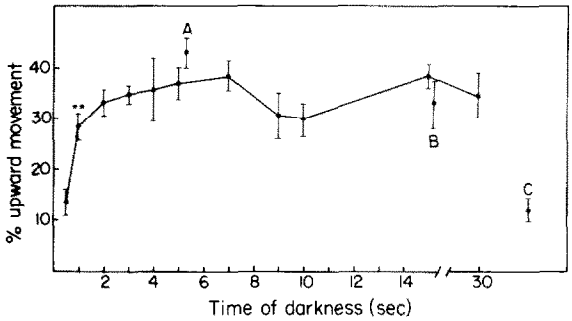

Fig. 6. Percent upward movement (ordinate) after interrupting light stimulation with variable periods of darkness (abscissa): the solid line is the percent response if darkness follows 10 s of stimulation; C indicates the combined percent upward movement 2 s before stimulation when the light is interrupted for 5 and 9 s; double asterisk indicates the shortest interruption time after which the mean percent upward movement is significantly (Dunnett's t-test) ($P < 0.01$) greater than the control level (C); A and B are the percents of upward movement following continuous stimulation for 60 s and then darkness for 5 and 15 s, respectively; the means and standard errors are plotted based on a minimum of five determinations for each condition.

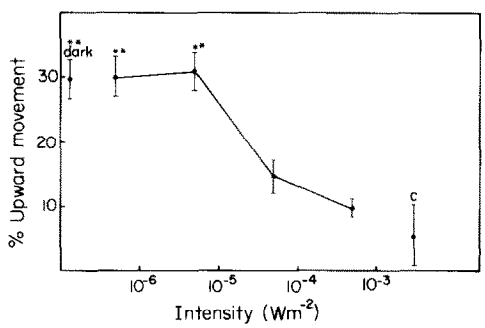

Fig. 7. Percent upward movement (ordinate) following light stimulation for 10 s and then a 10-s reduction in intensity to various levels (abscissa): the means and standard errors are plotted based on nine determinations for each experimental condition and four for the control; C is the control percent upward movement measured after 10 s in light; dark designates the response if the light is totally extinguished for 10 s; asterisks indicate that the mean percent response is significantly (Dunnett's t-test) ($P < 0.05$) greater than the control level (C).

in phototaxis are exposure to a light intensity below the lower phototactic threshold for at least 1 s.

RESPONSES IN SAND TO LIGHT AND PRESSURE

In their natural habitat, the amphipods are buried in sand at the upper edge of the wave uprush during rising tide. They ascend from the sand as a wave passes over them.

Considering this sequence of events, the question is whether light will attract the animals out of the sand. In this and all subsequent experiments the procedure was to place 200 animals in the test chamber, which was filled to 1 cm depth with sand and the remainder with water. Under these conditions during the time of rising tide, most of the animals remained in the sand if they did not receive appropriate stimulation. The procedure was to position the chamber in the bath having the natural ALD. Animals were kept in darkness 1 min and then stimulated with light of different intensities for 20 s. Responsiveness was quantified by counting the number of animals, which ascended out of the sand and swam up at least 2 cm in the first 15 sec after the beginning of light stimulation. The control was to measure the number ascending this distance in the 15 s in darkness before stimulation. At no intensity is there a significant increase in upward movement (Fig. 8). Thus light alone does not attract the amphipods out of the sand.

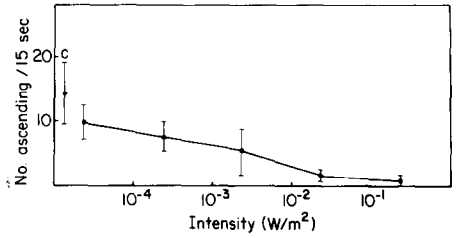

Fig. 8. The number of animals (ordinate) ascending from the sand in 15 s upon stimulation with a range of light intensities (abscissa): C is the control and shows the number ascending in darkness before stimulation; the means and standard errors are shown for four determinations; in no case is there a significant (Dunnett's *t*-test) increase in the number ascending.

Pressure increase is another possible stimulus that may induce an ascent from the sand. The pressure chamber was filled with water, 1 cm depth of sand and 200 animals. After 1 min in darkness, the animals were stimulated with an increase in pressure lasting 20 s. The number of animals ascending from the sand at least 2 cm in the 15-s interval after the stimulus began was used as a measure of responsiveness. The control was to measure the number ascending in the 15-s interval before stimulation. Fifteen seconds was used because most of the movement out of the sand had occurred by this time (Fig. 11).

The number of animals ascending increased as the pressure change increased (Fig. 9). The smallest change to evoke a significant increase in the number ascending was 7.5 mb. Thus an increase in pressure serves as a cue for the ascent from the sand, which represents a negative geotactic response.

The interaction between light and pressure was tested next. The procedure was to again place 200 animals in the pressure chamber with 1 cm of sand and water. The animals were left in darkness for 1 min and then stimulated with 50 mb pressure and light at a range of intensities. The number of animals ascending 2 cm above the sand

in the 15-s interval after the beginning of stimulation was again measured as an index of responsiveness. Two controls were also determined. First, the number of animals ascending in 15 s upon stimulation with only a 50 mb pressure increase was measured, and second, the number ascending in 15 s in darkness before the onset of pressure and light was determined.

The number ascending decreases as the light intensity increases (Fig. 10). At intensities of 2.5×10^{-3} W · m^{-2} and greater the response levels are significantly lower than that with pressure alone. A possible explanation is that at the higher intensities there is a reversal in phototactic sign from positive to negative before the end of 15 s, which reduces the total number of animals ascending in this time interval.

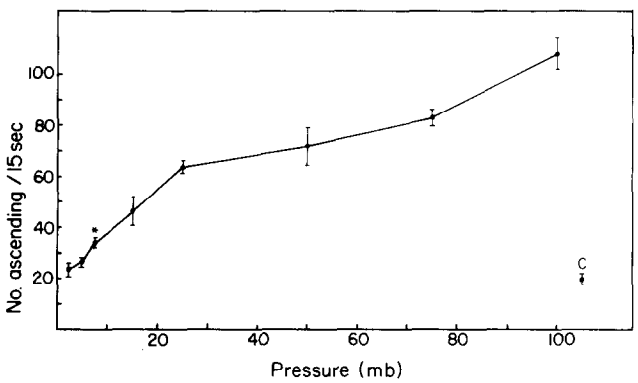

Fig. 9. The number of animals ascending (ordinate) in the 15-s interval after an increase in pressure (abscissa): C is the number descending in 15 s before the pressure change; the means and standard errors are plotted; there were four determinations for each experimental condition and 11 for the control; asterisk indicates the lowest pressure change at which the mean response is significantly (Dunnett's t-test) ($P < 0.05$) greater than the control level.

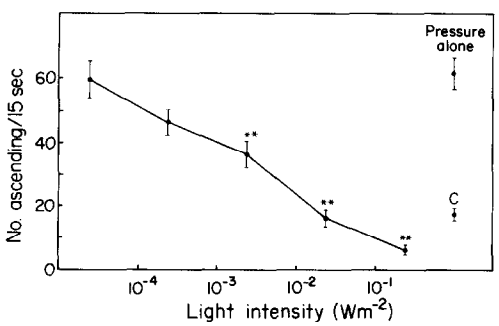

Fig. 10. The number of animals ascending (ordinate) in the 15-s interval after a simultaneous increase in pressure of 50 mb and stimulation with variable amount of light (abscissa): C is the number ascending in a 15-s interval in darkness while "pressure alone" shows the number ascending in this interval upon just a pressure increase; the means and standard errors are shown for 13 determinations under each condition; double asterisk indicates the intensities at which the response level is significantly lower (Dunnett's t-test) ($P < 0.01$) than that with pressure alone.

Visual observation of movements upon simultaneous stimulation with pressure and light indicated there was an initial ascent followed by a rapid descent. To assess the interaction of pressure and light intensity, responses were tested under three separate experimental conditions of (1) pressure alone at selected levels, (2) light intensity held constant at 5.0×10^{-3} W · m^{-2} and pressure varied, and (3) pressure held constant at 50 mb with variable light intensities. The procedure was to stimulate animals in the sand-filled pressure chamber with a particular pressure/light intensity combination for 45 s. To quantify the reversal from an ascent to a descent, the direction of movement was measured at 1-s intervals as timed from the beginning of stimulation. Directions were grouped into up and down categories. Movements in darkness before the beginning of stimulation were similarly measured as a control. A reversal from an ascent to a descent was considered to occur when there was a significant increase in the proportion (Z statistic for testing differences between two proportions; Walpole, 1974) of experimental animals descending and significant decrease in the proportion ascending as compared to control movements. In all but one case these significant changes occurred together. The time between the beginning of stimulation and the reversal was used to assess the interaction of light and pressure.

If the animals were exposed to a pressure increase alone, they did not ascend continuously and did eventually show a reversal. The time until the reversal varied with the amount of pressure increase (Fig. 11). The time increases at low pressures and seems to level off at pressures of 25 mb and larger at a value of about 20 s.

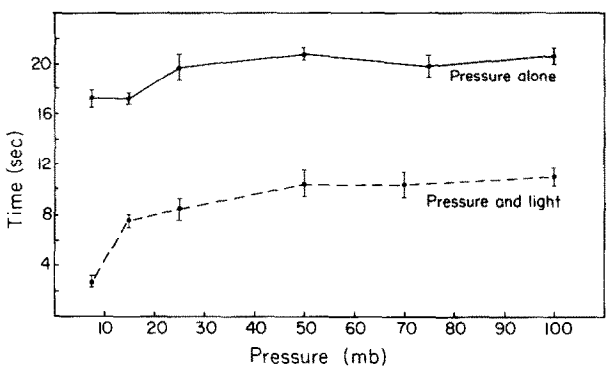

Fig. 11. The time (ordinate) until there was a reversal from an ascent to a descent after exposure to various pressure increases (abscissa) and exposure to the increase in pressure plus stimulation with a light intensity of 5.0×10^{-3} W · m^{-2}; the means and standard errors of an average of four determinations for each condition are shown.

If the animals were simultaneously exposed to a light intensity of 5.0×10^{-3} W · m^{-2} and pressure increases, the curve has the same form as with pressure alone but the reversals occurred at significantly (t-test for paired comparisons; $P < 0.01$) earlier times (Fig. 11). The times level off at about 10 s for pressures of 25 mb and greater.

When the pressure was held constant at 50 mb and the animals stimulated with different light intensities, the time until reversal was always significantly (Dunnett's t-test; $P < 0.01$) shorter than times with pressure alone (Fig. 12). The times decrease

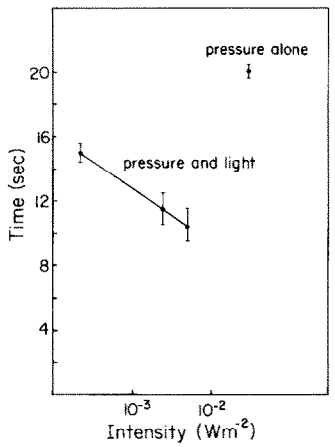

Fig. 12. The time (ordinate) until there was a reversal from an ascent to a descent after exposure to a pressure of 50 mb and different light levels (abscissa): "pressure alone" shows the response to a pressure increase of 50 mb; the means and standard errors of four determinations for each condition are shown.

as the intensity increases. Thus these results indicate that the duration of the ascent from the sand upon a pressure increase is shortened by the reversal in the phototactic sign from positive to negative.

LIGHT TRANSMISSION THROUGH SAND

Light transmission through sand from the beach inhabited by *Synchelidium micropleon* was measured to determine the approximate depth of the lower intensity threshold for phototaxis. The mean attenuation coefficient was 1.89 per mm (SD = 0.25; n = 10). The light intensity at any depth in the sand can be calculated using the equation $I_d = I_0 e^{-kd}$. I_0 is the surface intensity, I_d the intensity at depth d and k is the attenuation coefficient. The absolute lower threshold for phototaxis upon dark adaptation during rising tide was about 10^{-5} W · m^{-2} (Forward, 1980). Assuming a daylight intensity of 10^{2} W · m^{-2}, animals would experience a light intensity of 10^{-5} W · m^{-2} at about 7 mm depth in the sand.

DISCUSSION

The pattern of phototaxis of the demersal zooplankter *Synchelidium micropleon* during rising and falling tides in a light field which simulates the underwater ALD was similar

to that observed upon stimulation with a more traditional highly directional light source (Forward, 1980). In both cases there was a negative response to high intensities and positive to low, which indicates phototaxis can occur in the animal's natural environment. This result makes *S. micropleon* the exception to results from past studies. Stearns & Forward (1984a,b) found the holoplanktonic copepod *Acartia tonsa* was only positively phototactic to a highly directional light but lacks any phototaxis when tested in conditions which simulate the underwater ALD. Meroplanktonic larvae of the crab *Rhithropanopeus harrisii* showed both positive and negative phototaxis to a directional light (Forward & Costlow, 1974). In conditions simulating the underwater ALD, however, only the negative phototactic response occurred (Forward, 1985).

The common characteristic in those cases where phototaxis occurs in the natural light field is that the photoresponse functions in natural situations in which the light intensity changes rapidly. Slow rates of changes in light levels, such as occur at sunrise and sunset, do not induce phototaxis in light-adapted *R. harrisii* (Forward, 1985) or *A. tonsa* (Stearns & Forward, 1984b). For *R. harrisii* larvae, however, phototaxis does occur during diel vertical migration (DVM) and a predator avoidance shadow response. During DVM the larvae descend at sunrise with the isolume having a light intensity near the lower threshold for phototaxis. The underlying behavioral responses are a negative geotaxis in darkness which causes an ascent and a negative phototaxis upon exposure to light intensity near the lower visual threshold. In this situation a rapid increase in light intensity occurs as the larvae ascend from an area of darkness to a depth where perceptible light is just present (Forward, 1985).

In the shadow response of *R. harrisii*, negative phototaxis is initiated upon a sudden decrease in light intensity as would occur when an object passes over a larva (Forward, 1986). Similarly for *S. micropleon*, phototaxis functions during the rapid increase in light levels which occurs as the animal exits from the sand and migrates up into a passing wave. Thus for future studies, phototaxis must be tested in a lighting situation having the underwater ALD before it can be accepted as an ecologically meaningful behavior. If phototaxis does occur, then it will be interesting to determine whether the responses function only during rapid changes in light levels.

As the tide rises, *S. micropleon* migrates up the beach as a distinct band near the upper edge of the wave uprush. Behavioral responses to light and hydrostatic pressure interact to produce this migration. Let us first consider light responses. If stimulated suddenly with light, there is an initial positive phototaxis which reverses quickly to negative. The reversal is completed within 8 s and the animals remain negatively phototactic upon further light stimulation. A reversal in phototactic sign, however, will reoccur if they are first exposed to light below their lower phototactic threshold for as little as 1 s. Thus darkness serves to "reset" the response.

Considering the light transmission characteristics of beach sand, the only way the animals could be exposed to darkness during the day is to burrow to a depth of at least 7 mm. There are no measurements of the vertical distribution of the animals in sand. Nevertheless given cephalothorax lengths of 1–2 mm for adult females (Enright, 1961a),

which were used in the present experiments, and qualitative observations of their burrowing behavior, I consider it likely that they reach this depth.

If the animals remain in the sand at this depth for at least 15 min, they will develop the dark-adapted phototactic pattern because the light intensity is below 2.0×10^{-3} W · m^{-2}. In this state the phototactic pattern changes, and they become negatively phototactic to all intensities. The only other reported case where the phototactic pattern changes dramatically upon light and dark adaptation is for larvae of the crab *Rhithropanopeus harrisii* (Forward, 1974; Forward & Costlow, 1974). Coincidentally for this species the pattern upon dark adaptations occurred upon adaptation to similar light intensities of 1.6×10^{-3} W · m^{-2} and lower (Forward & Cronin, 1977). Both species respond phototactically to light intensity which induces the dark-adapted phototactic pattern, and thus exposure to total darkness is not required. It is not apparent why the phototactic pattern should change at this intensity level.

For *S. micropleon* the change in phototactic pattern may be functionally significant for conditions when the animals are stranded for any length of time above the wave uprush area. A negative phototaxis to low light levels would move them deeper into the sand. By this movement the chance of desiccation and overheating is reduced.

Once in the sand, the change in negative phototaxis upon dark adaptation would tend to keep the animals in the sand. In addition, experiments indicate that even if they are not dark adapted, light will not attract them out of the sand. Thus another cue must cause the animals to move out of the sand. A sufficient cue is an increase in hydrostatic pressure, which occurs as a wave passes over the animals. The ascent results from a negative geotaxis which is a common response among zooplankton upon an increase in hydrostatic pressure (Rice, 1964; Knight-Jones & Morgan, 1966; Sulkin, 1984). The lower pressure change threshold for *S. micropleon* as measured in the present study was 7.5 mb, which is in the range of thresholds (5 to 12 mb) reported for this species by Enright (1961a, 1962). This threshold is low when compared with those for other invertebrates (Morgan, 1984). On a sand beach the animals experience sudden changes in water depth from several cm to about 1 m as the waves overwash them. Their high sensitivity (7.5 cm water) to pressure change probably reflects the relatively low pressure levels they will experience in this environment.

Even though the pressure-induced responses will cause the amphipods to exit from the sand, the initial exit is probably brief because the light-induced negative phototaxis directs the animals into the substratum. However, light adaptation takes less than 30 s, after which the animals show the positive/negative phototactic reversal. The initial positive phototaxis upon exposure to light will then assist the animals in ascending into the waves. The length of time for this ascent is controlled by the magnitude of the light intensity and pressure increase. Large increases in pressure and low light levels cause longer ascent times. This relationship provides a method for the regulation of the position of the animals in the uprush zone.

Ideally the animals migrate to the upper edge of the wave uprush. If, for example, an animal is located in this zone, the pressure increase by a wave will be small, since only

the upper edge of a wave reaches this area. The light intensity upon exiting from the sand will be high because the overlying water is shallow. Hence the time until the reversal in phototactic sign is short. In this way the animal will not ride the wave far and avoids being swept back down the beach as the wave recedes.

In contrast, if the animal is low on the beach, deeper waves pass over it inducing larger increases in pressure. Since the water depth is greater, the light intensity upon exiting from the sand is low. The time until the phototactic reversal will be longer and the animals will ride the waves further up the beach toward the edge of the wave uprush. Thus responses to pressure and light regulate migration up the beach.

A final consideration is the way in which light functions during the migration of *S. micropleon*. Light may act as a controlling, an initiating or an orienting cue for vertical movements (Bainbridge, 1961). Control occurs through light adaptation. Once dark-adapted amphipods migrate into the sand while upon light adaptation they show the positive/negative phototactic reversal. The phototactic reversal is initiated by the onset of light, which occurs as the amphipods exit from the sand upon a hydrostatic pressure increase. Finally, light serves as a directional cue for vertical orientation through positive and negative phototaxis.

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