

The University of North Carolina  
at Greensboro

JACKSON LIBRARY



*CR*

*no. 1383*

UNIVERSITY ARCHIVES

BARDOLPH, MARK. Polarized Light Sensitivity in the Zoea of the Rock Crab (Panopeus herbstii). (1976)  
Directed by: Dr. Robert Hans Stavn. Pp. 28

This study was undertaken to investigate polarotaxis (orientation to the e-vector of polarized light) in the zoeae of the xanthid crab, Panopeus herbstii. Several lines of behavioral evidence indicate that arthropods are able to perceive polarized light as a stimulus distinct from light pattern (image) perception. The optical environment in this study was made as natural as possible to allow more realistic extrapolations from orientational behavior to the ecological relations of this group.

Panopeus herbstii larvae were cultured at 25°C in 25/00 filtered sea water, and on a 12:12 hour light:dark cycle. The first and second stage zoeae were tested for their orientation in different light intensity distributions both with unpolarized light or polarized beams from the sides (the e-vector horizontal or 20° off the horizontal). This was accomplished by placing the animals in an experimental apparatus which consisted of a glass cuvette suspended within a large supporting structure on which lamps, filters, camera, and electronic flash heads were mounted. Thus, the animals could be photographed while being subjected to a variety of light regimes. An enlarger was used to project the images from the photographic negatives onto a piece of paper. The orientations of the animals' rigid dorsal spine could then be drawn, measured, and tabulated. The Mann-Whitney (Wilcoxon) U test was used for location comparisons; dispersions about the median orientation were compared using the Siegel-Tukey test.

All experimental conditions of light intensity patterns and polarization planes resulted in a primarily vertical orientation of the dorsal

spine in both the first and second zoeal stages. Thus, gravity is probably the strongest stimulus for orientation. Although first-stage zoeae had primarily a vertical orientation of the dorsal spine under the conditions of no polaroid vs. horizontal e-vector, there was a significantly smaller degree of angular deviation of the vertical orientation of the dorsal spine in the horizontal e-vector population. This suggests the possibility of more precise orientation for swimming when polarized light is present. The fact that first-stage zoeae do shift the orientation of the dorsal spine off the vertical when presented with a  $20^{\circ}$  tilt of the e-vector also indicates polarized light is an orienting stimulus. Second-stage zoeae deviated somewhat from the above relations but still seemed to be sensitive to polarized light.

Thus, in combination with gravity and the light intensity pattern, polarized light may function as an orienting stimulus for the larva enhancing the accuracy of orientation for maintenance of position in the habitat.

POLARIZED LIGHT SENSITIVITY IN THE ZOEAE

OF THE ROCK CRAB (PANOPEUS

HERBSTII)

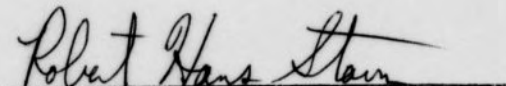
by

Mark Bardolph

A Thesis Submitted to  
the Faculty of the Graduate School at  
The University of North Carolina at Greensboro  
in Partial Fulfillment  
of the Requirements for the Degree  
Master of Arts

Greensboro  
1976

Approved by

  
Thesis Adviser

APPROVAL PAGE

This thesis has been approved by the following committee of the Faculty of the Graduate School at the University of North Carolina at Greensboro.

Thesis Adviser

Robert Hans Starr

Committee Members

Paul E. Lutz

Cheryl A. Leger

January 30, 1976  
Date of Acceptance by Committee

#### ACKNOWLEDGMENTS

The author would like to express his deepest thanks and appreciation to Dr. Robert H. Stavn for his advice during the progress of this study as well as for the critical reading of this thesis, and also Dr. Paul E. Lutz and Dr. Cheryl A. Logan for a critical review of the thesis. The use of facilities of the Department of Biology, UNC at Greensboro, and the laboratory of Dr. Richard B. Forward, Duke University Marine Laboratory, Beaufort, N.C. is also gratefully acknowledged.

TABLE OF CONTENTS

	Page
APPROVAL PAGE . . . . .	ii
ACKNOWLEDGMENTS . . . . .	iii
LIST OF TABLES . . . . .	v
LIST OF FIGURES . . . . .	vi
INTRODUCTION . . . . .	1
MATERIALS AND METHODS . . . . .	4
RESULTS . . . . .	9
DISCUSSION . . . . .	22
LITERATURE CITED . . . . .	27

LIST OF TABLES

Table	Page
1 . . . . .	14
2 . . . . .	15
3 . . . . .	16
4. Angular orientations of second-stage zones. Experimental optical conditions of a horizontal polarization plane and equal light intensity from each side and above the crystal . . . . .	17
5. Angular orientations of second-stage zones. Experimental optical conditions of a horizontal polarization plane tilted 30° off the horizontal and equal light intensities from each side and above the crystal . . . . .	18
6. Angular orientations of second-stage zones. Experimental optical conditions of unpolarized light and equal light intensities from each side and above the crystal . . . . .	19
7. Angular orientations of second-stage zones. Experimental optical conditions of a horizontal polarization plane and equal light intensities from each side and above the crystal . . . . .	20
8. Angular orientations of second-stage zones. Experimental optical conditions of unpolarized light and the light intensity from above 1.5 times the intensity from each side of the crystal . . . . .	21
9. Angular orientations of second-stage zones. Experimental optical conditions of a horizontal polarization plane and the light intensity from above 1.5 times the intensity from each side of the crystal . . . . .	22
10. Angular orientations of second-stage zones. Experimental optical conditions of the polarization plane tilted 30° off the horizontal and the light intensity from above 1.5 times the intensity from each side of the crystal . . . . .	23



LIST OF FIGURES

Figure	Page
1. Second-stage zoea oriented for approximately vertical swimming or for hovering. Dorsal spine oriented $85^\circ$ ( $\Theta$ ). See text for explanation of orientation angles . . . . .	10
2. Angular orientations of first-stage zoeae. Experimental optical conditions of unpolarized light and equal light intensities from each side and above the cuvette . . . . .	17
3. Angular orientations of first-stage zoeae. Experimental optical conditions of a horizontal polarization plane and equal light intensities from each side and above the cuvette . . . . .	18
4. Angular orientations of first-stage zoeae. Experimental optical conditions of the polarization plane tilted $20^\circ$ off the horizontal and equal light intensities from each side and above the cuvette . . . . .	19
5. Angular orientations of second-stage zoeae. Experimental optical conditions of unpolarized light and equal light intensities from each side and above the cuvette . . . . .	20
6. Angular orientations of second-stage zoeae. Experimental optical conditions of a horizontal polarization plane and equal light intensities from each side and above the cuvette . . . . .	20
7. Angular orientations of second-stage zoeae. Experimental optical conditions of the polarization plane tilted $20^\circ$ off the horizontal and equal light intensities from each side and above the cuvette . . . . .	20
8. Angular orientations of second-stage zoeae. Experimental optical conditions of unpolarized light and the light intensity from above 2.5 times the intensity from each side of the cuvette . . . . .	21
9. Angular orientations of second-stage zoeae. Experimental optical conditions of a horizontal polarization plane and the light intensity from above 2.5 times the intensity from each side of the cuvette . . . . .	21
10. Angular orientations of second-stage zoeae. Experimental optical conditions of the polarization plane tilted $20^\circ$ off the horizontal and the light intensity from above 2.5 times the intensity from each side of the cuvette . . . . .	21

## INTRODUCTION

Directed reactions of animals to light have long been noted by researchers; perhaps the most common response is phototaxis in which the orientation path is directed toward (positive phototaxis) or away from (negative phototaxis) the stimulus without deviation to either side. This taxis may take the form of a phototropotaxis in which there is simultaneous comparison of intensities by eyes on the two sides of the head; with the body axis oriented so that there is a balance of stimulation in the two eyes. The orientation may also be accomplished by a telotactic reaction in which orientation in the direction of the source of stimulation occurs without balance, with the body axis being oriented so that a certain region in each eye (or just one eye) is directed toward the stimulus. Since this form of phototaxis does not require balance of stimulation in the two eyes there is no set angle of orientation of the body axis with respect to the direction of the stimulus as long as the "fixation region" of one or both eyes is maximally stimulated (Fraenkel and Gunn 1961). More recently, polarized light has been found to influence orientation. Most experiments using polarized light have been designed from a physiological standpoint in order to show that the plane of polarization is perceived as a discrete stimulus. Several lines of behavioral evidence indicate that arthropods are able to perceive polarized light as a stimulus distinct from light pattern (image) perception. For example the reversal of phototactic sign reverses light pattern responses but has no effect on polarized

light orientation (Jander and Waterman 1960). Umminger (1968a) has related polarotaxis to its ecological significance in copepods implicating it as a behavioral mechanism in vertical migration. The mechanism for polarized light perception in decapod Crustacea has been found to be intraretinal. This ability is based on dichroic pigment molecules on mutually perpendicular microvilli that are oriented in the direction of the vertical and horizontal axes of the animal's normal spatial orientation (Shaw 1966; Waterman and Horch 1966).

The phenomenon of polarization of submarine light is well established and is ascribed to scattering of directional light by water molecules. The degree of polarization decreases with an increase in the turbidity of the water due to scattering and diffraction of the light by small particles (i.e., diameter comparable to the wavelength of light). Maximum polarization occurs perpendicular to the refracted solar rays under the sea surface (Jerlov 1968). Since underwater light intensity patterns are usually highly directional, polarization patterns exist even to great depths. Near the surface the direction of maximum light intensity is correlated with the angle of the refracted solar rays. As depth increases the direction of maximum light intensity moves toward the vertical and also becomes more diffuse (due to scattering) but still remains highly directional. At great depths (or relatively near the surface in the case of very turbid water) a limiting angular distribution pattern of intensity is approached. This elongated oval pattern, symmetrical about a horizontal plane as well as about a vertical axis, was termed "characteristic diffuse light" by Whitney (1941) and has been more recently described by Jerlov (1968) as the "asymptotic radiance

distribution." With a completely clouded and diffuse sky or in turbid water this characteristic pattern may exist as shallow as at 3 meters depth due to the rapid loss in directionality of skylight (Whitney 1941). Therefore, the plane of polarization near the surface of the water will tilt in the direction of the sun's bearing maintaining its direction of maximum polarization at  $90^{\circ}$  to the direction of the refracted solar rays. The polarization plane will move toward the horizontal as the direction of most intense light becomes vertical with an increase in depth or in turbidity (Timofeeva 1962). The degree of polarization in the direction of maximum polarization also varies with depth and turbidity. Polarization values are 60% or more near the surface in clear waters and decrease to a 30% level in deep water (Ivanoff and Waterman 1958).

The present study was undertaken to investigate polarotaxis (orientation to the e-vector of polarized light) in the zoea of the xanthid crab, Panopeus herbstii. The e-vector represents the plane of vibration of the electrical vector of the electromagnetic light wave. The optical environment was made as natural as possible to allow more realistic extrapolations from orientational behavior to the ecological relations of this group (i.e., the utilization of polarized light sensitivity for the maintenance of optimum position in its habitat and perhaps for feeding behavior). Preparatory work (equipment construction, light calibration, etc.) was done under the guidance of Dr. R.H. Stavn, UNC at Greensboro. Experimental work was performed in the laboratory of Dr. R.B. Forward at the Duke University Marine Laboratory, Beaufort, N.C.

## MATERIALS AND METHODS

Ovigerous females of Panopeus herbstii were collected intertidally. Their larvae were cultured at 25°C in 25<sup>0</sup>/00 filtered sea water, and on a 12:12 hour light:dark cycle. Larvae were transferred to fresh sea water every 24 hours and fed on freshly-hatched brine shrimp nauplii. Although this crab possesses four zoeal stages, most experiments were performed using the first and second zoeal stages since increased mortality in later stages made it difficult to obtain sufficient numbers for experiments.

The experimental apparatus consisted of a glass cuvette (inside dimensions: 35mm x 35mm x 5mm) suspended within an experimental apparatus made of structural steel on which lamps, filters, camera, and electronic flash heads were mounted. Five microscope lamps were directed at the rectangular cuvette, one from each side and one from above. Each light source was a General Electric 100W-T8½ tungsten (CC13) filament microscope bulb, the light beam from the source partially collimated and focused by a lens and iris diaphragm incorporated into the lamp. In keeping with the attempt to make the animals' visual field as natural as possible, two Corning Glass Filters - blue (Glass Number 5900) and yellow (Glass Number 3060) - were fixed between each lamp and the experimental cuvette. Waxed paper was also placed between each lamp and the cuvette to act as a depolarizer. Both the absolute intensities and the spectral composition of the filtered lights were calibrated using an ISCO spectroradiometer which demonstrated that these filters, used in

conjunction with the tungsten filament bulb, do indeed produce a close approximation to the solar radiation spectrum. A small black box surrounding the cuvette with "windows" on all four sides and above effectively eliminated unwanted reflection and refraction of light off the sides of the cuvette. The box was painted flat black inside and out as was the structural steel of the experimental apparatus. It also served as a surface for mounting neutral density filters to change the intensity of the incident light and polaroid filters (type HN 38) to provide the polarized light component necessary for the experimental procedure. The intensities thus obtained within the box ranged from 1.2 to 1.6  $\mu\text{W}/\text{cm}^2$  (with an average value of 1.4  $\mu\text{W}/\text{cm}^2$ ) with neutral density filters and from 1.3 to 1.8  $\mu\text{W}/\text{cm}^2$  (average 1.6  $\mu\text{W}/\text{cm}^2$ ) with polaroid filters. The overhead light was used with two different intensity settings, 1.9  $\mu\text{W}/\text{cm}^2$  (approximately 1.3 times the average side light intensity) and 3.7  $\mu\text{W}/\text{cm}^2$  (approximately 2.5 times the average side light intensity).

A 35mm camera with a 55mm lens on an extension tube was positioned to one side of the cuvette, the images of the zoeae within the cuvette were then reflected into the camera lens from a beam-splitter mirror. This allowed both photography and illumination of the cuvette in the same axis. The camera was located about 15cm from the cuvette which permitted an approximate 0.75:1 image/object magnification on the negative. The film used was Kodak high-speed infrared (HIE 135). Photographic lighting was provided by a custom-designed flash head with a xenon flash tube (filtered with a Corning Glass infrared filter, Glass Number 2600) powered at 300 watt-seconds from a Thomastrobe electronic

flash power supply. Infrared was chosen for photographic lighting since light near this wavelength has been shown to neither induce nor alter light sensitive behavior (Forward, personal communication).

The experimental procedure consisted of filling the cuvette with water containing numerous zoeae (approximately 50). The cuvette was then taken into a darkened room and placed in the experimental apparatus. The five microscope lamps (either all of approximately equal intensity or with the overhead adapting lamp 2.5 times the intensity of the average side lamp) were turned on simultaneously and left on for thirty seconds to permit the larvae to partially adapt to the light intensity being used. The lamps were then turned off for 30 seconds to allow the animals to assume a random orientation distribution. The lamps were again turned on, and after a period of 15 seconds a photograph was taken. The apparatus was once again darkened for 30 seconds after which it was illuminated for 15 seconds and another photograph was taken. After the series of two pictures, the cuvette was emptied and refilled with more zoeae, and the procedure was repeated. This period of exposure to the lamps was not long enough to cause any increase in temperature of the water in the cuvette. The laboratory temperature was maintained at approximately 23°C, so the larvae experienced little temperature change during the time the experiments were performed. Each experimental run consisted of 20 pictures taken under the same conditions. Each experimental set consisted of 60 pictures: 20 pictures using neutral density filters on all four sides and above; 20 pictures using polaroid filters (e-vector horizontal) on all four sides and neutral density filter above; and 20 pictures with the e-vector 20° off the horizontal

on the front and back (image-forming sides for the camera), e-vector horizontal on the other two sides, and neutral density filter above. The neutral density filters (on the "windows" of the box) duplicated the reduction in intensity caused by the polaroid filters. The experimental set was repeated several times using zoeae from different hatches which allowed for replication and the investigation of the reactions of different zoeal stages. It may have been better to use neutral density filters in place of polaroid filters (e-vector horizontal) on the two non-image-forming sides of the box surrounding the cuvette in the  $20^{\circ}$  polaroid tilt experiments. To be as natural as possible, the direction of maximum polarization for the horizontally polarized light should be at an angle  $20^{\circ}$  above the horizontal on one side and  $20^{\circ}$  below the horizontal on the other side (Timofeeva 1962). Therefore, the polarization pattern as presented in these experiments may have provided two differing cues for orientation.

The film was developed in Kodak D-76 developer and the negatives were placed in an enlarger. The images were then projected onto a piece of paper, so the orientations could be drawn with an accuracy of at least  $1^{\circ}$ . The orientation angles were then measured and tabulated. It was noted that due to a peculiarity of the positioning of the electronic flash, larvae facing left formed clearer images on the negatives than those facing right. Results for only the population facing left were, therefore, presented in the following discussion. The statistical tests employed were the Mann-Whitney (Wilcoxon) U test for location comparisons (Owen 1962) and the Siegel-Tukey test for comparison of dispersions about the median (Siegel and Tukey 1960). The circular data were linearized



because orientations of the body axis in the vertical plane are unique and circular distributions are not required (Batschelet 1965). Critical values for the statistical tests and distribution-free 95% confidence intervals for the medians of each distribution came from a standard handbook of statistical tables (Owen 1962).

## RESULTS

Preliminary experiments using relatively high light intensities resulted in all but a few larvae sinking to the bottom of the cuvette. Therefore, low light intensity was used in all the experiments reported here. This does not necessarily mean that the zoeae in their natural environment are photonegative to intensities higher than those used in these experiments. Instead, this behavior may be due to the manner in which the stimulus was presented (relatively brief period of exposure to the lights).

All experimental conditions of light intensity patterns and polarization planes resulted in primarily a vertical orientation in both the first and second zoeal stages (Figures 2-10). In the following discussion an angular orientation of  $90^{\circ}$  was defined as the orientation assumed by a left-facing zoea with the basal portion of its dorsal spine pointing vertically upward (Figure 1). The angle increases in a counter-clockwise direction. Larvae swimming upward vertically or hovering oriented with their dorsal spine at approximately  $90^{\circ}$ , head end in the horizontal plane.  $0^{\circ}$  would be horizontal backward swimming; dorsal spine in the horizontal plane, head end vertically upward. This would be a highly unstable orientation and was never observed. The orientation angles were seldom less than  $70^{\circ}$ .  $180^{\circ}$  would be horizontal forward swimming; dorsal spine in the horizontal plane, head end vertically downward. Actually, roughly horizontal forward swimming occurs regularly at a much lesser tilt of the body axis (dorsal spine at

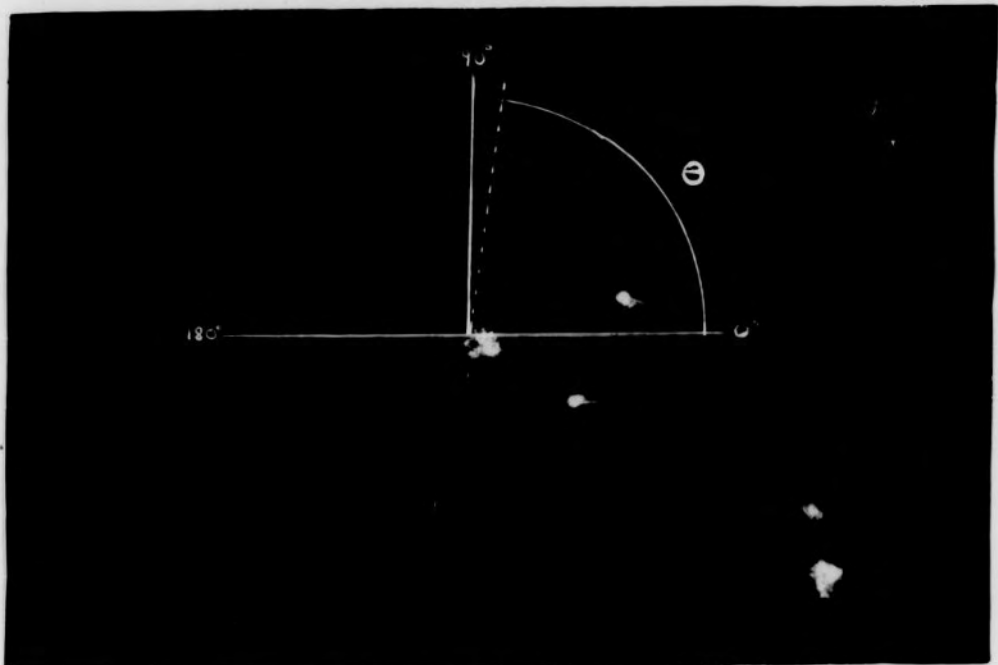


Figure 1. Second-stage zoea oriented for approximately vertical swimming or for hovering. Dorsal spine oriented  $85^\circ$  ( $\theta$ ). See text for explanation of orientation angles.

approximately  $120^{\circ}$ ). Orientation angles much greater than  $120^{\circ}$  were normally observed only during passive sinking when a forward roll took place (this was usually followed by a rapid righting maneuver after which active swimming resumed). First stage zoeae under the conditions of approximately equal light intensities from each side and from above showed no significant difference in location (median orientation angle) between the population presented with a horizontal e-vector and that in which no polarized component was present (Tables 1 and 2; Figures 2 and 3). There was, however, a highly significant difference in dispersion about the median. The population presented with a horizontal e-vector showed less dispersion about the primary orientation position. Comparison of the population with no polarized component to the population with the plane of polarization tilted  $20^{\circ}$  downward to the left off the horizontal showed no significant difference in location (Tables 1 and 2; Figures 2 and 4). However, comparison of horizontal e-vector and  $20^{\circ}$  tilt e-vector populations showed a highly significant difference in location. The population subject to the  $20^{\circ}$  tilt of the e-vector shifted its orientation counterclockwise, the direction of the tilt of the plane of polarization (Tables 1 and 2; Figures 3 and 4). The shift in the location of the population was between  $1^{\circ}$  and  $5^{\circ}$  (95% confidence interval) off the location of the horizontal e-vector population. Values for the 95% confidence interval for the shift ("slippage") of the population came from a modification of the Mann-Whitney (Wilcoxon) U test. This method involved the trial and error usage of a "treatment effect", i.e., a certain number of degrees was added to each angular orientation of the horizontal e-vector population. In this way a range

of values was found which, when added to the angular orientation values of the one population, would cause the two populations to have distribution locations which were statistically the same (Kraft and VanEeden 1968).

The results from these statistical tests are in agreement with results obtained using Chi-square tests. Comparison of the no polaroid population to the one with a horizontal e-vector gives a significant difference in the two populations using the Chi-square test (Table 3). The Chi-square test, however, gives no information concerning whether this difference comes from differences in location or differences in dispersion about the median. There was no significant difference between the no polaroid population and the  $20^{\circ}$  e-vector tilt population when comparing them with the Chi-square test. There was a significant difference between the horizontal e-vector and the  $20^{\circ}$  tilt e-vector population. The Chi-square test was not used for the second stage data because numbers within each  $3^{\circ}$  sector used for the tests were often low. This would necessitate a large amount of combining of groups to meet the requirement of the test that all expected frequencies be five or greater. Such combining of sectors would result in a definite loss of information contained in the data.

Insufficient numbers for proper statistical testing were obtained in the first-stage zoea experiments using the increased overhead light intensity. This was because the season during which ovigerous females could be found was ending and there was mass mortality of the first-stage zoeae.

Second-stage zoeae under conditions of approximately equal intensities from each side and from above showed no significant difference

in location or dispersion between the population with no polarized component and the horizontal e-vector population. Comparison of the population with no polaroid and that with the  $20^{\circ}$  e-vector tilt showed a significant difference in location. The  $20^{\circ}$  e-vector population shifted more toward the vertical rather than in the direction of tilt of the polarization plane. In the comparison of the locations of the horizontal e-vector and the  $20^{\circ}$  e-vector populations the  $20^{\circ}$  population again shifted its orientation toward the vertical and away from the tilt of the polarization plane (Tables 1 and 2; Figures 5-7).

Under conditions of increased intensity from above (overhead lamp 2.5 times the intensity of each side lamp) the results were the same. There was no significant difference between the locations or the dispersions of the no polaroid population and the population with the horizontal plane of polarization. There was a significant difference in location between the no polaroid and the  $20^{\circ}$  tilt e-vector populations, the  $20^{\circ}$  tilt population shifting toward the vertical. The difference borders on being significant in the comparison of the horizontal and the  $20^{\circ}$  tilt e-vector populations, the  $20^{\circ}$  tilt population again shifting toward the vertical (Tables 1 and 2; Figures 8-10).

A comparison of the first stage to the second stage zoea experiments (equal intensities for all five lamps) showed no significant differences in locations. Comparing the second stage zoea experiments with the increased intensity from above to those with equal intensities for all lamps demonstrated that a shift off the vertical (greater angles counter-clockwise) occurred for the populations exposed to increased intensity from above (Tables 1 and 2; Figures 5-10).

TABLE 1

	<u>Median Angle</u>	<u>95% Confidence Interval of the Median Angle</u>
<u>First Stage Larvae</u>		
(approximately equal light intensities all sides and above):		
no polaroid	97°	92°-100°
horizontal e-vector	92°	91°-94°
20° tilt e-vector	98°	94°-101°
<u>Second Stage Larvae</u>		
(approximately equal light intensities all sides and above):		
no polaroid	92°	89°-95°
horizontal e-vector	93°	89°-100°
20° tilt e-vector	90°	87°-93°
<u>Second Stage Larvae</u>		
(overhead lamp 2.5 times intensity of each side lamp):		
no polaroid	104°	92°-116°
horizontal e-vector	108°	97°-119°
20° tilt e-vector	99°	94°-105°

TABLE 2

<u>Populations compared</u>	<u>Probability that the decision that the locations are the same is true</u>	<u>Probability that the decision that the dispersions are the same is true</u>
<u>First Stage Larvae</u> (approximately equal light intensities all sides and above):		
no polaroid vs. horizontal e-vector	p=0.40	p=0.009
no polaroid vs. 20° tilt e-vector	p=0.32	
horizontal e-vector vs. 20° tilt e-vector	p=0.01	
<u>Second Stage Larvae</u> (approximately equal light intensities all sides and above):		
no polaroid vs. horizontal e-vector	p=0.64	p=0.69
no polaroid vs. 20° tilt e-vector	p=0.02	
horizontal e-vector vs. 20° tilt e-vector	p=0.03	
<u>Second Stage Larvae</u> (overhead lamp 2.5 times intensity of each side lamp):		
no polaroid vs. horizontal e-vector	p=0.58	p=0.87
no polaroid vs. 20° tilt e-vector	p=0.02	
horizontal e-vector vs. 20° tilt e-vector	p=0.13	
<u>Second Stage Larvae</u> (approximately equal light intensities all sides and above) vs. <u>Second Stage Larvae</u> (overhead lamp 2.5 times intensity of each side lamp):		
no polaroid	p=0.004	
horizontal e-vector	p=0.010	
20° tilt e-vector	p=0.00002	



TABLE 3

## Chi-Square Tests

First Stage Larvae

(approximately equal light intensities all sides and above):

no polaroid vs. horizontal e-vector

$$\chi^2 = 35.51 \quad df = 17 \quad 0.005 < p < 0.010$$

no polaroid vs. 20° tilt e-vector

$$\chi^2 = 24.18 \quad df = 18 \quad 0.10 < p < 0.25$$

horizontal e-vector vs. 20° tilt e-vector

$$\chi^2 = 32.09 \quad df = 17 \quad 0.01 < p < 0.026$$

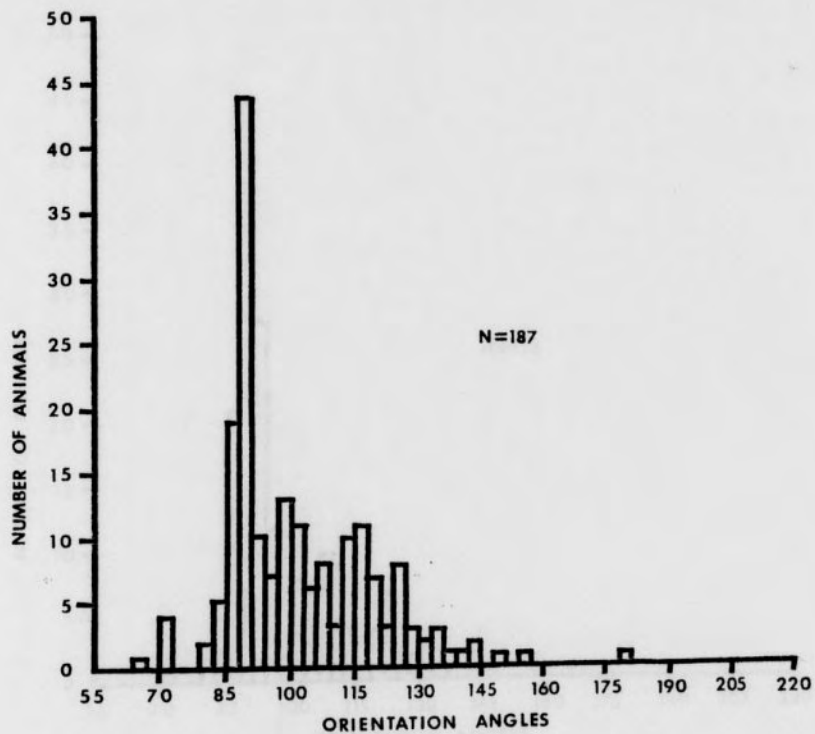


Figure 2. Angular orientations of first-stage zoeae. Experimental optical conditions of unpolarized light and equal light intensities from each side and above the cuvette.

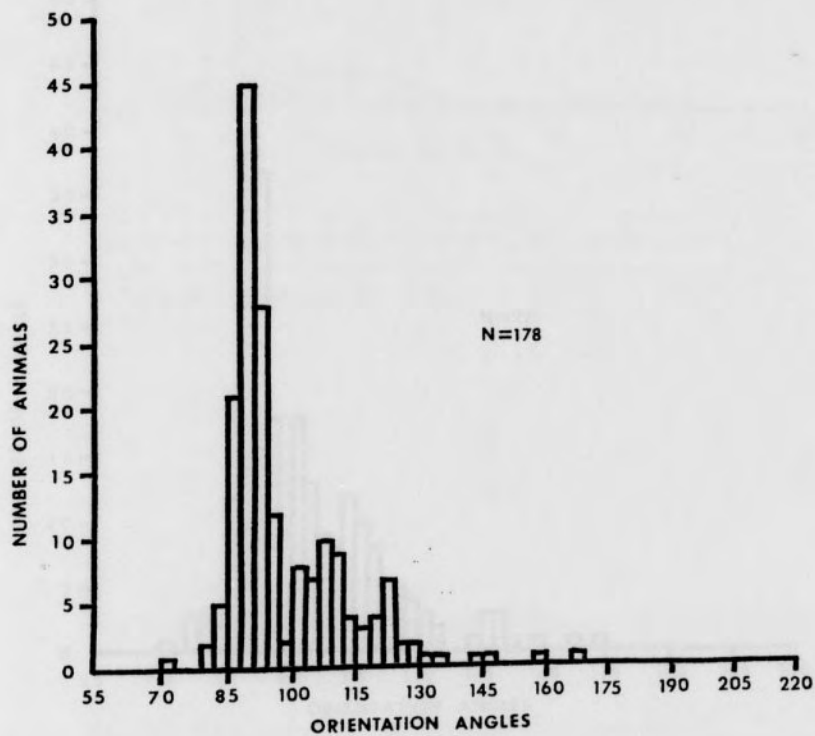


Figure 3. Angular orientations of first-stage zoeae. Experimental optical conditions of a horizontal polarization plane and equal light intensities from each side and above the cuvette.

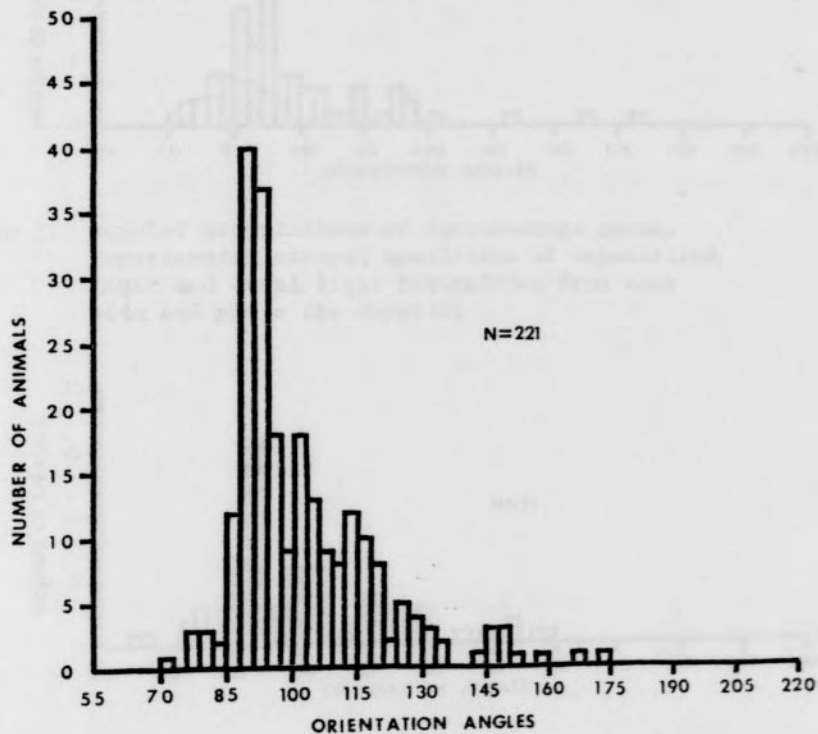


Figure 4. Angular orientations of first-stage zoeae. Experimental optical conditions of the polarization plane tilted  $20^\circ$  off the horizontal and equal light intensities from each side and above the cuvette.

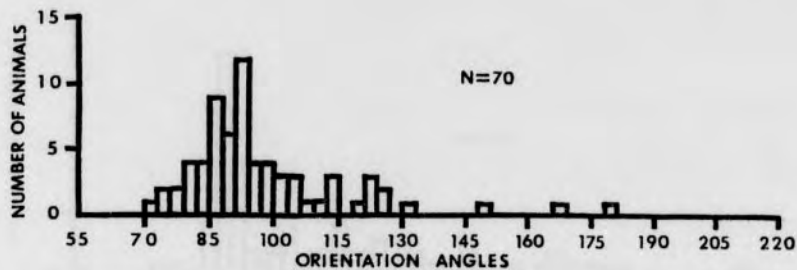


Figure 5. Angular orientations of second-stage zoeae. Experimental optical conditions of unpolarized light and equal light intensities from each side and above the cuvette.

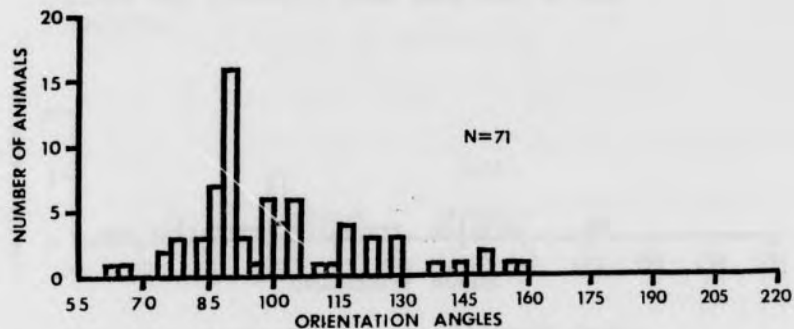


Figure 6. Angular orientations of second-stage zoeae. Experimental optical conditions of a horizontal polarization plane and equal light intensities from each side and above the cuvette.

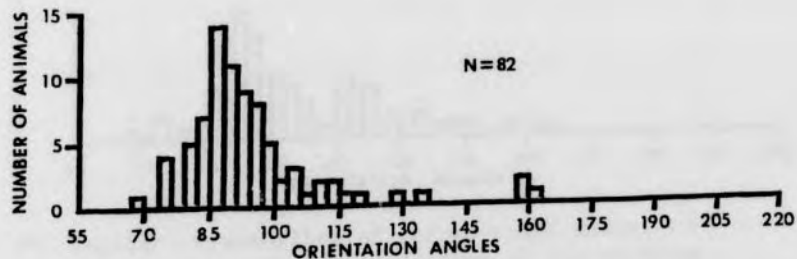


Figure 7. Angular orientations of second-stage zoeae. Experimental optical conditions of the polarization plane tilted  $20^\circ$  off the horizontal and equal light intensities from each side and above the cuvette.

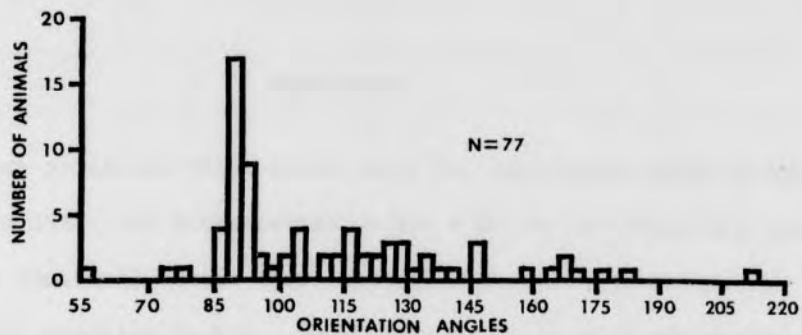


Figure 8. Angular orientations of second-stage zoeae. Experimental optical conditions of unpolarized light and the light intensity from above 2.5 times the intensity from each side of the cuvette.

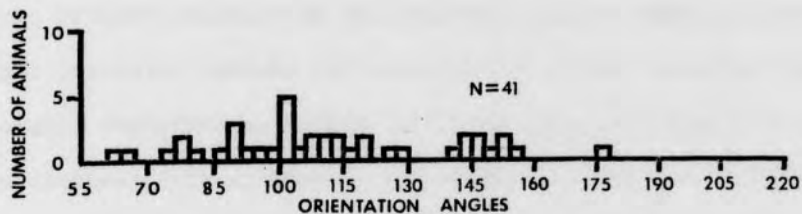


Figure 9. Angular orientations of second-stage zoeae. Experimental optical conditions of a horizontal polarization plane and the light intensity from above 2.5 times the intensity from each side of the cuvette.

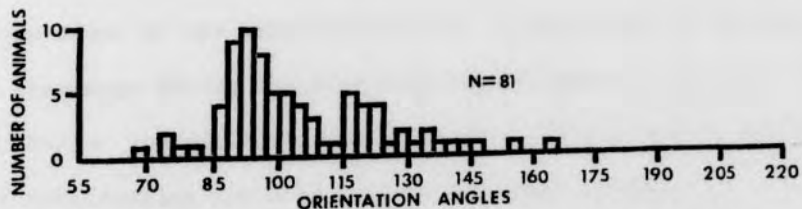


Figure 10. Angular orientations of second-stage zoeae. Experimental optical conditions of the polarization plane tilted  $20^\circ$  off the horizontal and the light intensity from above 2.5 times the intensity from each side of the cuvette.

## DISCUSSION

Although polarized light sensitivity has been demonstrated in many groups of animals, the presentation of the stimulus has often been done in a highly unnatural manner (i.e., polarized beam from above and/or with no other adapting light). By presenting the experimental animal with a more natural light field (i.e., a significant non-polarized light source at  $90^{\circ}$  to the direction of the e-vector), it is possible that more ecologically pertinent data may be obtained.

The zoeal primary orientation and swimming behavior under all conditions of light intensity pattern and polarization closely resembled the negative geotaxis described by Sulkin (1973) for Panopeus herbstii zoeae orienting in darkness. Thus, gravity is probably the strongest stimulus for orientation. Since decapod crustacean larvae do not develop statocysts until the last larval stage (Ringelberg 1969), there must be an alternative method of gravity detection. The negative geotaxis may be accomplished by a feed-back system involving a receptor organ at the base of the antennae or the maxillipeds which is stimulated by the position of the appendage during the free fall between swimming strokes (Hutchinson 1967). The present study shows that light intensity patterns and plane of polarization are also used as orienting stimuli.

First-stage zoeae showed no significant difference in angular orientations under the conditions of no polaroid vs. horizontal e-vector. There is, however, a significantly smaller degree of angular deviation from the upward swimming orientation in the horizontal e-vector population

which suggests the possibility of more precise orientation for vertical migration when polarized light is present. Umminger (1968a) has also implicated polarotaxis as a behavioral mechanism in vertical migration of copepods. The finding that first stage zoeae shift their orientation off the vertical when presented with a  $20^{\circ}$  tilt of the e-vector rather than a horizontal e-vector also indicates polarized light is an orienting stimulus. However, it is not as strong a stimulus as gravity (the plane of polarization was shifted  $20^{\circ}$  but the population's shift was only between  $1^{\circ}$  and  $5^{\circ}$ ). An angle of  $20^{\circ}$  off the horizontal was chosen for the tilt of the e-vector because several studies have found that there are four basic orientations assumed by most arthropods swimming in a vertical beam of polarized light. These orientation peaks occur parallel to, perpendicular to, and at  $45^{\circ}$  to the left and right of the e-vector (Jander and Waterman 1960). Had an angle of  $45^{\circ}$  off the horizontal been chosen for the tilt of the e-vector, a change in orientation of this population may, therefore, have been hard to detect. The choice of a  $20^{\circ}$  tilt is also in keeping with the attempt to stimulate natural conditions. The water in which the crabs live is often quite turbid which causes a rapid loss in directionality of the light in the bearing of the sun. This, in turn, results in less tilt of the polarization plane off the horizontal than would be found in clear water.

Second-stage larvae seem to have undergone an ontogenetic change in their reaction to polarized light. Under both intensity patterns there was no difference in location or dispersion between the population with no polaroid and that presented with a horizontal e-vector. This would seem to indicate that second stage larvae have become indifferent to the



plane of polarization. However, in both the no polaroid vs.  $20^{\circ}$  e-vector and the horizontal e-vector vs. the  $20^{\circ}$  e-vector comparisons (for both intensity patterns) a shift toward the vertical (away from the e-vector tilt) was found for the  $20^{\circ}$  e-vector population. This suggests that although second-stage larvae are capable of perceiving and orienting to the plane of polarization, the information rendered by the plane of polarization is used to shift the population away from orientation perpendicular to the e-vector (and thus away from the apparent direction of maximum light intensity as indicated by the direction of the e-vector). An alternative explanation for this shift away from orientation direction perpendicular to the e-vector might be postulated considering Umminger's (1968a) finding of an endogenous rhythm in polarotaxis in copepods with orientation peaks at "dawn", "midday", and "dusk". If Panopeus zoeae also undergo this rhythmic change in orientation to the plane of polarization, it may be that the time of day at which the experiments were performed (usually 2-4 hours after the beginning of the light cycle) is the determining factor in this seemingly aberrant orientation to the e-vector.

Although no locational differences were found between the first-stage populations vs. the second-stage populations (equal intensity from above), differences in location did occur between the second-stage larvae presented with equal intensities from above and each side as compared to those with 2.5 times the intensity from above. The greater contrast in intensity in all cases caused a shift from the vertical, indicating that the second stage larvae were less positively phototactic at this higher intensity. It should be noted that the presentation of equal

light intensities from each side and from above will result in an intensity distribution which is brighter in the upper hemisphere. This occurs because there was very little light reflected off the bottom of the cuvette. The bottom of the cuvette was coated with a mixture of carbon black and paraffin. The low overall light intensities used and the relatively small degree of contrast in the intensity patterns may be considered fairly representative of the actual light conditions in the animals' turbid coastal habitat. The more equal intensity pattern corresponds roughly to that found in an extremely turbid environment. The greater contrast intensity pattern is like that of a less turbid medium. In their natural environment the larvae might be expected to stay lower in the water column in less turbid water as a mechanism for avoidance of visually-oriented predators. The shift from the vertical upward position found in the contrast intensity populations would result in their assuming a lower level in the water column. This apparent shift in orientation for swimming direction may actually be just a decrease in swimming activity in the primary vertical orientation during their "hop and sink" swimming behavior. Any decrease in swimming activity would cause a greater occurrence of the forward roll. Animals which in the pictures appear to be swimming in a direction off the vertical may actually just be in the early stages of the forward roll after ceasing active swimming ("active swimming" is needed to maintain a vertical upward orientation even during hovering). If this is actually the case, it too would displace the animals downward in the water column.

In addition to the functioning of polarized light as an orienting stimulus, Lythgoe and Hemmings (1967) have demonstrated that polarized

light sensitivity enables distant objects to be seen more clearly and causes contrasts in intensity to be much sharper. This fact plus Umminger's (1968b) finding that polarized light perception is more prominent in predatory than in herbivorous species of copepods has led Umminger to postulate that polarized light sensitivity is useful to visually-oriented predators. This may also be true of zoeae which actively pursue their food (Welch and Sulkin 1974). Thus, polarized light may serve the dual purpose of aiding in food capture and enhancing the accuracy of orientation for maintenance of position in the environment or "ecological niche". Polarized light perception might also be useful during vertical migration if this is important for the previously mentioned functions. These two factors may be more closely related than is readily apparent. Casual observation of the larvae's feeding strategy in culture dishes revealed that upon capture of prey swimming ceases and the larvae sink to the bottom of their dish. In their natural habitat this would result in the animals sinking out of their "preferred" position in the water column, necessitating accurate orientation to return them to the depth where food is at an optimal concentration. Such postulates call for further experimental work in addition to field studies to determine the actual position of the larvae in the water column. Measurements in the habitat of such parameters as percent polarization, light intensities and the interaction of polarization and intensity, and food levels at different depths are sure to increase our understanding of the subject.

## LITERATURE CITED

- Batschelet, E. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. The American Institute of Biological Sciences, Washington, D.C. 57pp.
- Fraenkel, G.S. and D.L. Gunn. 1961. (Dover edition). The orientation of animals. New York. 376pp.
- Hutchinson, G.E. 1967. A treatise on limnology. Vol. II: Introduction to lake biology and the limnoplankton, pp. 763-765. Wiley, New York. 1115pp.
- Ivanoff, A. and T.H. Waterman. 1958. Factors, mainly depth and wavelength, affecting the degree of underwater light polarization. J. Mar. Res., 16: 283-307.
- Jander, R. and T.H. Waterman. 1960. Sensory discrimination between polarized light and light patterns by arthropods. J. Cell. Comp. Physiol., 56: 137-160.
- Jerlov, N.G. 1968. Optical oceanography. Elsevier, Amsterdam. 194pp.
- Kraft, C.H. and C. vanEeden. 1968. A nonparametric introduction to statistics, p. 135. Macmillan, New York.
- Lythgoe, J.N. and C.C. Hemmings. 1967. Polarized light and underwater vision. Nature, 213: 893-894.
- Owen, D.B. 1962. Handbook of statistical tables. Addison-Wesley, Massachusetts. 580pp.
- Ringelberg, J. 1969. Spatial orientation of planktonic crustaceans. 2. The swimming behavior in a vertical plane. Verh. int. Ver. Limnol., 17: 841-847.
- Shaw, S.R. 1966. Polarized light responses from crab retinula cells. Nature, 211: 92-93.
- Siegel, S. and J.W. Tukey. 1960. A nonparametric sum of ranks procedure for relative spread in unpaired samples. J. Amer. Statist. Assoc., 55: 429-445.
- Sulkin, S.D. 1973. Depth regulation of crab larvae in the absence of light. J. Exp. Mar. Biol. and Ecol., 13: 73-82.

- Tinofeeva, V.A. 1962. Spatial distribution of the degree of polarization of natural light in the sea. *Izv. Akad. Nauk S.S.S.R., Ser. Geofiz.*, 6: 1843-1851.
- Umminger, B.L. 1968a. Polarotaxis in copepods. I. An endogenous rhythm in polarotaxis in Cyclops vernalis and its relation to vertical migration. *Biol. Bull.*, 135: 239-251.
- Umminger, B.L. 1968b. Polarotaxis in copepods. II. The ultrastructural basis and ecological significance of polarized light sensitivity in copepods. *Biol. Bull.*, 135: 252-261.
- Waterman, T.H. and K. Horch. 1966. Mechanism of polarized light perception. *Science*, 154: 467-475.
- Welch, J. and S.D. Sulkin. 1974. Effect of diet concentration on mortality and rate of development of zoeae of the xanthid crab, Richironanopeus harrisi (Gould). *J. Elisha Mitchell Sci. Soc.*, 90(2): 69-72.
- Whitney, L.V. 1941. The angular distribution of characteristic diffuse light in natural waters. *J. Mar. Res.*, 4: 122-131.