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TITLE OF THESIS. A Comparative Study of the Photoreceptors of the Decapod Crustacean Pandalus Borealis, Kroyer .....

UNIVERSITY..... Dalhousie University, Halifax, Nova Scotia .....

DEGREE FOR WHICH THESIS WAS PRESENTED.... Ph.D. .....

YEAR THIS DEGREE GRANTED..... May 1972 .....

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DATED. June 14, 1972

NL-91 (10-68)

DALHOUSIE UNIVERSITY

Date November 3, 1971

Author Peter B. Eaton

Title A COMPARATIVE STUDY OF THE PHOTORECEPTORS OF THE DECAPOD CRUSTACEAN PANDALUS BOREALIS, KRØYER

Department or School Department of Biology

Degree Ph.D. Convocation - May Year 1972

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A COMPARATIVE STUDY OF THE PHOTORECEPTORS OF THE DECAPOD CRUSTACEAN *PANDALUS BOREALIS*, KRØYER

by

Peter B. Eaton

Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy at Dalhousie University.

November, 1971

Approved by:

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CONTENTS

ABSTRACT

The nauplius and compound eyes of the shrimp, Pandalus borealis, have been investigated using electrophysiological and behavioural techniques. Ocellar potentials were used to determine the spectral sensitivity of the nauplius eye and to compare its characteristics with those of the compound eye. A peak of maximum sensitivity for the nauplius eye was found at 490 nm for female P. borealis, while male specimens had a maximum sensitivity at 510 nm. A similar situation was found for the compound eye, although differences between male and female were not as significant. Comparative studies using females of Pandalus montagui revealed a 490 nm peak restricted to the nauplius eye and a 510 nm peak for the compound eye. Behavioural studies carried out on larvae of P. borealis showed a strong positive phototaxis with a peak of activity between 490 and 525 nm, and ocellar potentials recorded from the larvae revealed sensitivity peaks at 510 and 490 nm. The significance of these findings is discussed in relationship to the environmental changes through which pandalid shrimps progress during their development. A preliminary histological examination of the eye exposed some structural characteristics which are discussed in relation with nauplius eye function.

INTRODUCTION

Almost all crustaceans have a median eye (commonly known as the nauplius eye) usually situated close to the front of the brain. Elofsson has traced this organ throughout the Crustacea and described its morphology (Elofsson 1963, 1965, 1966[a]). He has also described the embryological development of the nauplius eye of decapods (Elofsson 1966[b]) and has studied its fine structure in Pandalus borealis (Elofsson 1966[c]). The fine structure of the eye of a copepod Macrocyclops albidus has been described by Fahrenbach (1964) who has also examined the nauplius eye of Balanus cariosus (Fahrenbach 1965).

This investigation deals with Pandalus borealis for three main reasons: 1.) It is the species that was used for a preliminary study by the author (Eaton 1968, Eaton and Boyd 1970). 2.) P. borealis is a typical deep water decapod which is fairly accessable in Nova Scotian waters and easily maintained in the laboratory. 3.) A good descriptive study of the nauplius eye of P. borealis exists (Elofsson 1963, 1966[b], 1966[c]). These reasons were background for the present study which was directed toward extending our information on the function of the nauplius eye.

The nauplius eye of P. borealis consists of two main parts: the nauplius eye proper (sensu stricto) and the dorsal frontal organ. The nauplius eye proper is made up of three "cups" each containing three photoreceptor cells. A single ventral cup is directed forward and down,

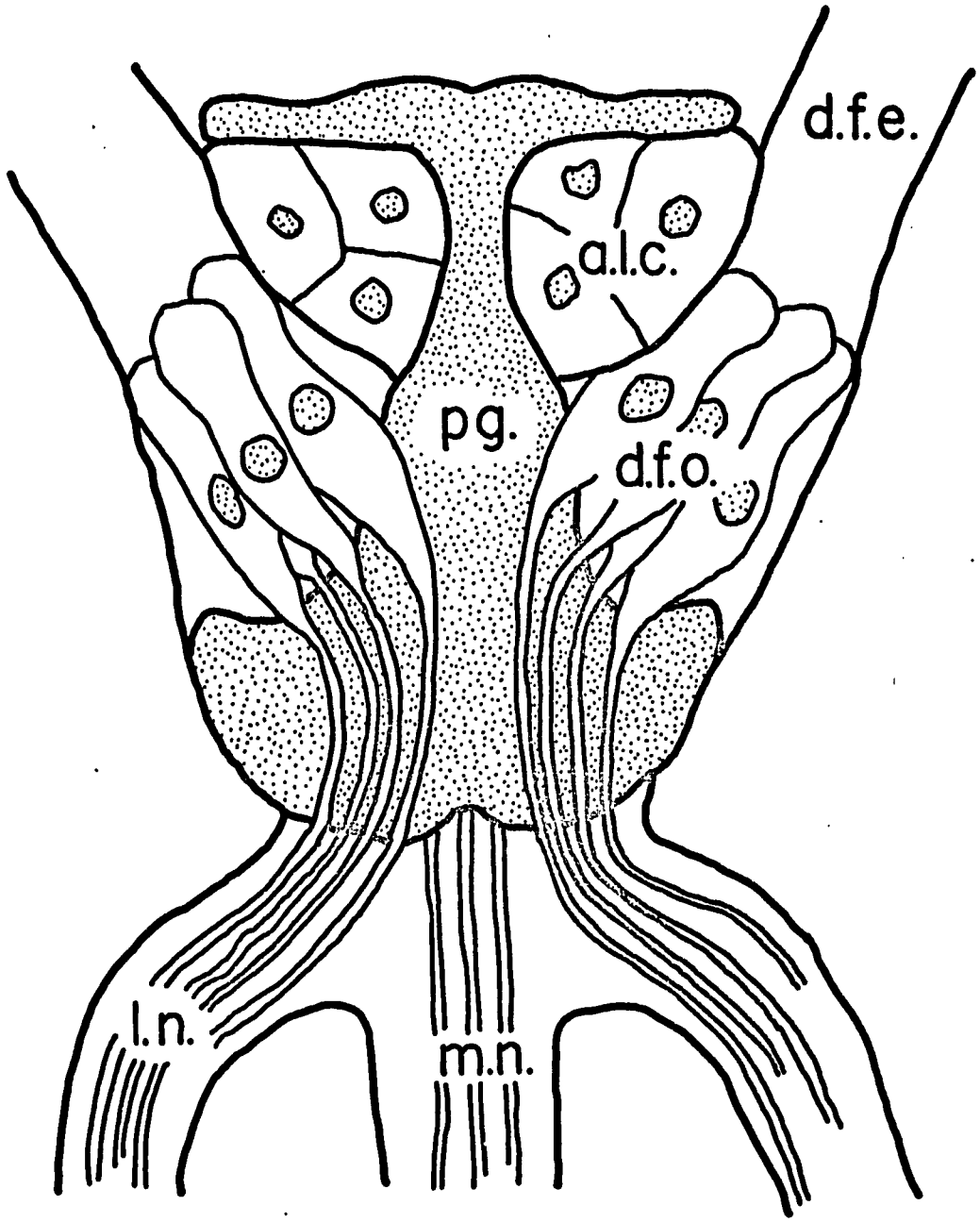
and two anterior lateral cups are directed dorsally and slightly forward. These three cups are connected to the brain by a median nerve containing nine axons, one for each photoreceptor cell. The dorsal frontal organ is a paired structure forming the lateral boundaries of the eye structure. Each half of the dorsal frontal organ is made up of a posterior lateral cup containing 30 photoreceptor cells in groups of three, and a curious extension which reaches from the posterior lateral cup forward and laterally to contact the integument of the "bec ocellaire" (Fig 1). The entire eye, including the extensions of the dorsal frontal organ, is sheathed in epineurium which is continuous with the brain covering.

The cells of the nauplius eye proper are imbedded in a black screening pigment as are the proximal parts of the cells of the dorsal frontal organs. Axons leaving the posterior lateral cup cells traverse the pigment and form two lateral nerves which enter the brain on either side of the median nerve. The eye itself is about 200 μ long not including the dorsal extensions or the connecting nerves. More will be said about the eye and its surrounding tissues in connection with results from the present histological study.

The photoreceptor cells themselves deserve some initial consideration. All sensory cells in the nauplius eye occur in groups of three and form a rhabdom where they come together, a feature which is common throughout invertebrates. The sensory cells are large (100 μ long) with a large irregularly rounded nucleus. At the surface contacting the other two

Figure 1.

Horizontal section through the nauplius eye of Pandalus borealis. (d.f.e.) dorsal frontal organ extension, (a.l.c.) anterior lateral"cup", (pg) shielding pigment, (d.f.o.) dorsal frontal organ, (l.n.) lateral nerve, (m.n.) median nerve.



NAUPLIUS EYE

HORIZONTAL SECTION

cells in a group, each cell develops a rhabdomere, a microvillous border with rather irregularly arranged and distributed microvillae. This feature contrasts with the highly organized arrangement of microvillae found in the compound eye of arthropods (Waterman 1966) which allows them to discriminate the plane of polorized light (Bainbridge and Waterman 1957, Shaw 1969).

The presence of sensory cells with rhabdomeres, screening pigment, and nervous connection with the supra-oesophageal ganglion certainly marks the nauplius eye as a receptor organ developed for light perception. Also, the lack of any signs of reduction or loss of the structures mentioned above attests to its functional usefulness as a sensory organ.

Because of the nauplius eye's similarity in basic cellular structure to many other simple photoreceptors, an extensive literature could be consulted for information on the electrical response of photoreceptor cells, and often the information so gleaned could be related directly to the nauplius eye. This was most useful due to the complete lack of previous physiological work on decapod nauplius eyes. One of the most closely related studies, apart from previous work carried out by the author (Eaton 1968), is that of Gwilliam (1963, 1965) who examined the shadow response of the barnacle as mediated by its nauplius eye. The electrical response of the eye and ocellar nerve of the barnacle appeared as a graded receptor potential uncomplicated by action potentials. Similar responses, characterized by a rapid voltage transient at the initiation of a light pulse, a sustained steady state depolarization during

the light pulse, and a rapid return to the polarized state at the cessation of the light pulse, have been recorded from individual photoreceptor cells of other arthropods. Information on the photoreceptors of Limulus far exceeds that of other arthropods, and a fairly complete understanding of the photoresponse of Limulus has been established. Smith, et al (1968) examined the light induced conductance changes of the photoreceptor cell membrane of Limulus and proposed that receptor depolarization was due to light induced changes in a metabolic sodium pump rather than direct changes in the conductance of the cell membrane. This hypothesis contradicted the more familiar view that depolarization was a result of a change in membrane conductance. New evidence to support the belief that membrane conductance changes were responsible for depolarazion was introduced by Brown et al (1969) for the photoreceptor cells of the barnacle, and by Millecchia and Mauro (1969), who demonstrated a similar light induced increase in membrane conductance for the ventral photoreceptor cells of Limulus, casting doubt upon the hypothesis of a metabolic sodium pump mechanism advanced by Smith et al (1968).

Bass and Moore (1970) have proposed a mechanism to explain the depolarization of Limulus photoreceptor cells in response to very small quantities of light. Their model involves the unique structural characteristics of retinular cells (i.e. the microvillae of the rhabdomere), and explains how the arrangement of these structures provides for the amplification necessary for the absorption of one photon of light to produce a depolarization of the receptor cell. Because of the universal occurrence

of microvillae in retinular cells of most invertebrates, the model of Bass and Moore (1970) might be applied throughout invertebrate photoreceptors. The present investigation was not intended to probe the detailed characteristics of photoreceptor cell membranes in Pandalus borealis, but rather, was directed at examining the spectral sensitivity of the photoreceptors. It is sufficient to note that the ocellar potentials of the nauplius and compound eyes of P. borealis resemble those of Limulus in basic shape.

Spectral sensitivity is one of the more important characteristics of photoreceptors since it usually bears a direct relationship with the existing or potential habitat of an animal and determines some of the animal's behavioural activity. The ability of some flying insects to seek out nectar-bearing flowers is dependent upon a sensitivity to the ultraviolet light reflected by the flower petals. Ultraviolet sensitivity has been found in the honeybee, a nectar feeder (Goldsmith 1960); however, it has also been found in Notonecta irrorata (Bennett and Ruck 1970), in the lateral eye of the scorpion (Machan 1968) and in the median ocellus of Limulus (Wald and Krainin 1963, Chapman and Lall 1967) in which cases its usefulness is obscure. Spectral sensitivity and habitat of marine fishes have been related by Wald, Brown and Brown (1957) who found that species living at greater depths had λ-max's at shorter wavelengths that those species living in shallow water. The peak spectral sensitivities of the Lobster, 515 nm (Wald and Hubbard 1957),and the crayfish, Orconectes, 565 nm (Goldsmith and Fernandez 1968), also reflect the coastal-marine and fresh water habitats of these two species. Fresh water bodies, because

of their relatively greater turbidity, tend to transmit longer wavelengths than water occurring in the open ocean (Tyler 1959).

The occurrence of two peaks of spectral sensitivity in arthropod eyes has been studied closely, especially in the median ocellus of Limulus. Two different photoreceptor cells, UV cells and Visible cells, have been postulated for the median eye of Limulus, based on intracellular recording (Nolte and Brown 1969, Lall 1970). Mote and Goldsmith (1971) have located UV and Visible receptor cells in a single ommatidium of the cockroach compound eye by a technique of recording and marking intracellularly. Although most multiple visual systems revealed in arthropods have pigments that are widely separated spectrally (i.e. UV and Visible), there is one species in which pigment maxima occur close together Euphausia pacifica. Boden and Kampa (1965) discovered a trimodal spectral sensitivity curve for this euphausiid with peaks at 465, 495 and 515 nm, a detail which should be considered in relation to the results of this study.

The earlier study by the author (Eaton 1968) provided some interesting results from an electrophysiological investigation of the nauplius eye. Stainless steel electrodes were used to record ocellar potentials (O.P.'s) from the nauplius eye of Pandalus borealis and film records of O.P.'s were measured. This study was one of the first attempts to detect photoreception in the nauplius eye of decapods by a physiological method. A major peak of maximum spectral sensitivity (λ -max) was found at 475 nm except in one individual, smaller than the others, where it appeared to be at 500 nm,

Further investigation into the significance of the latter finding was impossible at that time but it was suspected that size or age of specimens might have some influence on their λ-max. Compound eyes of Pandalus were not thoroughly investigated at that time either,and an adequate comparison between compound and nauplius eyes could not be made.

The present investigation was undertaken to extend the knowledge of photoreception in Pandalus borealis. Spectral sensitivity of a large size-range of shrimp was measured to determine whether the λ-max of the nauplius eye was related to size, age, or sex. The compound eye was further studied for comparison with nauplius eye results. Both the nauplius and compound eyes of an associated species, Pandalus montagui, were studied for comparison with results from P. borealis. Physiological and behavioral responses of Pandalus zoea to light were measured and results compared with those of adults. The use of more refined techniques, particularly in light control and data processing, allowed greater accuracy in recording and hence a higher level of confidence in results than was possible in the previous study (Eaton 1968).

MATERIALS AND METHODS

(A) Collection and Maintenance of Animals

Pandalus borealis and P. montagui, used in this study, were collected using an otter trawl in 50 to 100 m of water off Blacks Harbor in the Bay of Fundy. Shrimps were transported to the laboratory in seawater-filled styrofoam coolers, where they were transfered to shallow plastic tanks (56 x 36 x 14 cm) and maintained at $5^{o}C$. Each specimen received a 0.5 cm^3 fragment of frozen fish once a week and water was changed once or twice a week. In this manner pandalids can be maintained 12 months in the laboratory in a state of apparent good health.

Collections were carried out in February 1970 and 1971 and provided many gravid females. Eggs started to hatch in the laboratory by mid-March and larvae were present in the tanks during March and April. A number of P. borealis stage I zoea were isolated in a 10 gallon glass aquarium containing aerated seawater. These larvae were fed newly hatched Artemia and some larvae survived as long as 5 months, reaching stage V zoea. Larvae from this population were used in behavioral and electrophysiological experiments.

(B) Histological Preparation

Standard histological methods were used to prepare nauplius eye material for light microscopy. The bec ocellaire, removed from freshly killed animals, was fixed in Bouin's solution and dehydrated in alcohol. The material was then mounted in paraffin, sectioned, stained with aldehyde fuchsin, and counterstained with Halmi's stain. This preparation provided a good resolution of nervous and connective tissue, and although some problems were encountered in sectioning the calcareous exoskeleton, a number of good serial sections were obtained.

Attempts to observe fresh tissue with phase-contrast microscopy were rather unproductive due to the disorientation of the various parts of the nauplius eye during extirpation and mounting of the fresh tissue. These attempts were made to assess the feasibility of using microspectrophotometry to identify pigments in the nauplius eye. Because photoreceptor cells could not be readily identified, microspectrophotometry was not attempted.

(C) Experimental Treatment

(1) Behavioural Studies

(i) Adults

Several attempts were made to establish the photo-induced behavioural responses of adult shrimp. It was thought that by observing the animal's response to light, before and after systematic occlusion of individual photoreceptors, one could ultimately determine the receptor organ or organs involved. Adults of Pandalus borealis were observed under red illumination in aquaria of varying sizes to determine their normal movements, intra-species contact, and behavioural response to food. The response of shrimp to sudden illumination with white light was also examined. A 60 watt incandescent bulb, operated on a variable resistance to allow regulation of intensity, was used in these experiments.

An attempt was made to determine the existence of a photo-tactic response in adult shrimp. Four P. borealis were placed in one of the shallow plastic tanks painted flat black inside and equipped with lighting devices allowing for the illumination of either end from above. The positions of the animals were checked hourly over a period of four days with the tank lighted at alternate ends on alternate days. Animals located in the middle of the tank were considered "no-choice" and were not counted.

Because this method, with non-continuous position counts, might have lacked sufficient resolution to demonstrate a phototactic response,

some method of recording activity continuously was needed. The recording of continuous activity can be approached in two ways; electrically (Heusner and Enright 1966, Fielder and French 1970) and mechanically (Naylor 1958). After an unsuccessful attempt at following Heusner and Enrights' electrical design, a simple mechanical activity meter was devised (Fig. 2) which proved to be similar to that used by Naylor (1958) in his study on Carcinus. The device, a glass plate pivoted at the center and free to move about one centimeter at each end, operated on the principle of a balance. The weight of a shrimp depressed one end of the glass plate and caused a stylus, attached to the plate, to move against a rotating smoked drum. When the shrimp moved to the other end of the glass plate a tilt in that direction moved the stylus away from the drum. In this way one could obtaine a continuous record of the shrimp's position without subjecting either the observer or the animal to a regime of frequant observations. In practice the device was enclosed in a dark box and activity in darkness monitored for 24-36 hrs. A dim white light was then turned on at one end of the aquarium for about 48 hrs. and moved to the other end for another 48 hrs. The amount of time the shrimp spent at each end of the tank during the 5-6 day trial was measured and the percentage time spent at each end determined.

The lack of any overt light response made simple behavioural observation useless in assessing light perception; thus an attempt was made to produce a conditioned response to light using electric shock or food. Electric shock was tried but rejected in lieu of food as a conditioning

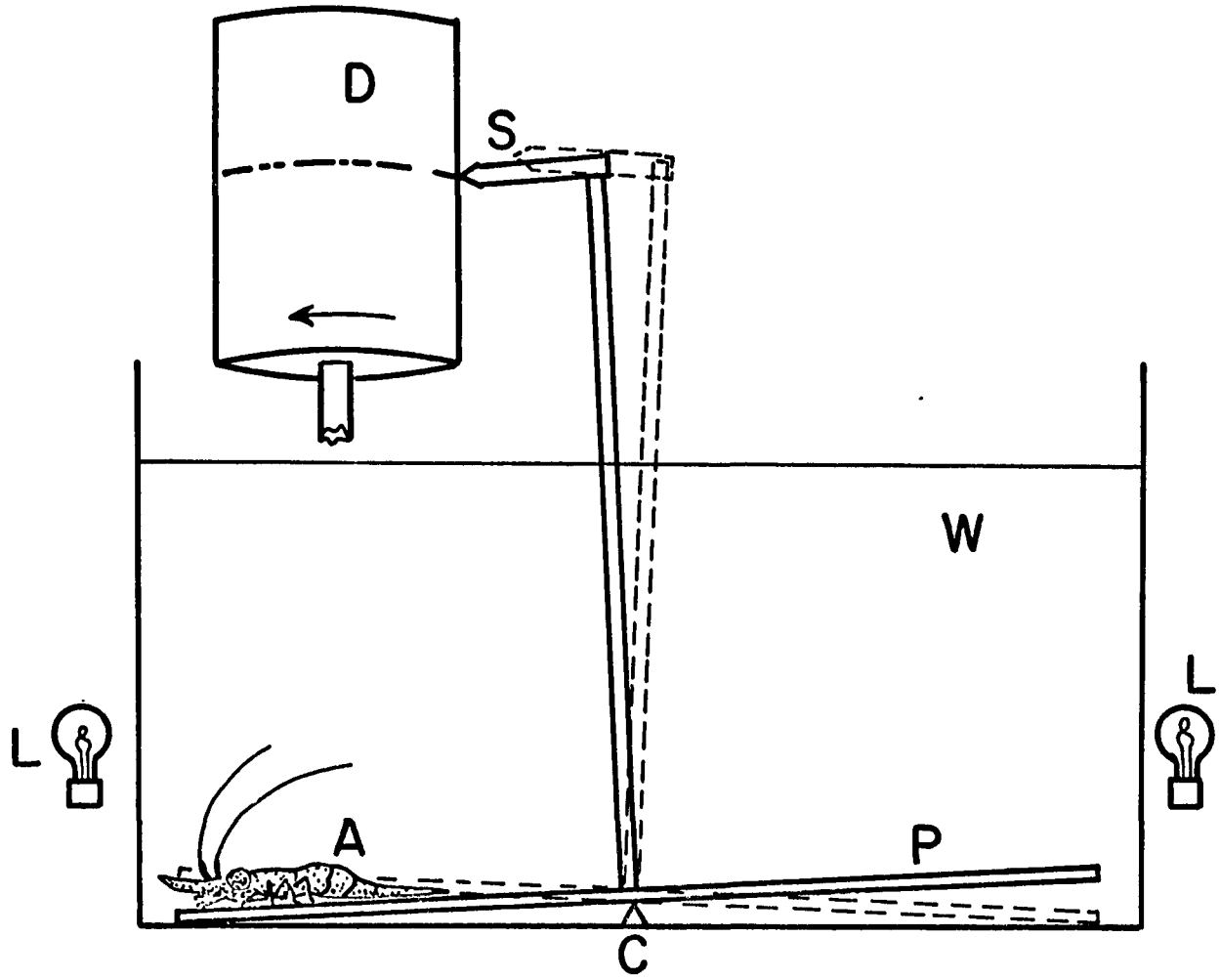


Figure 2

Diagram of activity meter. D, rotating smoked drum; S, stylus; P, glass plate balanced at the center, C; W, small (8 L) glass aquarium filled with sea water; A, experimental animal; L, small 6 V lights.

stimulus. A single adult shrimp in an 8 l aquarium was enclosed in a dark box fitted with red illumination and an observation port. A 40 watt incandescent bulb mounted above the aquarium acted as the light stimulus. The conditioning stimulus, food (a very small fragment of fish), was introduced into the aquarium near the animal and the light turned on at the shrimp's first reaction to the food. The light remained on until the food pellet was contacted. This process was repeated every 5 minutes, 6-12 times, then the effect of the stimulus light without food was tested and the response noted. An attempt was made to train five P. borealis to respond to light by this method.

(ii) Larvae

Larvae of P. borealis, on the other hand, possess a well developed positive phototaxis. This feature was used to examine the spectral response of larvae in three different experimental arrangements.

In the first setup, a small 8 l aquarium was shielded from stray light and illuminated from both ends. A red overhead light provided additional illumination when needed. Ten larvae were released simultaneously at the center of the tank and presented with a choice of swimming toward one of two lights of different spectral quality but of equal intensity. The positions of the larvae were recorded 5 minutes after their release. Three trials were performed for each of the 8 spectral regimes tested (Table 1). One major drawback to this setup was loss of visual contact with larvae due to their small size, their relative transparency, and the low levels of light intensity employed.

Table 1. Aquarium experiment with larvae of P. borealis.

Test	Filters	Distribution	No Choice	Lost	Chi-square	Significance
1	Light Red Deep Red	19 1	--	10	16.20	.001
2	Yellow Orange	15 5	1	9	5.00	.025
3	Light Green Green	6 9	4	12	0.60	N.S.
4	Light Red Blue	0 24	--	6	24.00	.001
5	Light Red Light Red	9 16	4	3	1.96	N.S.
6	Light Green Blue-green	21 41	--	--	6.45	.01
7	Yellow Orange	27 10	--	--	7.81	.005
8	Light Red Light Red	18 22	--	--	0.40	N.S.

The second setup attempted to follow the movements of larvae in a small "Y" chamber where they were presented with the choice of two differently coloured lights of equal intensity. Here again, the animals became hidden and insufficient data were obtained to report in this experiment.

In the third experimental setup (Fig. 3), 5 larvae at a time were placed in a glass tube (59 x .38 cm. diameter) filled with seawater and stoppered at both ends. The tube was clamped in a horizontal position in a dark room at $5^{o}C$ and illuminated by a dim white light placed at the center of the tube. After two minutes, this center light was turned off and the tube was illuminated from the side by two lights of different colour but of equal intensity placed near opposite ends of the tube. An opaque partition, placed between the two lights, reduced the overlap of the lights to a small region near the middle. After 10 minutes, the positions of the larvae were recorded, the tube reversed end for end, and the process repeated 4 or 5 times. This setup proved to be the most successful since the larve were easily seen in the tubes at all times.

In all the behavioural experiments light was provided by one or two 40 watt incandescent bulbs. Different wavelengths were provided by combinations of pyrex glass filters tested for spectral transmission prior to the experiments (Table 2). The intensity of the light transmitted by the filters was measured with an Eppley Thermo-pile and regulated to equalize the intensity of the two lights in use at any one time.

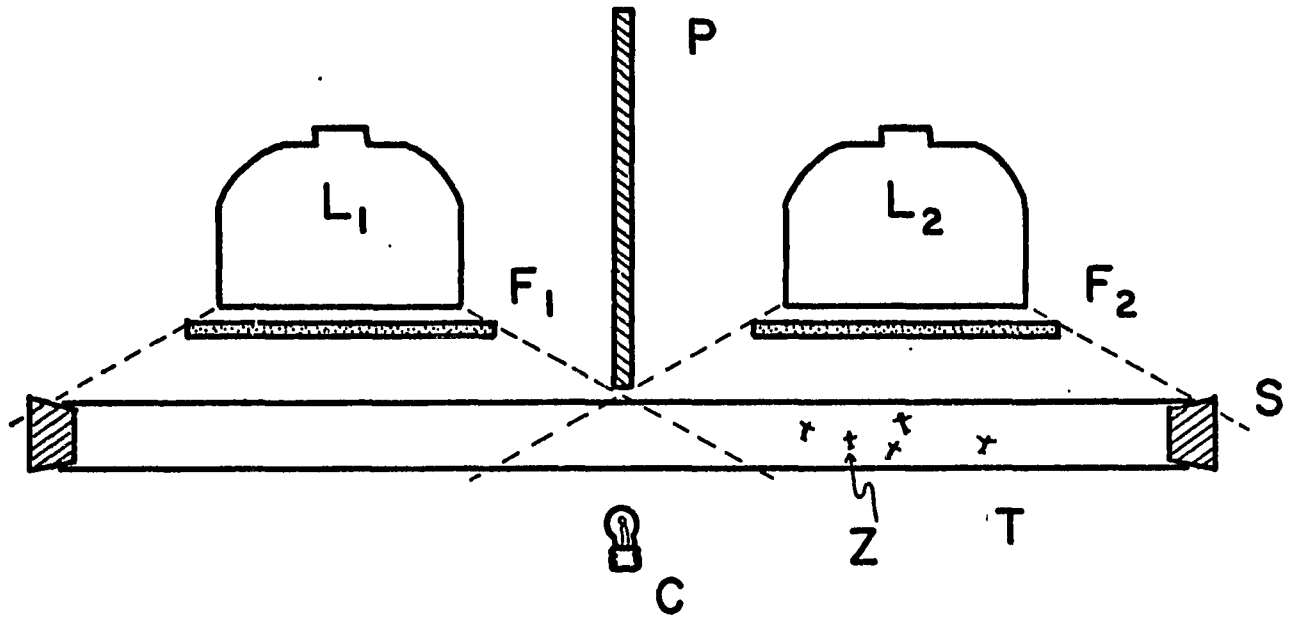


Figure 3

Diagram of selection tube apparatus used to test the preference of pandalid larvae for light of different spectral quality. L_1 and L_2,40 W incandescent lights; P, opaque partition; F_1 and F_2, coloured filters; S, black rubber stopper; T, glass tube (58 x .38 cm diam.); Z, P. borealis larvae; C, centering light.

Table 2. Spectral characteristics of the chromatic glass filters used in behaviour study. Determined by spectrophotometry.

Filters	Range	Peak of Trans. (nm)
Deep Red	(630 ----)	
Light Red	(610 ----)	
Orange	(570 ----)	
Yellow	(550 ----)	
Green	(380 - 560)	500
Light Green	(400 - 570)	500
Blue-green	(360 - 540)	430
Blue	(360 - 500)	430
Yellow + Blue	(540 - 580)	560
Blue-green + Orange	(570 - 590)	580
Blue-green + Blue	(370 - 510)	410

(2) Electrophysiological Studies

The major source of data for this investigation was the analysis of ocellar potentials (O.P.'s) recorded from the nauplius and compound eyes of Pandalus borealis and P. montagui in response to photic stimuli. The basic techniques for stimulation and recording are fairly standard (Graham and Hartland 1935, Wulff and Pandazzi 1951, Ruck 1957, Fuortes 1958, Kennedy 1958), but are described here because the various techniques that exist produce slightly different data.

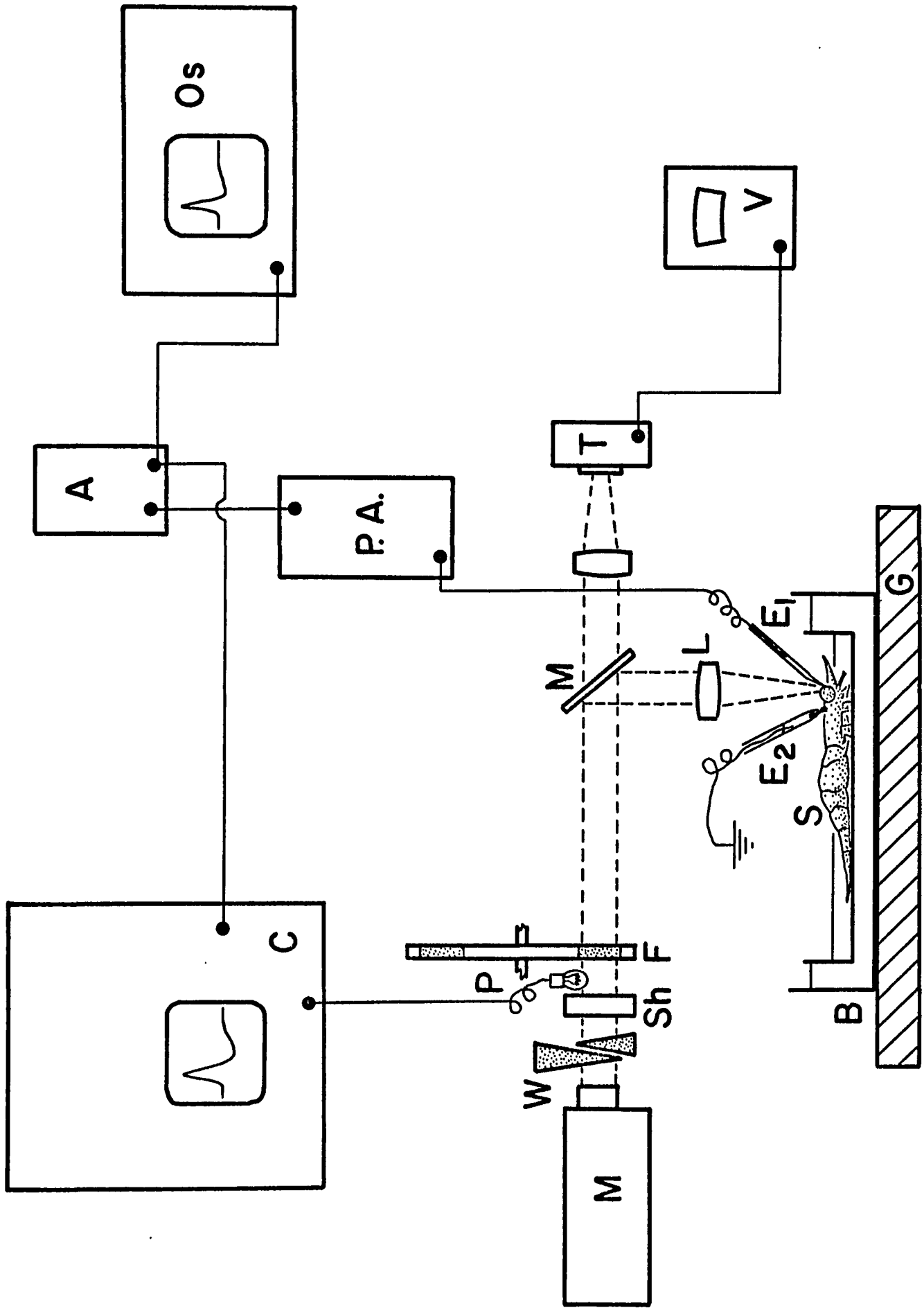
(i) Stimulation System

A Bauch and Lomb grating monochromator (33-86-25-01), fitted with a Xenon lamp, provided monochromatic light over a continuous range from 200-700 nm. The light beam was intercepted near its source by a neutral density optical wedge to allow fine adjustments in intensity. It was then passed through a collimating lens, a Kodak Compier shutter, and a neutral density step filter providing transmittances of 63,50,32,25 and 16 percent plus a reference transmittance. The light beam was reflected by a first-surface mirror through a concentrating lens onto the preparation (Fig. 4).

The light source was calibrated using an Eppley Thermo-pile placed in the position to be occupied by the preparation. The setting of the neutral density wedge was determined for each wavelength so that the quantal energy (intensity) of the light could be maintained constant throughout an experiment, even though different wavelengths were selected.

Figure 4

Diagram of apparatus used in recording O P's from adult shrimp. M, monochromator; W, optical neutral density wedge; Sh, shutter; F, neutral density step filter; P, photocell; M, mirror; L, concentrating lens; E_1, pipette electrode; E_2, reference electrode; S, specimen; B, ice-water bath; G, heavy steel plate; T, thermopile; V, micro voltmeter; P.A.,preamplifier; A, attenuator; Os, oscilloscope; C, computer.


Os
V
T
A
P.A.
G
E_1
L
M
E_2
S
B
C
P
F
Sh
W
M

(ii) Recording System:

One of the more difficult problems in this study was the development of a dependable electrode for recording O.P.'s from the nauplius eye. A wide range of electrodes including stainless steel, Platinum-Iridium, Silver-Silver Chloride, and a commercial electrode manufactured by Transdyne General Corp. (Microtrode 200 and 250 B.) was tried with limited success. Finally, a glass pipette filled with 2 M NaCl was found to be the most dependable type of electrode (Goldsmith 1960, Trevino and Larimer 1969). These were produced by hand pulling 1 mm glass capillary tubing down to a tip diameter of 30-50 μ, and allowing them to fill with 2 M NaCl by capillary action. Electrical contact with the NaCl was achieved with a fine Ag-AgCl wire, freshly prepared prior to each experiment. The Ag-AgCl wire was prepared by washing cleaned silver wire in 95% ethanol and immersing it in a chloride solution. A 1.5 volt current was passed through the solution between the silver wire (+ pole) and a carbon rod (- pole) for 15-30 seconds after which the wire, distinctly coated with a whitish layer, was washed with distilled water.

The reference electrode consisted of a cotton wick held in a tapered glass tube filled with 2 M NaCl. Here again, electrical contact with the NaCl was made with a Ag-AgCl wire. Reference electrodes could be used repeatedly, whereas the pipette electode could only be used once or twice before replacement.

The very small electrical signal (0.05-0.5 mv) picked up from the photoreceptors was amplified 10,000 times by two pre-amplifiers (Tektronix

122) with upper and lower band-pass filters set at 10 kc and 0.8 cps respectively. Because of its variability the amplified signal was led into an attenuator so that maximum amplitudes could be kept below 1.5 V, the maximum voltage accepted by the computer.

The signal, after attenuation, was monitored by an oscilloscope and fed into the imput channel of a LINC 8 Computer (Digital Equipment Corporation) programmed to average a certain number of O.P.'s (usually 8) and calculate standard deviations. The program was started by a photocell which intercepted the light beam distal to the shutter. The data from each series of replicated O.P.'s was recorded on magnetic tape. Upon completion of a series of experimental runs, which would consist of a number of flashes of light of varying intensity and wavelength on one animal, the stored information was read and measured using a READIT program.

(iii) Treatment of Adults

Healthy adults of Pandalus borealis were taken from the holding tanks, secured with Plasticene in a trough partly filled with seawater, and placed in an ice-water bath (Fig. 4). Compound eyes and rostrum were removed and the dorsal surface of the bec ocellaire was dissected to expose the nauplius eye. A small cut was made in the carapace just posterior to the margin of the compound eye socket to allow implantation of the reference electrode, while the active electrode was manipulated into contact with the anterior lateral part of the nauplius eye. During experiments, either the entire room was darkened or the preparation was enclosed in a dark box.

Animals were subjected to two experimental procedures: one in which the intensity of the stimulating light remained constant while the wavelength was changed ($\Delta\lambda$), and the other in which intensity was changed 5 times for each given wavelength (Δ I) (Goldsmith 1960, Kennedy and Bruno 1961). In both procedures a reference unit at a set intensity and wavelength initiated each run, and was repeated every 6th unit allowing the intervening units to be related to one another in case of drift in electrode contact. One unit of a run usually consisted of eight 10 msec. flashes of the stimulus light separated by one-second time intervals. Nine seconds elapsed between one unit and another while the computer calculated and stored the recorded information during which time either wavelength, intensity, or both were changed in preparation for the next unit. Each photoreceptor examined was exposed to 3 or 4 runs using the $\Delta\lambda$ method and one or two runs using the Δ I method.

When compound eyes were examined the excised eye was placed in the depression made at the anterior end of a shrimp by removal of the dorsal surface of the bec ocellaire. The cut eyestalk was directed posteriorly and placed in contact with the refrence electrode. The active electrode was placed against the corneal surface which sometimes had to be scraped or punctured before electrical contact could be effected. Compound eyes were stimulated following the same procedure used for nauplius eyes.

(iv) Treatment of Larvae

Three stage I zoea of P. borealis were subjected to experimental treatment with the electrophysiological setup. A larva was placed on the surface of a seawater-agar pad chilled to 3^{o}C and cooled during

experimentation by an ice-water bath (Fig. 5). A fine No. 00 insect pin was used to remove the rostrum and anterior dorsal carapace to expose the nauplius eye. No attempt was made to remove the compound eye.

A reference electrode was placed near the abdomen of the larva while the active electrode was manipulated into contact with the nauplius eye or, as in one case, the compound eye. Receptor stimulation and treatment of resultant O.P.'s were identical to that described for adult shrimp.

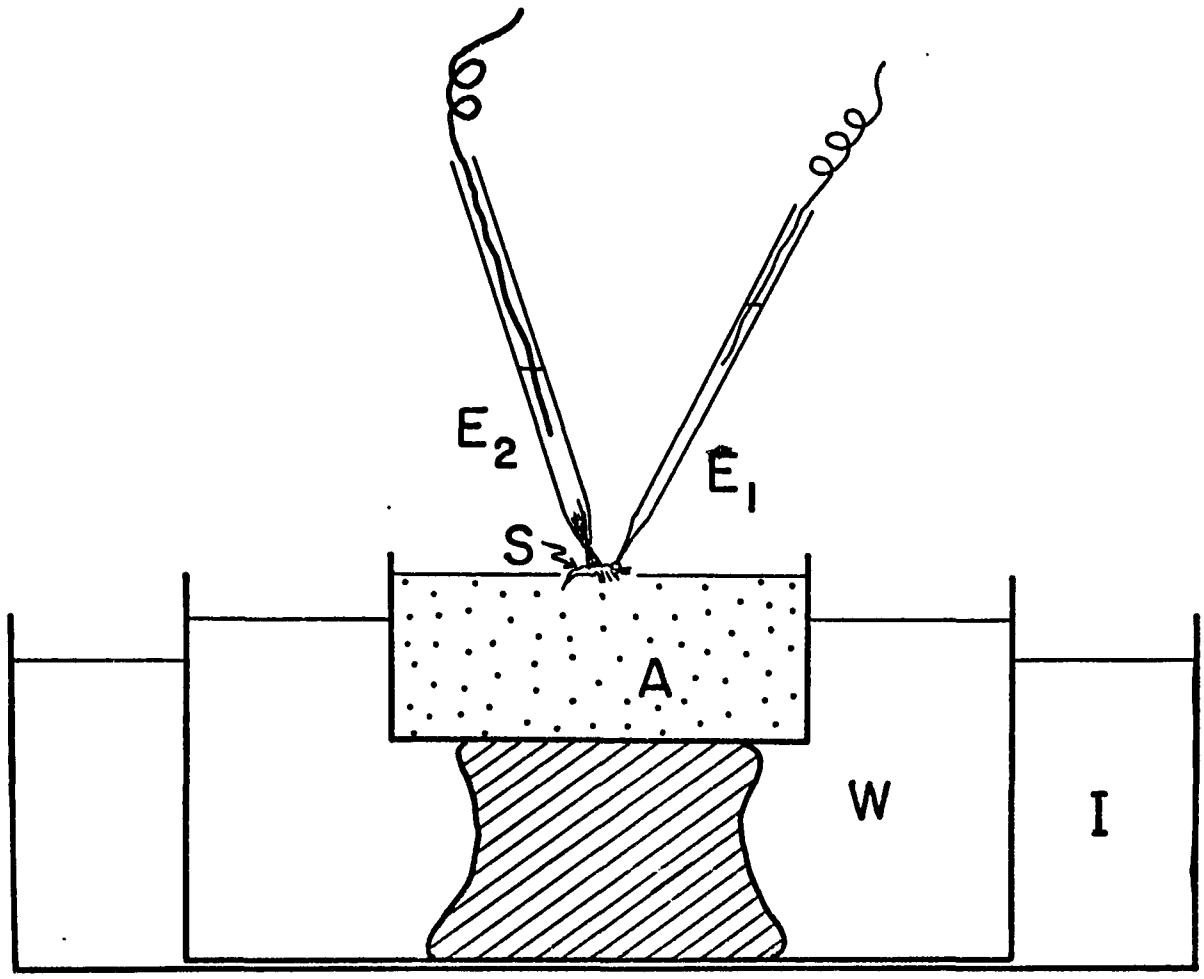


Figure 5

Diagram of apparatus for recording O P's from larval pandalids. E_1, active electrode (pipette); E_2, reference electrode (cotton wick); A, agar-sea water gel; W, water bath; I, ice water cooling bath; S, specimen.

RESULTS

(A) Behavioural Study:

(1) Adults

The activity of adult shrimp was erratic and unpredictable except in the presence of food. When adult Pandalus borealis were exposed to sudden illumination by white light at various intensities a twitching of the antennules and, occasionally, leg movements were observed, but the occurrence of these reactions was not predictable.

Attempts to demonstrate phototaxis of adults were also unsuccessful. Animals tested in a plastic tank (56 x 36 x 14 cm), lighted at one end or the other, showed a more or less random distribution with no significant selection for the lighted or darkened end. The activity meter showed similar results for three specimens tested, two with white light and one with blue light. There was no significant preference for either the lighted or darkened end of the aquarium; also, the previous photo-orientation experiments in the larger tanks would tend to confirm the lack of an obvious phototaxis.

The response of pandalids suddenly exposed to food in the water is quite spectacular. Immediately upon detection of the food a rather frantic search is initiated, characterized by rapid walking movements and searching activity of the feeding appendages. This continues until the food is contacted where-upon the contacting leg pulls the food toward the body by a rapid flexure. This behavioural pattern was consistent as

long as the animals were moderately hungry and it was hoped that this easily detectable response could be conditioned by some sort of photic exposure. After 10-15 synchronous exposures to food and sudden illumination, with good feeding responses each time, the shrimp was exposed to the light alone. Although the reaction to the light may have been slightly more overt than in previous experiments using light exposure alone, the response was far from that exhibited upon exposure to food. After several such attempts with similar disappointing results, attempts to use adults for behavioural experiments were discontinued.

(2) Larvae

Observation of newly hatched Pandalus larvae in the tanks with adults, and larvae kept in a separate tank, revealed the presence of a definite positive phototaxis. Larvae were invariably attracted, either to the regular lights in the 5°C cold room, or to any observation light placed near the aquarium. A light turned on close to the aquarium elicited a rapid swimming movement in the direction of the light and resulted in clumping of larvae at the position of the light. This characteristic of pandalid larvae was used to examine their spectral sensitivity.

The aquarium setup described above was designed to test the ability of larvae to discriminate between lights of different spectral quality but of equal intensity. The results of 8 tests carried out in the aquarium setup are shown in Table 1. The larvae appear to avoid longer wavelengths and move toward shorter wavelengths in the area of blue or blue-green.

Unfortunately,the tendency of larvae to seek out corners and to hide necessitated the search for a more accurate method of testing their spectral selection.

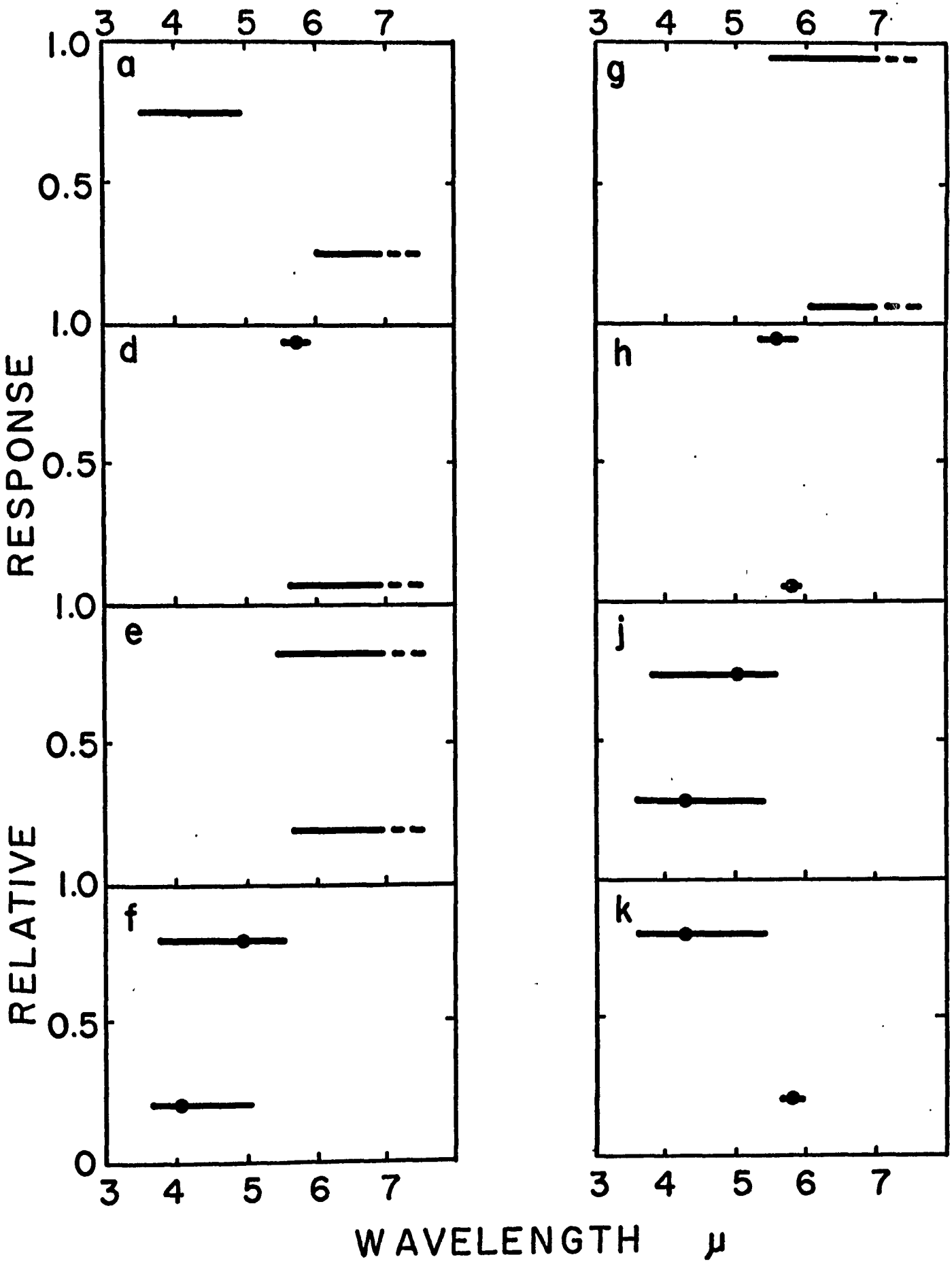
The "selection tube experiment" avoided the problem of larvae becoming hidden and eliminated the possibility of light reflection which was an undesirable feature of the glass aquarium. The results of this experiment have been tabulated (Table 3) and are represented graphically (Fig. 6). Again, a tendency to move toward blue or blue-green is evident, with a peak of maximum positive phototaxis probably between 500 and 520 nm. It is impossible to describe any relative preference for other wavelengths i.e. to construct a spectral sensitivity curve from these results. Table (2) should be consulted for information on the spectral ranges and maxima of the filters used in these experiments.

Table 3. Selection tube experiment on larvae of P. borealis.

Test No.	Filters	Distribution of Animals	Chi-square	Significance
a	Light Red	5	5.00	.025
	Blue	15		
b	Yellow	17	0.86	N.S.
	Orange	12		
c	Light Red	11	0.36	N.S.
	Light Red	14		
d	Orange	1	16.20	.001
	Blue-green + Orange	19		
e	Yellow	20	10.28	.0015
	Orange	4		
f	Green	16	7.20	.007
	Blue-green + Blue	4		
g	Yellow	37	30.65	.001
	Light Red	2		
h	Blue-green + Orange	1	16.20	.001
	Yellow + Blue	19		
i	Light Green	25	0.16	N.S.
	Blue-green	23		
j	Blue-green	8	5.66	.019
	Green	21		
k	Blue-green	32	14.40	.001
	Blue-green + Orange	8		
l	white light	29	8.10	.004
	dark	11		

Figure 6.

Graphic representation of the results from the "selection tube experiment". The results of each significant test (see Table 3.) are displayed. Heavy black bars indicate the spectral range covered by the filters used. Where dots appear in the bars they indicate the peak of transmittance. The horizontal positions of the bars indicate the relative number of animals which chose that particular colour.


3 4 5 6 7
1.0
0.5
1.0
a
g
d
h
e
j
f
k
RESPONSE
RELATIVE
0.5
1.0
0.5
0
3 4 5 6 7
3 4 5 6 7
WAVELENGTH μ

(B) Electrophysiology Results

(1) The Ocellar Potential

Considerable time was spent in the search for a dependable electrode system before the present NaCl filled pipettes were chosen (Chapman and Lall 1967). A number of examples of the O.P. recorded using the system described in the previous section are seen in Fig. 7.

In most recordings from nauplius and compound eyes the O.P. was characterized by a rapid negative deflection of the ocilloscope trace following the onset of the light pulse, followed by a slightly more gradual return to zero and a small positive deflection. The trace sometimes swung slightly negative before gradually returning to zero again. The shape of the various components of the O.P. depended quite strongly on the position of the electrode, the duration of the stimulus flash, and the state of adaptation, whereas the stimulus wavelength and intensity (within limits) appeared to influence only the amplitude of the O.P.

In using the O.P. to determine an eye's sensitivity to light it is conventional to measure the amplitude of the initial negative wave of the O.P. and use this measurement as an indication of the eye's response (Goldsmith 1960, Chapman and Lall 1967, Stratten and Ogden 1971). The amplitude of the O.P. is influenced by the degree of dark adaptation of the eye, thus, to minimize this effect background illumination was reduced and stimulus duration and interval were held constant throughout. Electrode position also influences the shape and amplitude of the O.P., and although a single position was maintained throughout a run, minute

Figure 7.

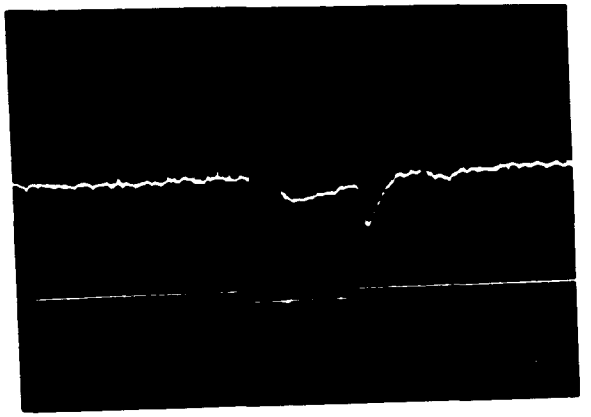
(A) A single O.P. resulting from a 1 second light pulse. "on" and "off" components of the response can be easily distinguished. Time scale, 0.5 sec./large division. Amplitude scale, 0.1 mV/large vertical division. Upward deflection indicates electrode negative in all photographs. Lower trace shows the response of a photocell to the light pulse.

(B) Ten O.P.'s superimposed on one photograph. Light pulse (lower trace) 1/50 sec. every 1 sec. Time scale, 20 msec/large division. Amplitude scale, 0.1 mV/large division.

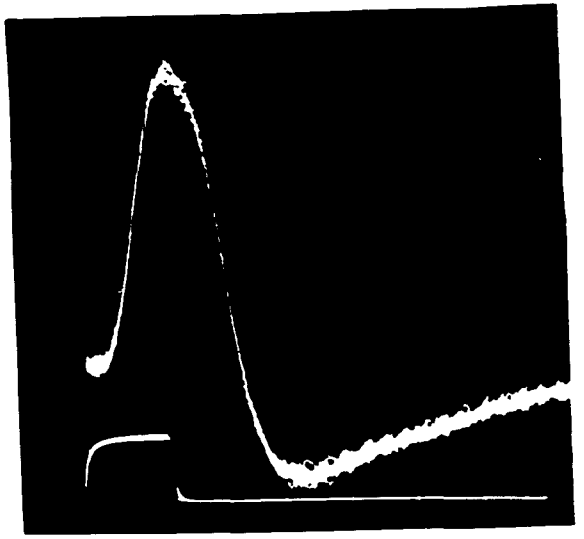
(C) Computer display of data stored on magnetic tape. Solid line represents the mean of 8 O.P.'s. Dots indicate $\pm$ 2 standard deviations. Numbers in upper right corner indicate the position of a cursor used to measure position and amplitude of peaks. Numbers at center bottom indicate the block of magnetic tape at which the data are stored.

(D) A series of O.P.'s recorded by exposing the nauplius eye of an adult of P. borealis to pulses of light of increasing intensity. Light intensities used are: (top to bottom) 0.5×10^{13}, 2.5×10^{13}, 5×10^{13}, 10×10^{13}, and 20×10^{13} quanta/sec./cm^2. Time scale, 50 msec/large division. Amplitude scale, 0.1 mV/large division.

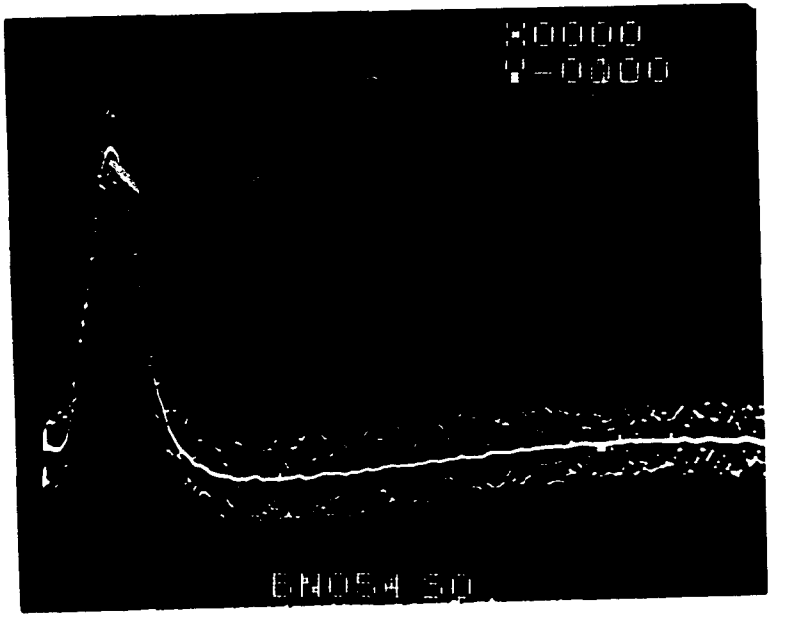
(E) A series of O.P.'s recorded from a larva of P. borealis which was exposed to light pulses of 400, 450, 500, and 550 nm, top to bottom. Particularly notable in this instance is the downward or positive peak of the O.P., opposite in polarity to the O.P. usually recorded.



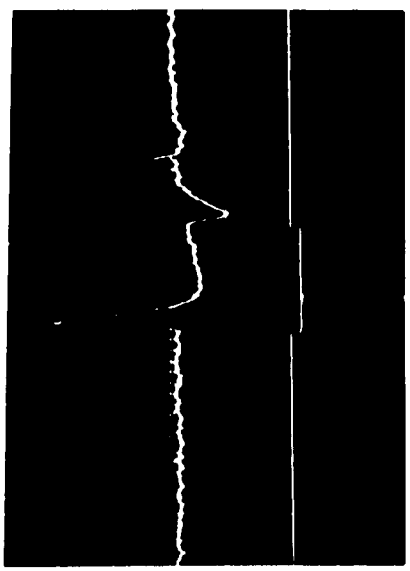
A



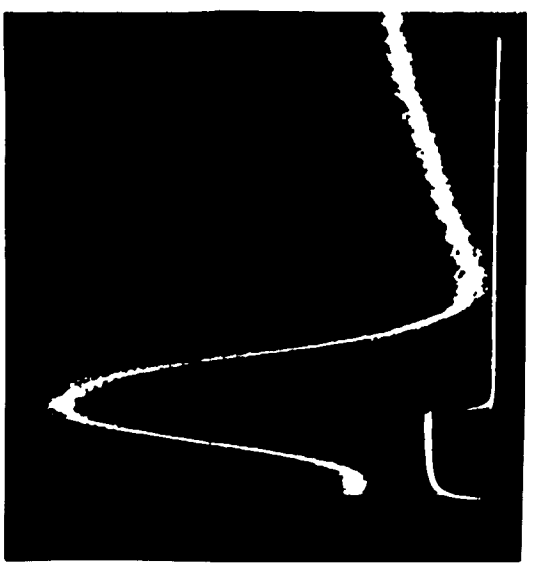
B



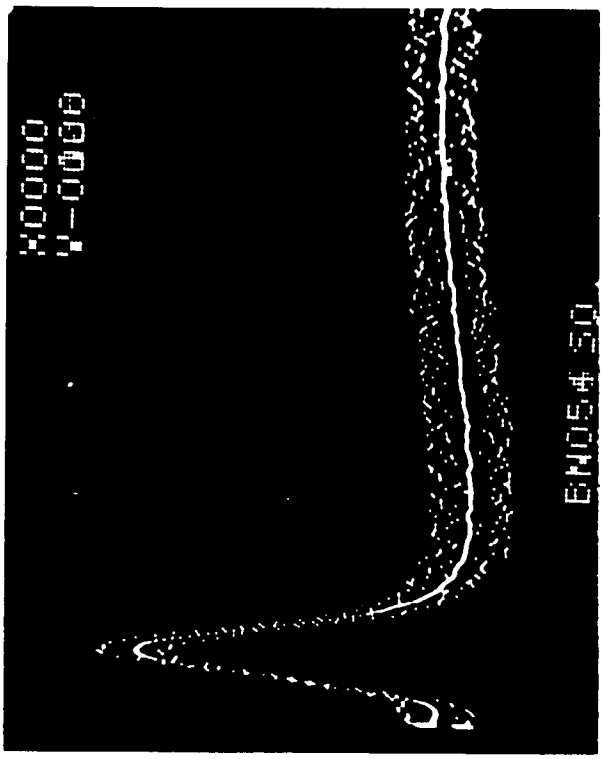
C



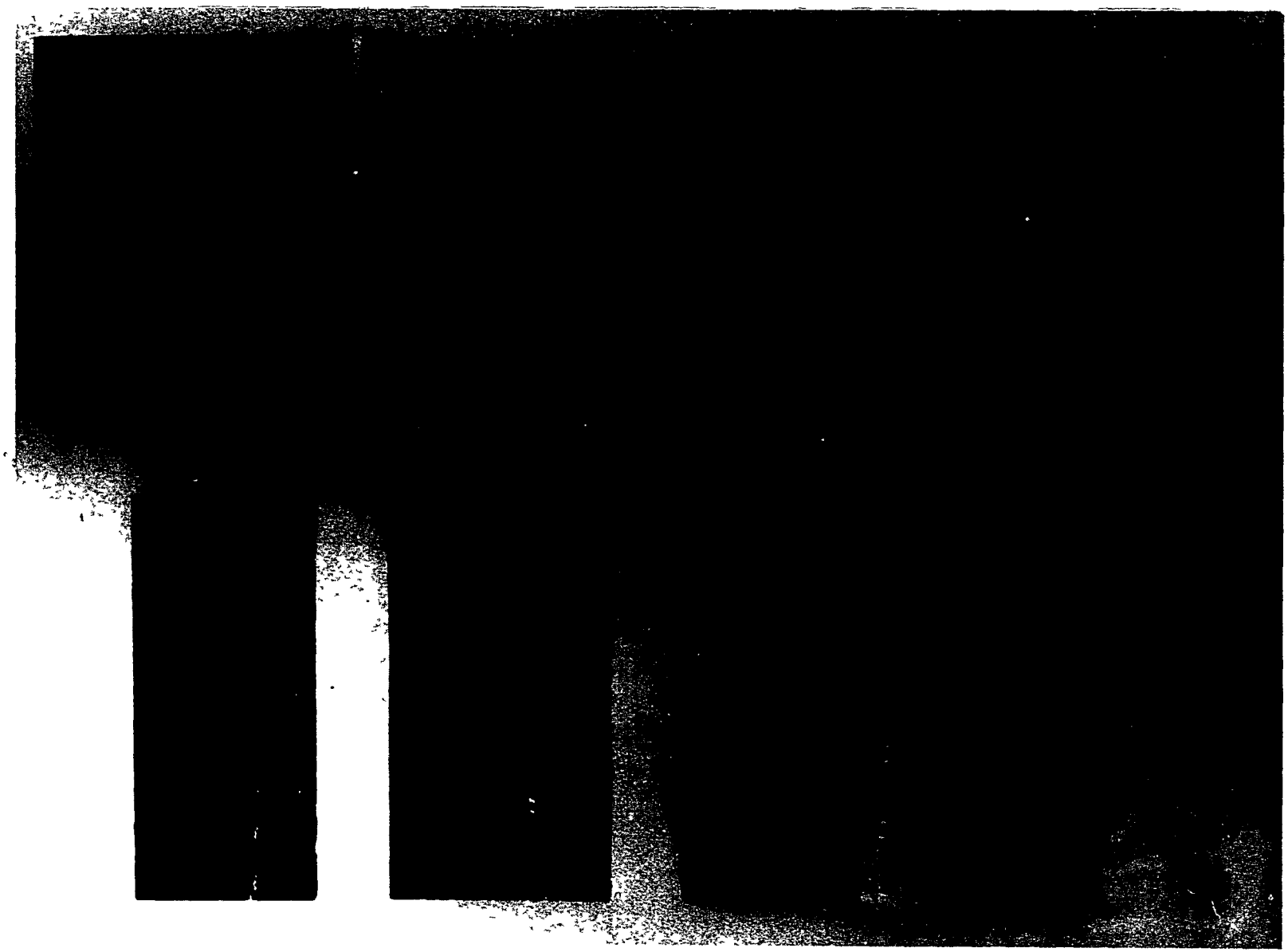
A

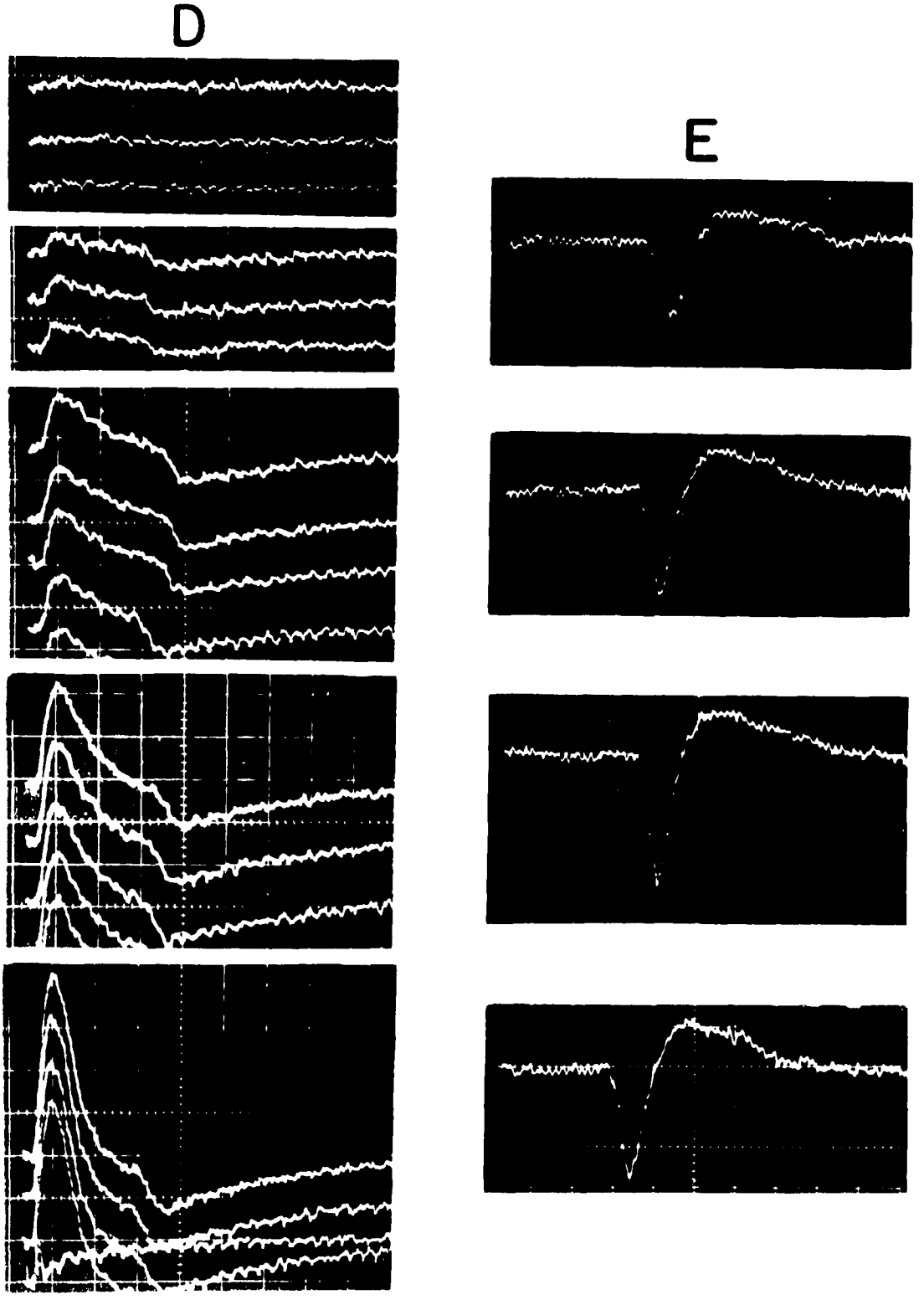


B



C




D
E

changes in electrode/tissue contact resulted in some drifting in O.P. amplitude. In general, extraneous influences on the O.P. of the eye were eliminated or held constant so that it could be assumed that any differences in amplitude arose from the eye's discrimination of wavelength and intensity.

(2) Treatment of Data

Raw data, resulting from the electrophysiological procedure and measured from the storage tape, consisted of groups of 5 measurements each, separated by a reference measurement. During any one run the reference measurements were found to vary due to the continuously fluctuating electrode pick up mentioned above. A series of consecutive exposures at a constant wavelength and intensity showed that electrode sensitivity drift between reference measurements was approximately linear; thus a corresponding reference value could be calculated for each of the 5 interim experimental measurements by dividing the difference between any two adjoining reference measurements by 6 and adding or subtracting, depending on direction of drift, the appropriate fraction of the difference to or from the first of the two references. All the experimental measurements could then be related to one another on an equal basis by dividing each measurement by its calculated reference. Thus the reference value for any one run was 0.1 and the experimental measurements were proportional to this value.

From this point on, the data obtained from the two methods ($\Delta\lambda$, and ΔI) were treated quite differently. The variability of

amplitudes from one experimental animal to another made it necessary to show results from each $\Delta\lambda$ experiment as proportions of the maximum response of each run. This problem was encountered by Stratten and Ogden (1971) and handled by them in the same way.

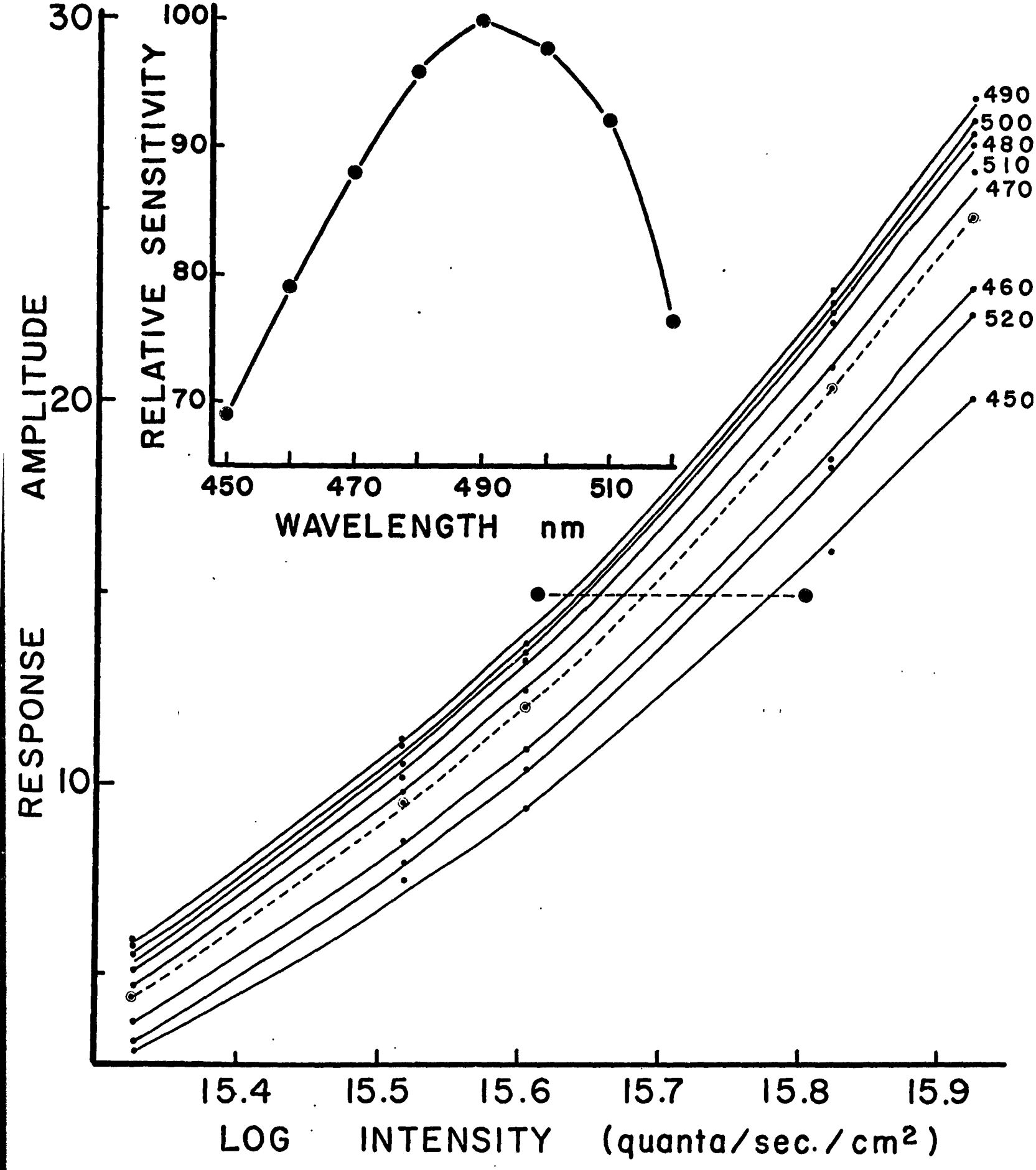
The Δ I method produced sets of measurements representing 5 different intensities for each wavelength tested (Fig. 8). These measurements were plotted against the log of intensity, and for each wavelength a curve was fitted by eye to the values for that particular wavelength. A curve drawn through the average of all the values was used as a pattern in drawing the eye-fitted curves. Stratten and Ogden (1971) employed a similar procedure in their graphic representation of λ/I curves for the ocellar nerve of the barnacle.

Relative spectral sensitivity of the eye was established by using the λ/I curves to indicate the number of quanta necessary to elicit a response of a given magnitude for each wavelength. This was done by taking the reciprocal of the log I for each λ/I curve where it crossed a chosen response amplitude and representing these values as proportions of the maximum value. In other words, the wavelength that required the least quanta to produce the chosen response was represented as 100% while other wavelengths, requiring more quanta to produce the same response, ranged somewhere below 100% (Fig. 8 insert).

According to Kennedy and Bruno (1961) the $\Delta\lambda$ method produces a relationship between wavelength and response amplitude which is not a function of overall sensitivity, whereas spectral sensitivity can be determined using the Δ I method. Both methods were used here, but since

Figure 8.

An example of data from an experiment using the variable intensity method (ΔI). Eight wavelength/intensity curves are shown relating retinal response to stimulus energy for the eight wavelengths indicated. The broken curve was constructed from the average of all the data shown and was used to determine the shape of all the other curves (see text). The insert shows a spectral sensitivity curve constructed by plotting the reciprocals of the quanta/sec./cm^2, required to produce a certain response (broaken horizontal line), as proportions of the highest value (i.e. 490 nm in this case).


30
20
10
RESPONSE AMPLITUDE
100
90
80
70
RELATIVE SENSITIVITY
450
470
490
510
WAVELENGTH nm
490
500
480
510
470
460
520
450
15.4
15.5
15.6
15.7
15.8
15.9
LOG INTENSITY (quanta/sec./cm²)

very little difference was found between results obtained with them these results were combined and all subjected to the same statistical treatment which will be discussed later.

(3) Pandalus borealis

Examination of the data for the nauplius eye of Pandalus borealis revealed a discrepancy in the position of λ-max. Some specimens had λ-max at 490 nm while others peaked at 510 nm and a few at 500 nm. Previous research by the author had revealed the occurrence of a λ-max displaced toward the longer wavelengths for a shrimp which was considerably smaller than the others. At that time it was impossible to examine a possible size / λ-max relationship, however this was one of the intensions of the present investigation.

A relationship between size and λ-max was noted. Most P. borealis less than 20 mm (carapace length) had λ-max 510, whereas most larger shrimp had λ-max 490 nm (Table 4). This relationship did not hold true in a number of cases however, so another hypothesis was advanced to explain the λ-max discrepancy. Due to the protandrous hermaphroditic nature of pandalid shrimp, size, age, and sex are closely related (Allen 1959, Butler 1964, Couture and Trudel 1969); thus, a correlation was looked for between sex and λ-max instead of size and λ-max. The sex of experimental animals was determined by the presence or absence of the male copulatory organ on the 2nd pleopod. Specimens with partially degenerated male organs were classified as intersex. The proportions of

Table 4. Data on the size, sex and λ-max of all the adults of *P. borealis* used in this study.

Size (mm) carapace lgth.	Sex	Occurrences of λ-max (nm) 490	500	510
Nauplius Eye				
17.3	male	1		1
18.0	intersex	3		
18.7	male	1	3	2
19.0	intersex	3		
23.0	intersex	2	4	
24.8	female	1		
25.7	female	3		
26.0	female	5	1	
26.5	female	3	1	
27.0	female	2	1	
27.9	female	2	1	
Compound Eye				
16.6	male			4
17.3	male		3	2
17.8	intersex		2	2
18.0	intersex		3	2
19.0	intersex		2	2
19.0	male	2	1	
26.2	female	1	1	
27.9	female	2		1
	Totals	31	23	16

P.borealis in each group; male, female, and intersex, were then plotted against λ-max for nauplius and compound eyes (Fig. 9). A larger proportion of males had λ-max 510 and 500 while the λ-max of a much larger proportion of females was 490, with none occuring at 510 nm. This pattern held true for the nauplius and compound eyes.

It is obvious from the results that the peaks of the spectral sensitivity curves are rather broad, and an actual λ-max cannot be stated precisely. Curves for the compound and nauplius eyes of males of P. borealis have a peak mean at 510 nm, however, the actual λ-max might fall between 500 and 515 nm. Similarly, for females of P. borealis, the λ-max might fall between 485 and 500 nm. For convenience, however, the peak means alone will be used when refering to λ-max.

The Mann-Whitney U test (Sokal and Rohlf 1969) was applied to the data for the nauplius and compound eyes of males and females of P. borealis at 490 and 510 nm. The object of this test was to determine whether or not a significant difference existed between males and females at the peaks of the spectral sensitivity curves, and to test the difference between 490 and 510 nm in individual curves. The results of this test are tabulated in Table 5.

All the data accumulated from the electrophysiological experiments to determine the spectral sensitivity of the nauplius and compound eyes existed in the form of percentages, some of which were values derived from the λ/I curves of the ΔI method. Since all these values were to be averaged, and the means determined for each wavelength, it was necessary to preform an arcsine transformation on the data (Mather 1943, Schefler 1969, Sokal and Rholf 1969). Means derived from the transformed data were

converted back to proportions and are displayed as percentages. Here again the data from the three groups, male female and intersex, were treated separately and appear as three curves in each of the Figures 10 and 11 for the nauplius and compound eyes. 95% confinence limits were calculated for the data used in constructing the curves shown in Figures 10 and 11 (P. borealis), 13 (P. montagui), and 14 (larvae of P. borealis) (see Table 6). These limits were determined using the transformed data and then converted back to percent. To illustrate the distribution of the data, the spectral sensitivity curve for the nauplius eye of females of P. borealis has been drawn with the the range of the data displayed. (Fig. 12).

Figure 9

Graphic representation of the distribution of λ-max's of all the adults of P. borealis examined in this study. Results are proportioned for each sex group -- males, females, and intersex, and are divided into nauplius eye and compound eye. Chi-square tests of the significance of differences of the various groups appear in Table 5.

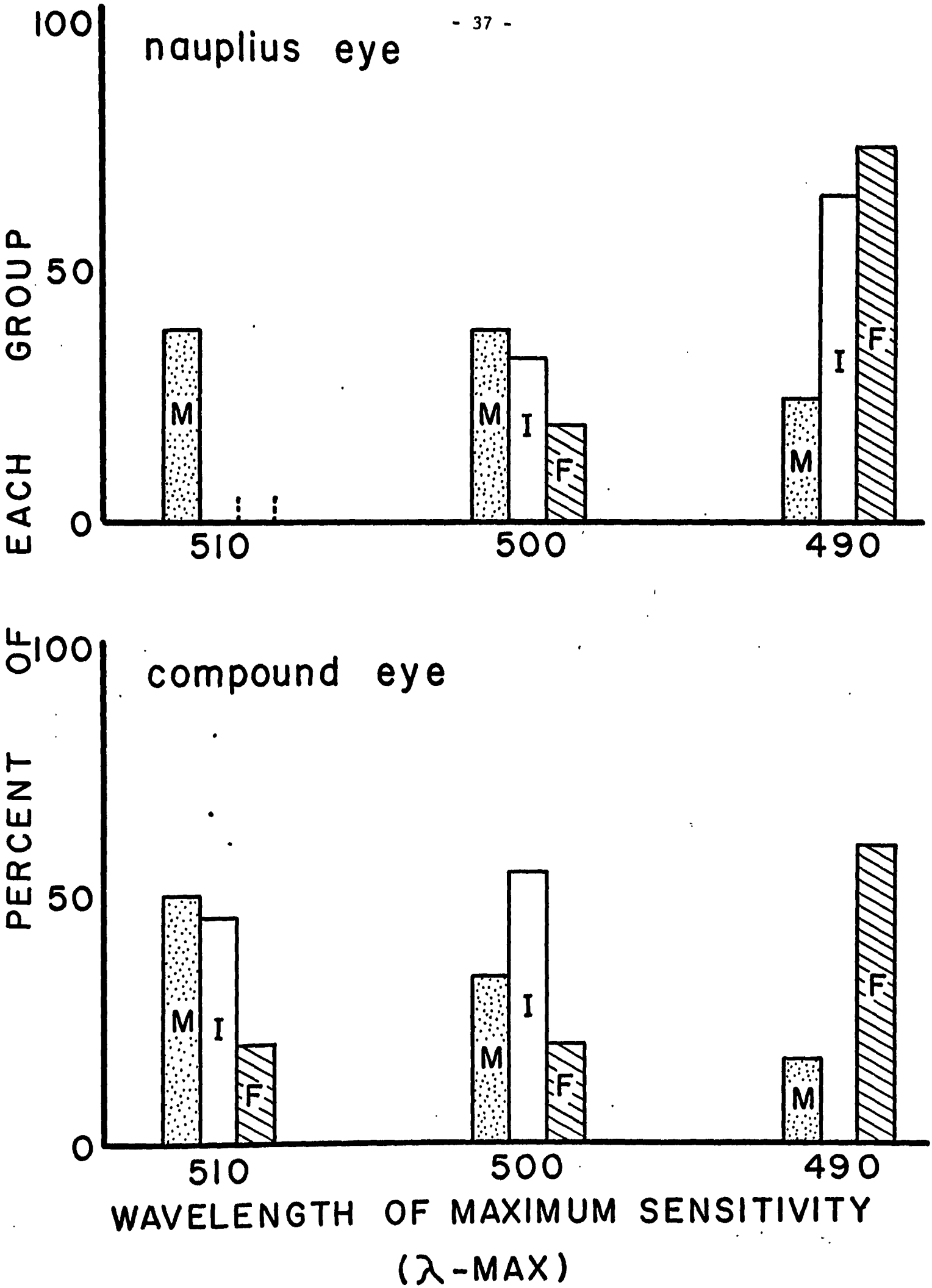

nauplius eye
100
50
0
PERCENT OF EACH GROUP
M
M
I
F
M
I
F
510
500
490
compound eye
100
50
0
PERCENT OF
M
I
F
M
I
F
M
F
510
500
490
WAVELENGTH OF MAXIMUM SENSITIVITY
(λ-MAX)

Table 5. Mann-Whitney U test on the data used to construct the spectral sensitivity curves for males and females of P. borealis. The curves are tested at the critical peak points.

Components tested	n_1	n_2	U value	Probability
Nauplius eye				
Females vs. Males (at 490nm)	20	8	113	0.05
Females vs. Males (at 510nm)	20	8	140	0.001
Males 490nm vs. 510nm	8	8	38.5	N.S.
Females 490nm vs. 510nm	20	18	354.5	0.001
Compound eye				
Males vs. Females (at490nm)	10	5	35.5	0.10
Males vs. Females (at 510nm)	10	4	32	0.05 0.10
Males 490 vs. 510nm	10	10	75.5	0.05
Females 490nm vs. 510nm	5	4	13.5	N.S.
Females 490nm vs. 480nm	5	5	22	0.05

Probability indicates the percentage probability that the observed differences in the components tested occured by chance.

Table 6. Display of data means and the upper and lower 95% confidence limits for larvae and adults of P. borealis , and adults of P. montagui. Calculations were preformed in the arcsin transform and are converted back to percentages.

Wavelength (nm)	Mean (%)	Upper limit	Lower limit
Pandalus borealis			
Nauplius Eye			
(Intersexes)			
450	72.17	77.52	66.44
460	80.64	93.30	66.61
470	90.83	98.47	77.52
480	96.95	100.00	87.74
490	99.63	100.00	93.98
500	99.20	100.00	92.68
510	96.46	100.00	86.33
520	83.72	88.19	78.68
(Females)			
450	69.91	75.60	63.78
460	79.73	89.08	68.41
470	87.72	97.33	72.39
480	93.81	99.62	81.74
490	99.57	100.00	94.15
500	98.00	100.00	89.40
510	94.90	97.39	91.65
520	80.92	93.47	63.78
(Males)			
450	71.93	79.53	63.45
460	80.76	86.69	74.09
470	90.65	94.71	85.60
480	93.83	96.92	89.61
490	97.94	99.67	94.63
500	98.33	99.78	95.46
510	98.88	99.93	96.68
520	87.31	93.53	75.60
Compound Eye			
(Intersexes)			
450	65.79	73.63	57.48
460	73.99	79.81	67.43
470	84.97	89.08	80.37
480	89.78	98.06	76.05
490	96.18	99.98	86.57
500	99.68	100.00	94.94
510	99.41	99.99	97.92
520	88.48	97.55	74.09

Table 6. (Contd.)

Wavelength	Mean (%)	Upper limit	Lower limit
(Females)			
450	66.49	75.90	56.44
460	76.53	83.84	68.24
470	86.85	92.40	79.95
480	93.43	97.04	88.41
490	98.77	100.00	95.39
500	98.61	99.76	96.23
510	97.24	99.71	98.83
520	90.55	94.47	85.60
(Males)			
450	69.80	77.52	61.42
460	79.03	84.48	73.01
470	84.86	90.24	78.68
480	91.89	95.61	87.39
490	97.11	98.80	91.84
500	98.72	99.81	96.62
510	99.25	99.98	85.23
520	89.94	93.73	85.23
Larvae			
450	76.36	85.48	65.95
460	78.49	86.33	69.38
470	86.83	92.95	79.11
480	93.43	97.22	88.08
490	98.05	99.84	94.15
500	97.59	99.46	94.15
510	99.04	99.93	97.16
520	86.74	93.04	78.68
Pandalus montagui			
Nauplius Eye			
450	75.39	82.01	68.08
460	83.11	87.62	77.96
470	93.36	96.92	88.64
480	96.75	99.08	93.21
490	99.90	100.00	98.20
500	98.28	100.00	90.96
510	94.76	98.34	89.40
520	81.26	87.85	73.63
Compound Eye			
450	60.05	68.24	51.57
460	68.04	73.63	62.10
470	76.88	83.20	70.02
480	83.73	89.51	76.94
490	91.44	96.03	85.23
500	95.41	97.97	91.84
510	99.90	100.00	98.01
520	90.83	94.47	86.33

Figure 10.

Spectral sensitivity curves for the nauplius eye of males, females, and intersexes of P. borealis. Numbers of observations are: males 8, females 20, and intersexes 11.

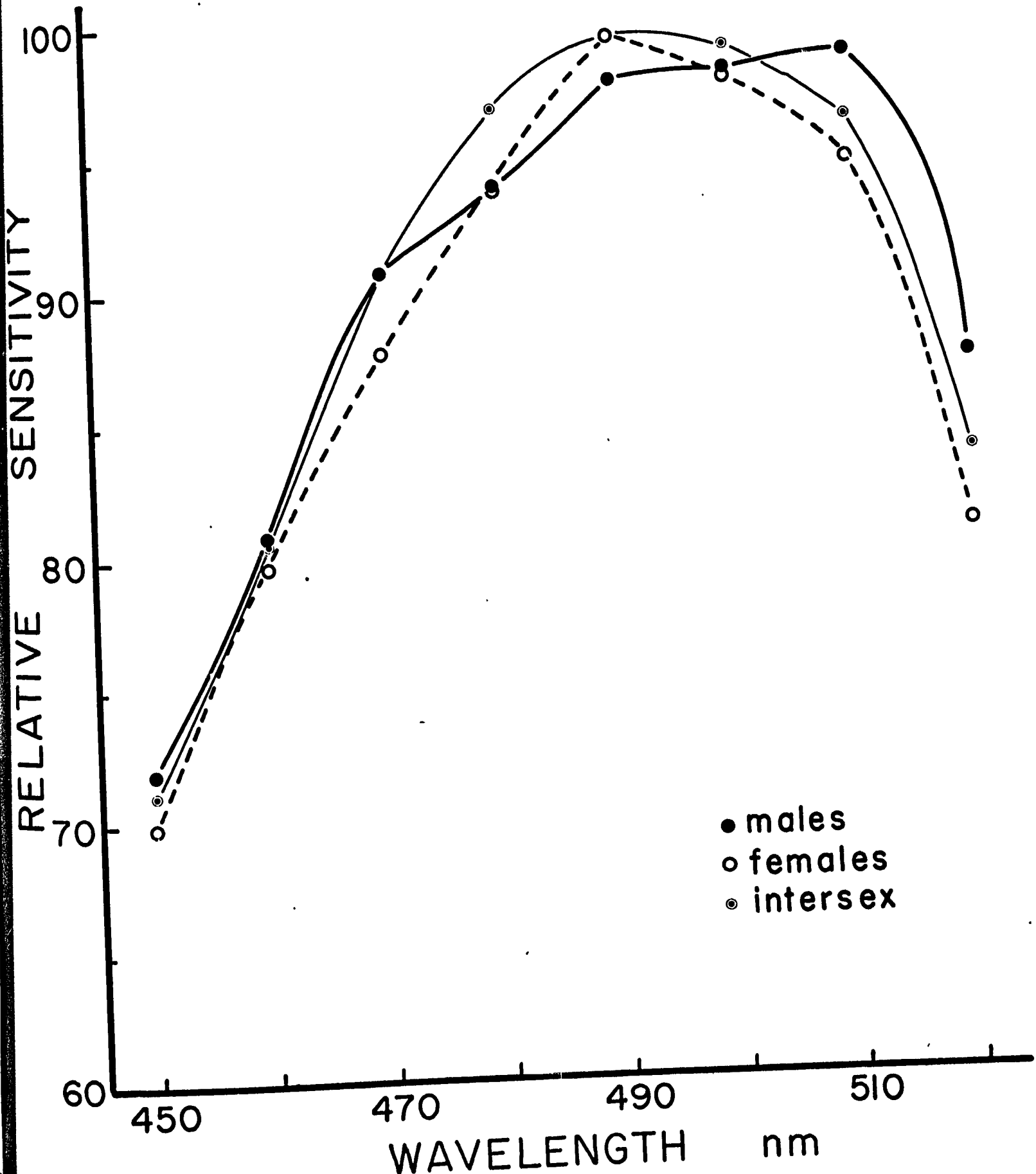
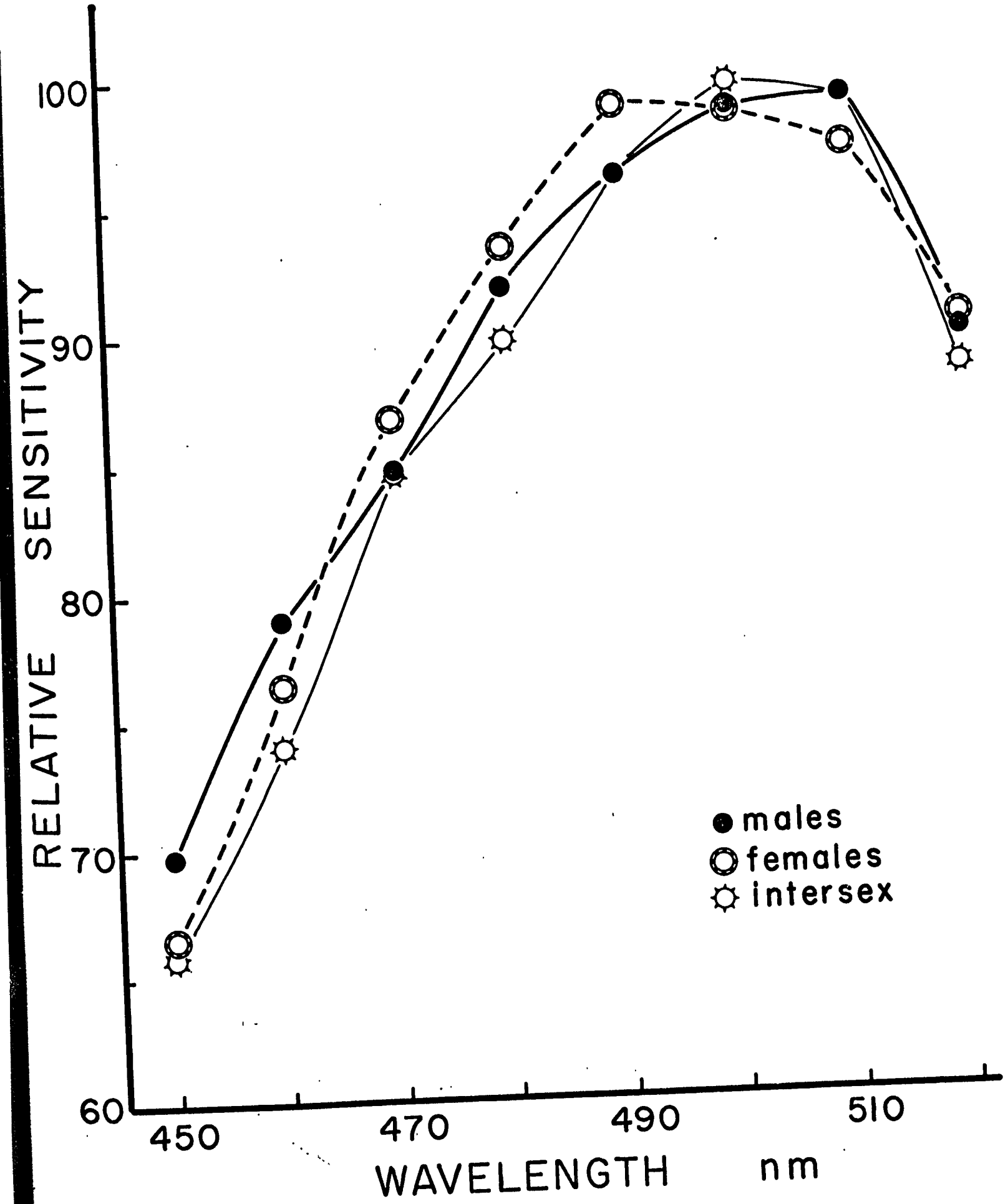

100
90
80
70
60
RELATIVE SENSITIVITY
450
470
490
510
WAVELENGTH nm
males
females
intersex

Figure 11.

Spectral sensitivity curves for the compound eyes of males, females, and intersexes of P. borealis. Numbers of observations were: males 10, females 5, and intersexes 10.


RELATIVE SENSITIVITY
100
90
80
70
60
450
470
490
510
WAVELENGTH nm
males
females
intersex

(4) Pandalus montagui

Only three specimens of Pandalus montagui were examined, all of them females. Two nauplius eyes and two compound eyes were investigated with electrophysiological methods and the results are displayed in graphic form in Fig. 13. These data were also treated with the arcsine transformation described for P. borealis. A striking difference exists between the spectral sensitivity curves for the nauplius and compound eye both in λ-max and shape. The compound eye λ-max occured at 510 nm while the nauplius eye λ-max was 490. Unfortunately no specimens of male or intersex were available, however, the data obtained provide a rather interesting comparison with P. borealis which will be discussed later.

(5) Pandalus Larvae

Only three larvae of P. borealis were available for examination with electrophysiological technique. The small size of these specimens (8-10 mm total length), and the unity of the photoreceptive organs made separate recording from the nauplius or compound eye impossible with the electrodes and technique available. Compound eyes of stage I zoea have not yet developed stalks and therefore cannot be removed easily (Berkeley 1939). This meant that any O.P. recorded from the head of the larvae was probably composed of activity from both the nauplius and compound eye. The variability in results from the three individuals, both in O.P. configuration (Fig. 7 E) and shape of spectral sensitivity curves, attests to the uncertainty of O.P. source. The positive component of

Figure 12.

The spectral sensitivity curve for the nauplius eyes of females of P.borealis drawn with the range of data indicated. This serves to exemplify the variability of data encountered in this study. Means are indicated by circled stars.

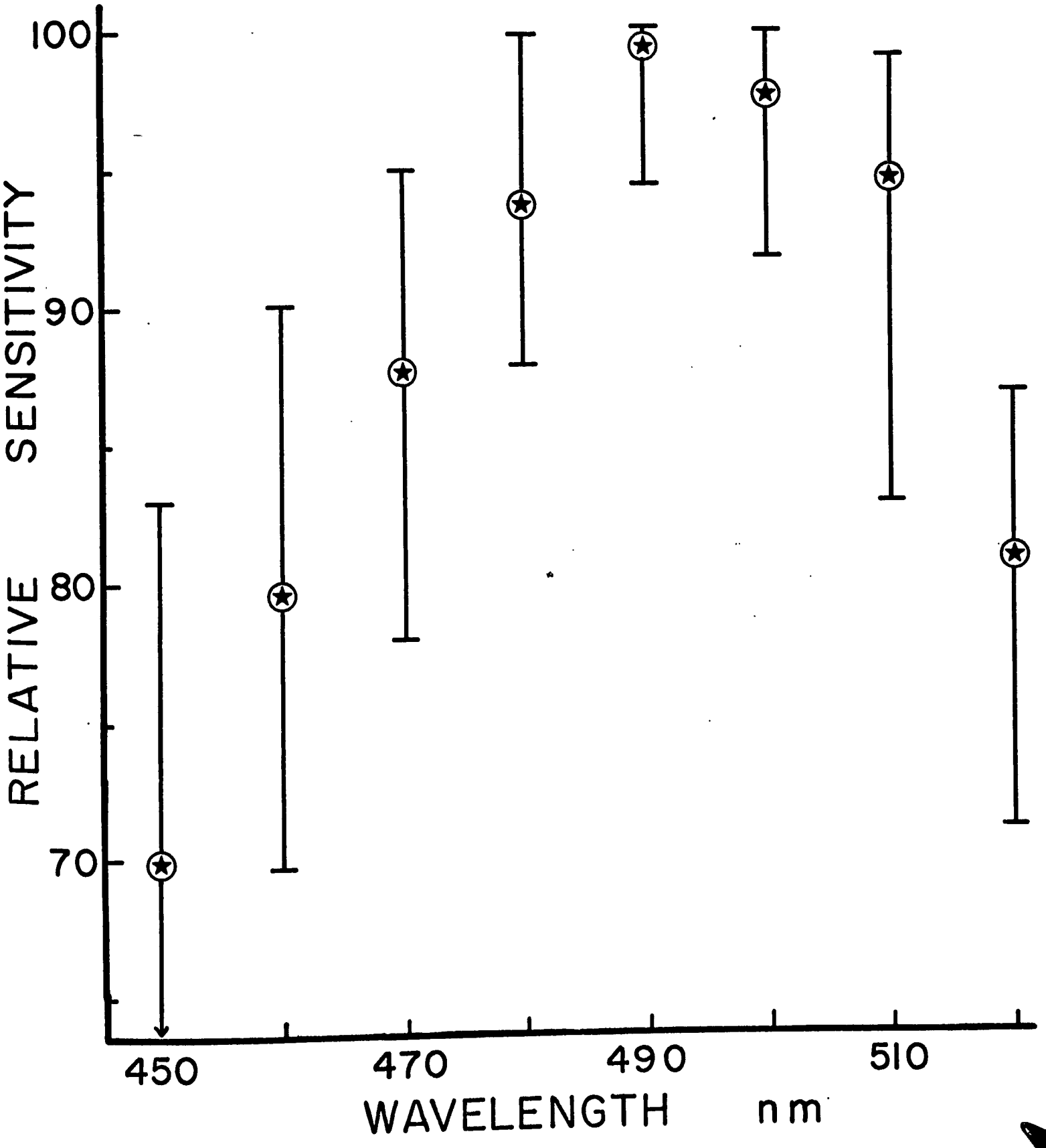
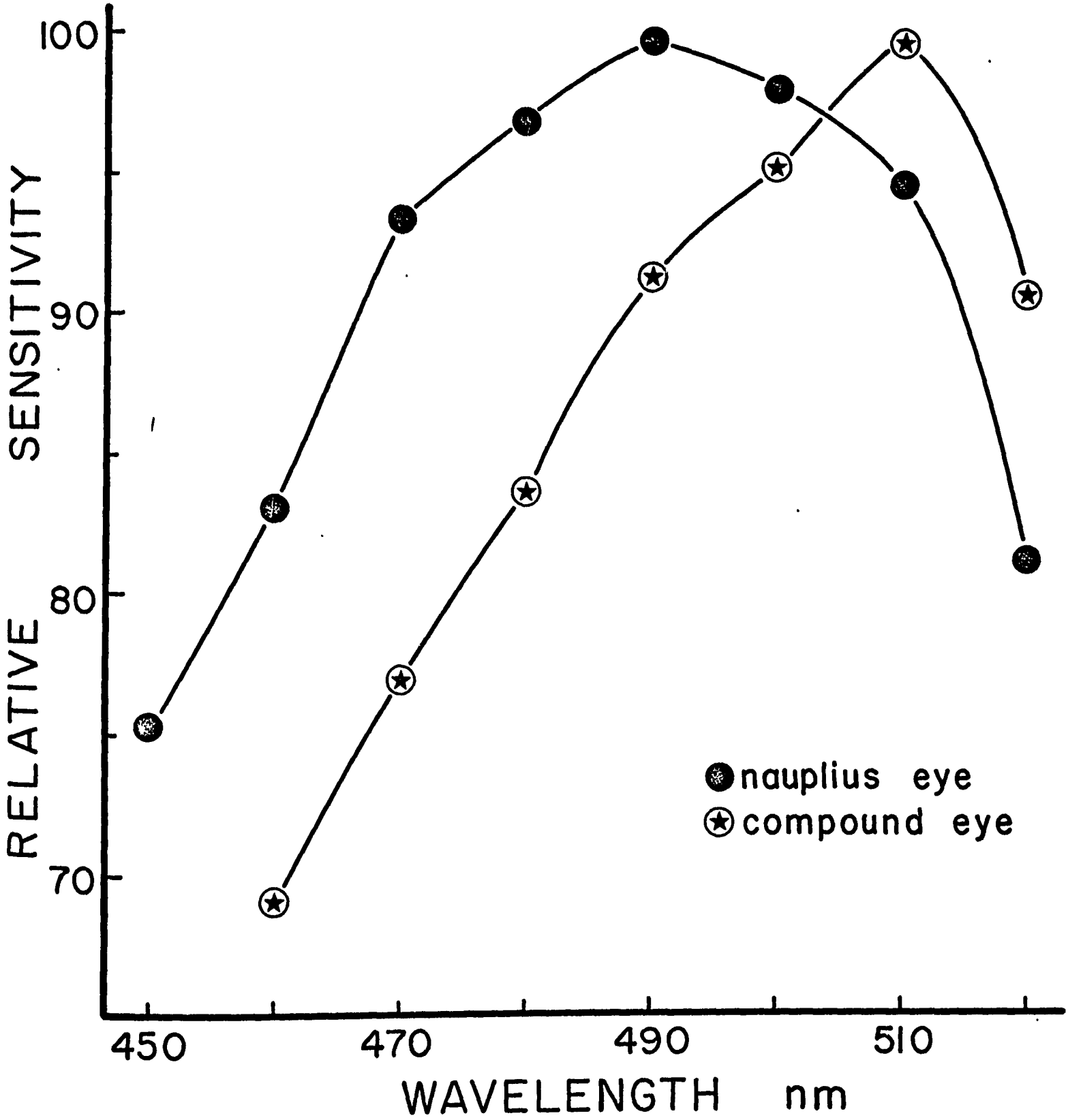

100
90
80
70
RELATIVE SENSITIVITY
450
470
490
510
WAVELENGTH nm

Figure 13.

Spectral sensitivity curves for the nauplius and compound eyes of Pandalus montagui derived from data for two adult females.

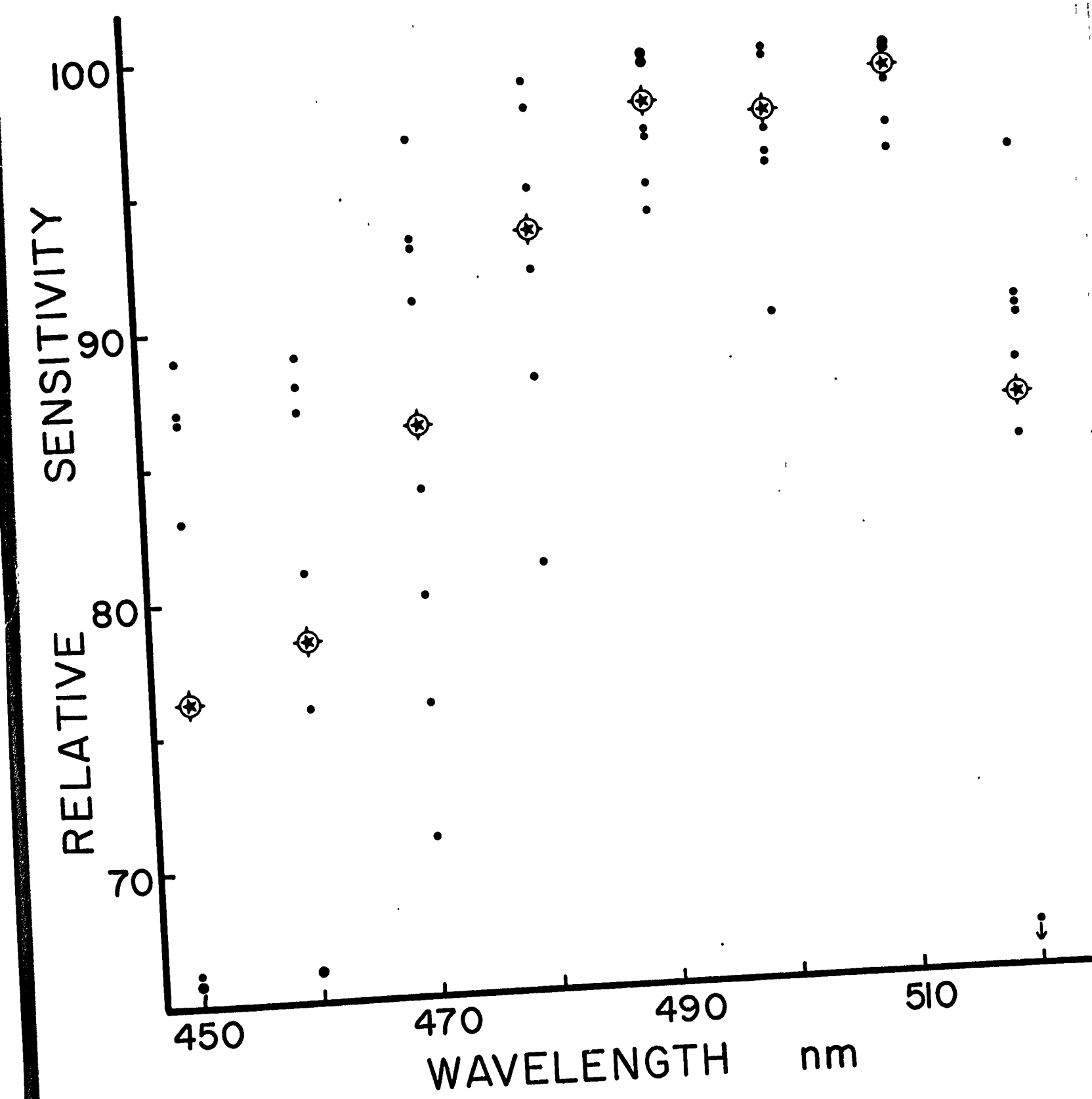

100
90
80
70
RELATIVE SENSITIVITY
450
470
490
510
WAVELENGTH nm
nauplius eye
compound eye

the larval O.P. was considerably larger than that previously observed for adults. In several instances the negative component was non-existent or very much reduced, leaving only the positive wave. The shape of the O.P. is greatly dependent on the relationship of the electrodes to the active photoreceptor cells. Advancement of an electrode through a layer of photoreceptor cells has been observed to result in an abrupt change in the polarity of O.P.'s in Planaria (Brown and Ogden 1968), and a change in potential in insect compound eyes (Burt and Catton 1964). Ruck (1962) describes a situation in which responses of opposite polarity could be recorded from insect photoreceptor systems by varying the location of the recording electrode. Effects such as those which occured in the experiments on larvae were probably the result of variations in the electrode position, combined with the complication of two separate photoreactive centers.

Measurements of the O.P. were made and the data combined and treated as previously described for adult shrimp. A spectral sensitivity curve has been constructed (Fig. 14) which shows the combined results of the larval experiments. One curious feature of some of the individual curves was their bimodal character with peaks at 510 and 490 nm (Fig. 15). Both peaks showed up very strongly in these 3 runs; 3 of the remaining runs showed strong peaks at 510 nm only, while one run showed a broad peak between 490 and 510 nm.

Figure 14.

Spectral sensitivity curve for the larvae of P. borealis derived from analysis of 0 P's from the cephalic photoreceptors. The results from the individual runs have been displayed to indicate some of the variability encountered. Means of the combined data are displayed.


100
90
80
70
RELATIVE SENSITIVITY
450
470
490
510
WAVELENGTH nm

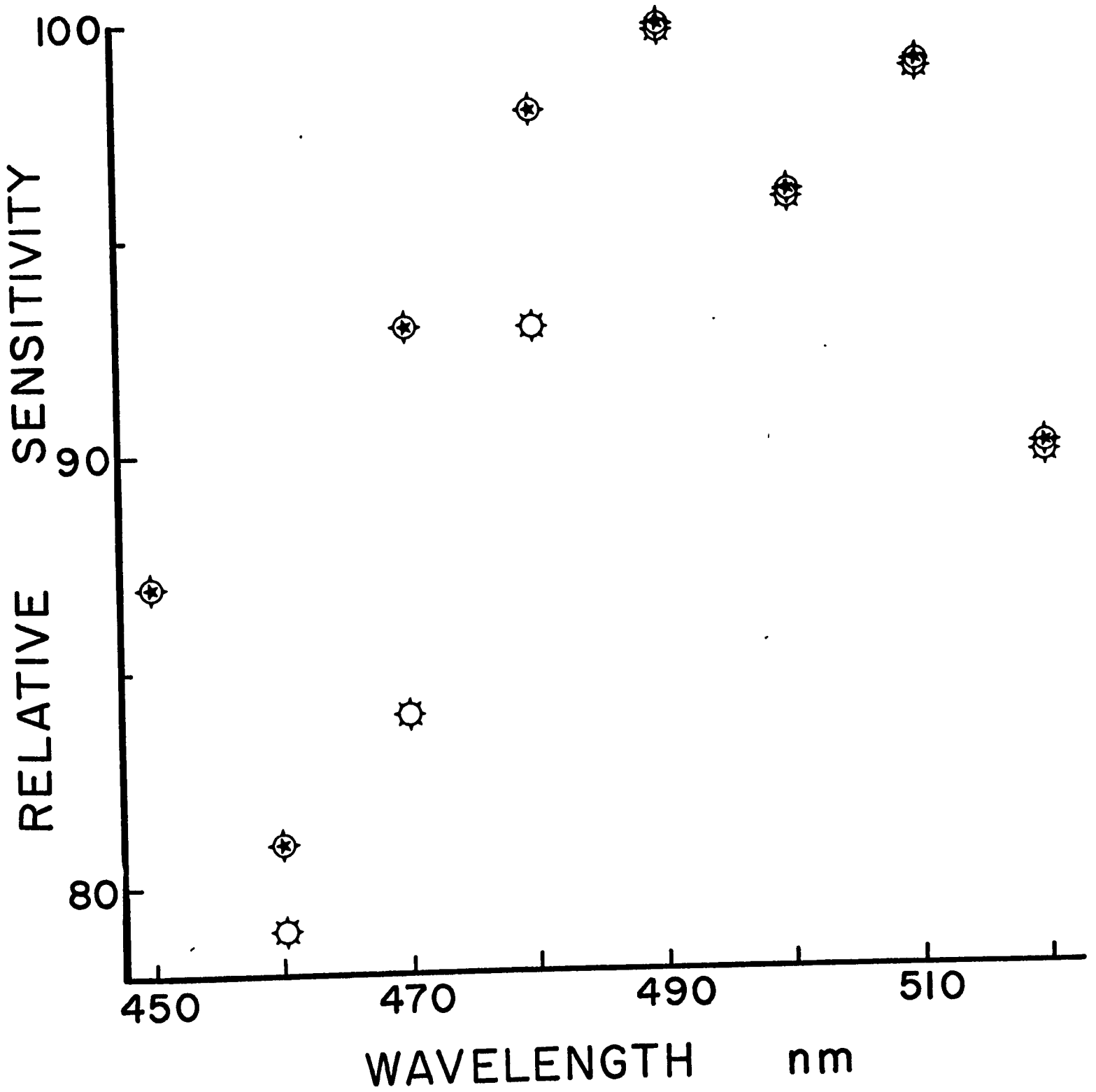


Figure 15.

Bimodal spectral sensitivity curves from two larvae of P. borealis.

(C) Histological Results

Examination of a complete set of serial cross sections through the bec ocellaire of P. borealis revealed some interesting features associated with the nauplius eye which had not been mentioned in previous histological work (Elofsson 1963, 1966[a]). From anterior to posterior along the bec ocellaire, structures described by Elofsson (1963) were observed and identified. The anterior terminations of the dorsal frontal organ extensions (Fig. 16 A) were found and their curious connection with the integument observed. The tube-like extension of the dorsal frontal organ meets and penetrates the integument so that direct open contact with the exterior appears to be made. Posterior to this, the extensions converge on the nauplius eye and merge into the eye itself at which point they become the dorsal frontal organs or posterior lateral cups of the eye. At this point, the integument dorsal to the nauplius eye forms a deep depression or dorsal pit which seems to act as the attachment for the heavy lateral compressor annulus muscles described by Elofsson (1963). Directly below the ventral margin of the dorsal pit is a sequence of structures which may be of great significance but go unmentioned by Elofsson.

A layer of connective tissue, only about 2 cells thick, encloses the loose spongy tissue which fills most of the space within the bec ocellaire. At a point directly beneath the dorsal pit this layer thickens to 5 times its normal width and forms a lens-like structure. Directly ventral to this, a funnel-shaped structure reaches down and expands to

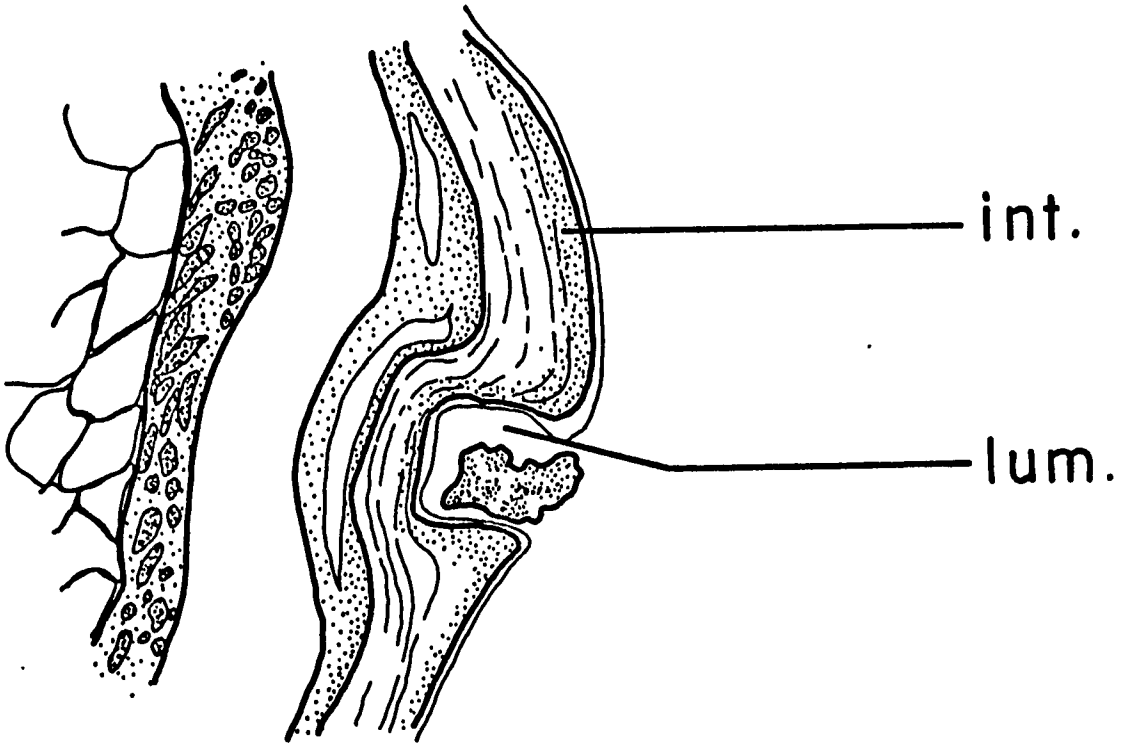
Figure 16.

(A) Cross-section of anterior lateral wall of the bec ocellaire at the point where the extension of the dorsal frontal organ penetrates the integument to make contact with the external medium. (int.) integument, (lum.) lumen of dorsal frontal organ extension. (100X)

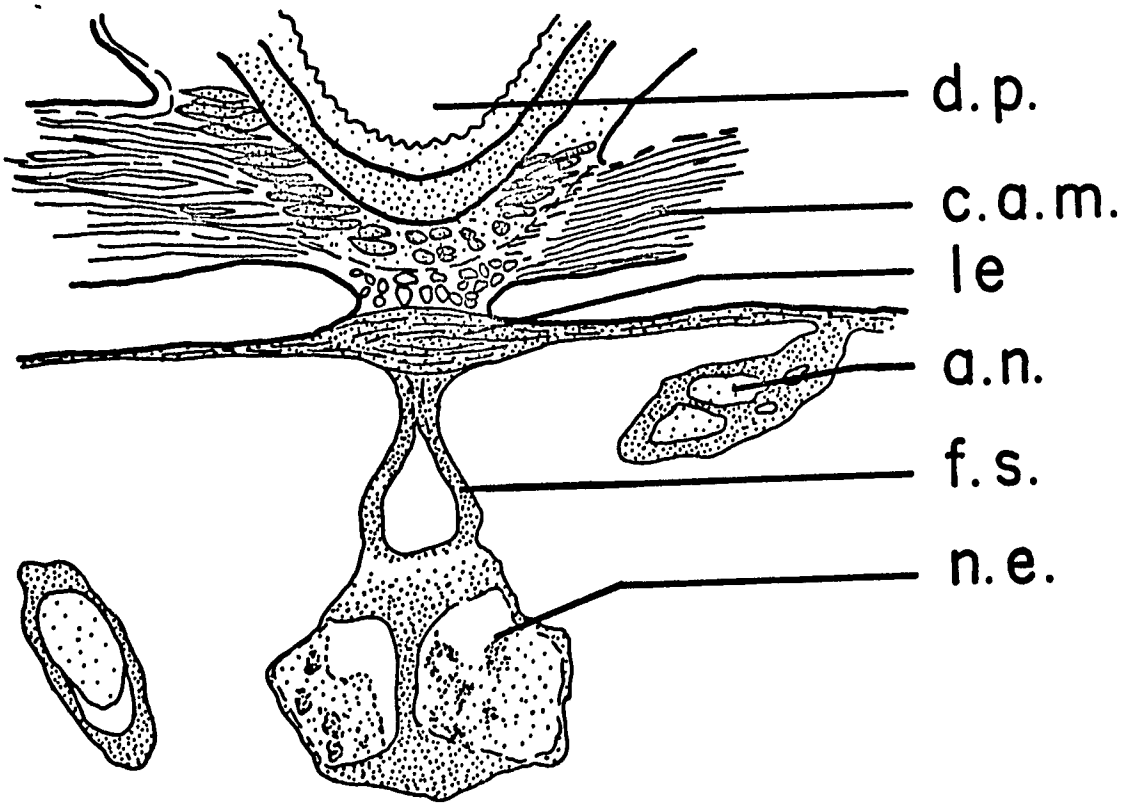
Figure 16.

(B) Cross-section of the bec ocellaire at the position of the dorsal pit. (d.p.) dorsal pit, (c.a.m.) compressor annulus muscle, (le) lens-like thickening of thin connective layer, (a.n.) apical nerve, (f.s.) funnel-shaped structure, (n.e.) nauplius eye. (100X)

A



B



make contact with the nauplius eye (Fig. 16 B). Elofsson (1963) has described this structure and suggests that it serves as part of the suspension of the nauplius eye. This structure is quite rigid and has been observed to remain intact even when the nauplius eyes of preserved specimens of P. borealis were exposed by dissection.

These structures, from the dorsal pit, through the thickened connective layer and the funnel-shaped apparatus to the nauplius eye, seem to represent more than just supporting structures; in fact, they have some of the characteristics of a dioptric apparatus. These structures are positioned directly above the central part of the nauplius eye and could function in directing light from above onto the eye.

DISCUSSION

(A) Electrophysiology

This study has reaffirmed the occurrence of electrical activity in the nauplius eye in response to light, first found in P. borealis during previous research by the author (Eaton 1968). In the earlier study, extracellular stainless steel electrodes were used to record ocellar potentials which were evaluated from oscilloscope photographs. The technique used in this investigation had two main advantages over the earlier work: measurement and analysis of data was performed by computer and stimulus parameters were better controlled. In addition a new electrode was employed and a Farady cage shielded the preparation more effectively from electrical interference.

The O.P.'s recorded in this investigation displayed the same shape and temporal characteristics as those recorded during the previous study. With A.C.-amplification, onset of light resulted in a rapid negative phase which was followed by a more gradual return to base line. Termination of the light pulse produced a rapid positive deflection, smaller in amplitude than the "on" response, with gradual return to base line (Fig. 7A). The latency of the "on" response was approximately 7-10 msec, while the transient negative phase took about 20 msec to reach its peak. Kirschfeld (1966) demonstrated a latency of about 20 msec and a rise time of 20 msec from single cells in the compound eye of the fly Musca, whereas Brown and Ogden (1968) showed a latency of 30 msec and rise time of 15 msec from single cells in the planarian ocellus.

When stimuli are short (10-20 msec) the "off" response becomes part of the initial response so that the positive "off" wave follows the negative "on" wave without interruption (Fig. 7B). The return to base line after the positive wave is a slow process which usually takes about 0.3-0.5 sec. All the characteristics of the O.P. recorded here have been described for other invertebrates such as planarians (Brown and Ogden 1968), spiders (De Voe et al 1969), insects (Goldsmith and Ruck 1958, Kirschfeld 1966, Yinon and Auerbach 1969), Limulus (Wald and Krainin 1963, Chapman and Lall 1967, Millecchia and Mauro 1969, Nolt and Brown 1969), crustaceans (Kennedy and Bruno 1961, Gwilliam 1963, 1965, Boden and Kampa 1965, Stratten and Ogden 1971). In all cases, the O.P. takes the general form described above, although it may differ slightly in some minor characteristics.

The standard measure of O.P. response - amplitude of voltage change from base line to peak of initial negative wave - was used in all cases except one where the O.P. from a larva of P. borealis was reversed in polarity, and the peak of the positive wave was measured. Reversal of polarity has been observed in the planarian ocellus when a micro-electrode is advanced through the eyecup (Brown and Ogden 1968), and is probably caused, in both the Planaria and Pandalus, by the electrode recording current activity distal to the active part of the receptor cells and therefore opposite in direction.

(B) The λ -max.

The spectral sensitivity results of this study are of particular interest in that they differ somewhat from results demonstrated in the previous work on P. borealis (Eaton 1968). In the earlier study, data indicated that the λ -max of the nauplius eye of P. borealis was 475 nm, with the possibility of a shift to 500 nm in small or young animals. In the present study, the λ -max fell between 490 and 510 nm; those of females were concentrated at the lower wavelength and those of males at the upper one. Often, if the λ -max of a pigment is known some of its other characteristics can be discerned. Dartnall (1953) pointed out the similarity in shape of absorption spectra of visual pigments in general. He has devised a nomogram, using the absorption spectrum of visual purple as a basis, which allows the construction of a spectral absorption curve for any pigment of known λ -max. Dartnall's nomogram has been used, successfully, to describe pigments extracted from Palaemonetes with λ -max 496 and 555 (Goldsmith, Dizon, and Fernandez 1968) and to describe sensitivity curves derived from O.P. analysis of Limulus (Nolte and Brown 1969), Balanus (Stratten and Ogden 1971), and a wolf spider (De Voe et al 1969). When the Dartnall nomogram is applied to a spectral sensitivity curve, discrepancies can be expected due to physical factors such as selective reflection of a tapetum, light-adaptation of the eye, of failure to base sensitivity measurements on quantized light. With the above factors considered, the Dartnall nomogram should fit the spectral sensitivity curve for any single photosensitive pigment.

The Dartnall nomogram has been fitted to the spectral sensitivity curves for the nauplius eyes of males (λ -max 510) and females (λ -max 490) and to curves from the compound eye of P. borealis (Figs. 17 and 18). The fit is obviously far from perfect. In the data from the females (λ -max 490) the left-hand limbs of the curves correspond with the nomogram fairly well but the right limbs diverge above 490 nm. The reverse is true of results from male nauplius and compound eyes; here the right-hand limb of the curve corresponds better while the left limb diverges greatly from a nomogram curve centered at 510 nm. Obviously, nomograms for single pigments with λ -max 490 and 510 are not sufficient to describe the curves observed here. A single curve derived from the nomogram centered mid-way at 500 nm almost follows the curves for males and females but tends to be too narrow, particularly in the case of the nauplius eye.

Two explanations for the observed lack of fit of Dartnall's nomogram are possible. 1) The results could represent variations of a spectral sensitivity curve with a λ -max at 500 nm and with some factor obscuring the 500 nm peak. 2) Variation of a screening pigment or inherent differences in dark adaptation between males and females could produce differences in the curves observed for the two sexes. The obscuring of a λ -max at 500 nm seems most unlikely since spectral absorption of the integument or screening pigment of most arthropods is non selective between 400-700 nm (Machan 1968). The inability of Dartnall's nomogram to describe these results could also be explained by the presence of more than one photosensitive pigment in the photoreceptors of P. borealis.

Figure 17.

Spectral sensitivity curves for the nauplius eyes of males and females of P. borealis compared with Dartnall's nomograms for pigments with λ-max 510 and 490 nm.

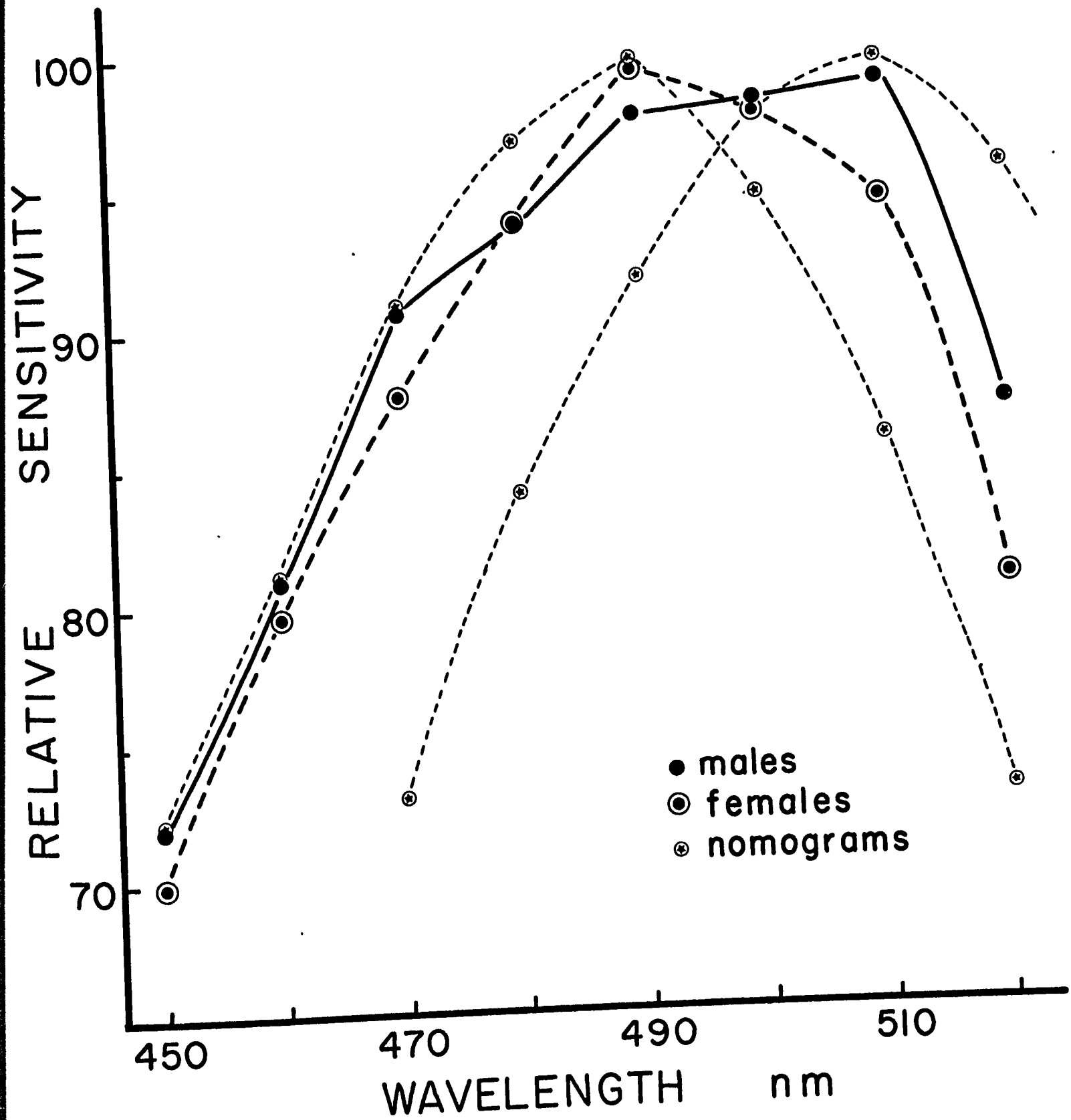
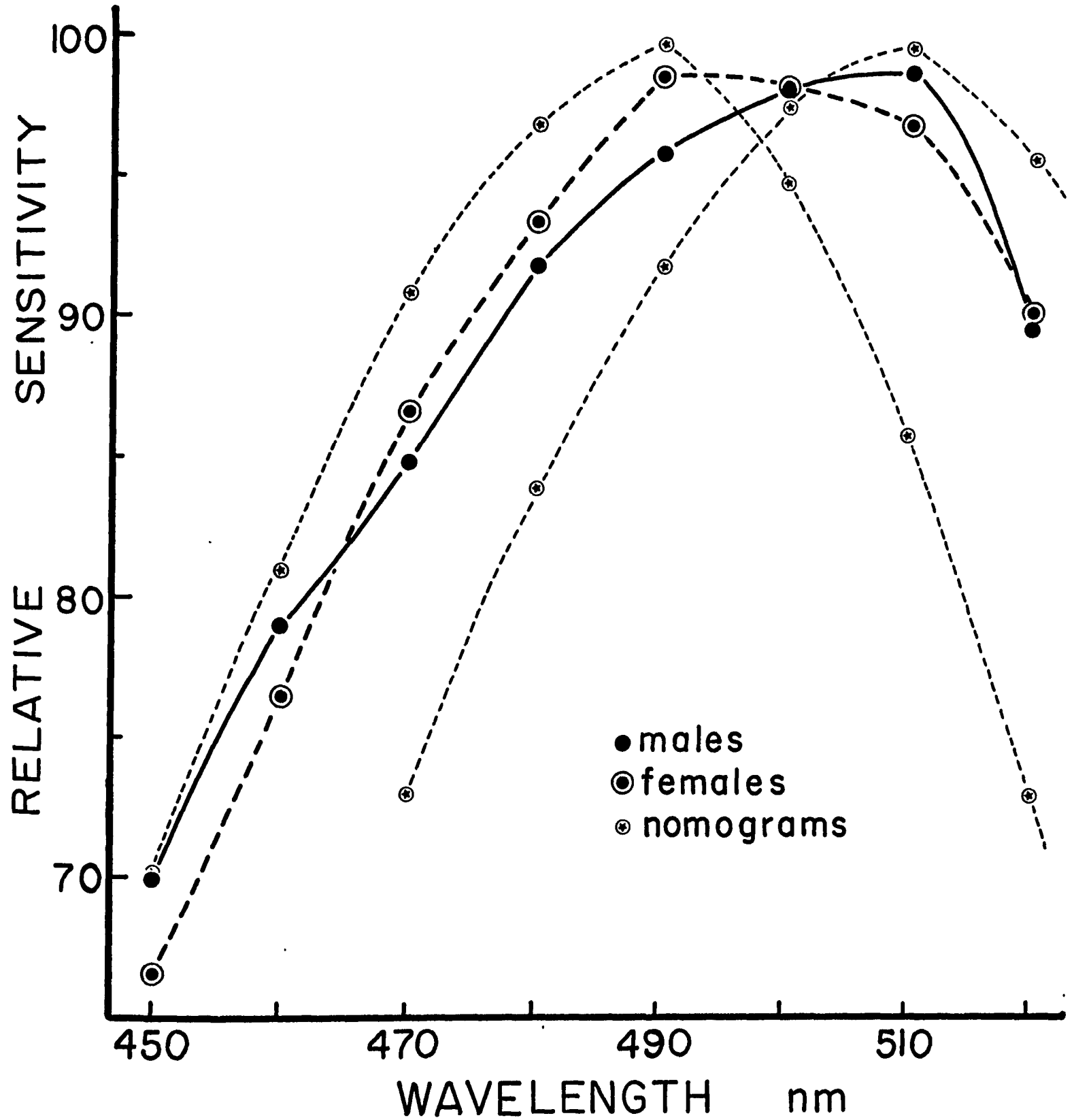

100
90
80
70
RELATIVE SENSITIVITY
males
females
nomograms
450
470
490
510
WAVELENGTH nm

Figure 18.

Spectral sensitivity curves for the compound eyes of males and females of P. borealis compared with Dartnall's nomograms for pigments with λ-max 510 and 490 nm.


100
90
80
70
SENSITIVITY
RELATIVE
450
470
490
510
WAVELENGTH nm
males
females
nomograms

Since Dartnall's nomogram is based on a pure, single pigment it would be incapable of describing a mixture of two pigments acting together. It seems likely from the results presented here that the photoreceptors of P. borealis contain two closely related photosensitive pigments, one with λ -max 490 and the other λ -max 510. The concentrations of these two pigments seem to vary according to the age or sexual status of the shrimp, or to environmental conditions. If so, young male shrimp must have a greater concentration of the 510 nm pigment while older female shrimp have a greater concentration of the 490 nm pigment. This explanation would account for the width of the spectral sensitivity curves since the curve for the 510 nm pigment would be expanded at shorter wavelengths by the effect of the 490 nm pigment and vice versa.

Evidence for the occurrence and interplay of more than one photopigment has been shown in some euphausiids which have a trimodal spectral sensitivity curve with peaks at 460, 495 and 515 nm (Boden and Kampa 1965). The λ -max of these euphausiids varies between 460 and 495 nm according to habitat, and the spectral sensitivity curve is very wide, expanded on both sides by the 460 and 515 nm pigments. The occurrence of three pigments in Euphausia lends support to the two-pigment hypothesis for P. borealis. Many arthropod eyes, particularly those of insects, commonly contain at least two pigments, one in the visible range(350-700 nm) and one in the ultraviolet (Goldsmith and Ruck 1958, Chapman and Lall 1967, Goldsmith, Dizon and Fernandez 1968, Wald and Seldin 1968, De Voe et al 1969, Nolte and Brown 1969, Sontag 1971). Although eye pigments occuring in the same animal are usually more widely separated spectrally (e.g. 496

and 555 nm in Palaemonetes or 490 and 340 nm in the honeybee), the occurrence of the three pigments in Euphausia indicates the possibility of closely spaced photosensitive pigments.

Differences between the spectral sensitivity of the nauplius and compound eyes were marginal. The O.P. appeared similar in both cases. The occurrence of λ-max was the same for males (510) and females (490) in the nauplius and compound eyes; however, the difference between nauplius and compound eyes of intersex shrimp was significant (Table 5). The spectral sensitivity curves show a slight difference in shape between nauplius and compound eyes. The difference between the male and female spectral sensitivity curves is smaller in the compound eye than in the nauplius eye as indicated by the Mann-Whitney U tests on the data (Table 5).

The results for the larvae of P. borealis are most interesting in the light of the above hypothesis since they indicate a bimodal spectral sensitivity curve (Figs. 14 and 15). Individual peaks show up at 490 and 510 nm, and may be a feature influenced by the fact that nauplius and compound eyes were recorded from simultaneously.

The results from P. montagui produce a different picture altogether. Peaks are again found at 490 and 510 nm, however, in this case the 490 λ-max occurs in the nauplius eye only and the compound eye has λ-max 510. Another salient factor is the apparent fit of Dartnall's nomogram to the sensitivity curve of the compound eye indicating the presence of a single photopigment in that eye. Unfortunately, the two specimens examined were of the same age (adult females) so that no comparisons could be made

between the various developmental stages of P. montagui; however, the contrast between P. borealis and P. montagui is sufficiently notable in itself.

(C) Behavioral Study

The behavioral work in this investigation served only to establish the approximate position of the peak of positive phototaxis of P. borealis larvae, which appears to be between 500 and 525 nm. This corresponds well with the electrophysiological results which place λ-max at 510 nm. The strong positive phototaxis observed in larval pandalids is a feature which could be expected since the larvae are planktonic during the first stages of development and would thus depend on positive phototaxis to maintain themselves in the upper water layers.

Surprisingly, adults of P. borealis showed little if any overt response to light. Jachowski and Myrberg (1968) studied the photo-orientation of the shrimp, Penaeus duorarum, and were able to make use of its preference for light to examine its spectral selection, however, this was not possible with adults of P. borealis. An undetected response may exist, and more rigorous experimental procedures might reveal it; however, it must be very small and unsuited for the purposes of this study.

(D) Ecological Significance

Determination of the functional and ecological significance of the nauplius eye was one of the purposes of this investigation. Some of the suggestions made in the earlier study by the author (Eaton 1968), concerning the function of the nauplius eye, received support from the present study. Certainly, the fact that the nauplius eye acts as a photoreceptor, with a photoresponse and sensitivity much the same as the compound eye, has been well established. Also, evidence is presented that the nauplius eye is associated with a series of structures - the dorsal pit, the lens-like connective layer, and a funnel-shaped structure - which might have light directing capabilities. If these structures do provide a dioptric apparatus its nature is rather crude, but, it could be very effective in selecting and conducting light from above. The increased ability of the eye in resolving light from the surface could increase its efficiency in performing functions such as the monitoring of light intensity, or the initiation of a shadow reflex. At the same time, it is impossible to think of the nauplius eye as an image-forming organ.

The locations of the λ-max's of the nauplius eye (490 and 510 nm) are not really surprising, although the difference between the λ-max of shrimp used in this study and those used in the previous work (λ-max 475 nm) is rather puzzling. Most of the animals used in the earlier study were collected in deep water off the North-East coast of Nova Scotia, whereas all the shrimps used in this investigation were collected from relatively shallow water in Passamaquoddy Bay in the Bay

of Fundy. The monochromator effect of water on sunlight is a well known fact (Clarke 1933, Strickland 1958, Tyler 1959, Boden, Kampa, and Snodgrass 1960, Kampa 1970[a], 1970[b]). Water tends to filter out wavelengths at the extreme ends of the visual spectrum and to transmit blue or blue-green light. The spectral transmittance of seawater is greatly influenced by suspended material and the range of transmitted wavelengths decreases with depth. In effect, shallow water and/or coastal water, containing a moderate load of particulate matter or sediment, has a broad irradiance spectrum with a peak at or above 500 nm, while clear oceanic water has a spectral transmission peak at 480 nm or lower and a band of irradiance which narrows considerably with depths greater than 50 m (Kampa 1970[a], 1970[b]). This exemplifies the possible variability in the spectral transmittance of sea water, a feature which seems to be reflected in the spectral sensitivity of most marine organisms and which may be the underlying reason for differences observed in the λ-max's of P. borealis investigated in these studies.

The visual pigments of several marine fishes have been shown to be correlated with their habitat (Wald, Brown and Brown 1957). Summer flounders and scup inhabit shallow coastal waters at depths less than 20 fathoms and have λ-max 503 and 498, whereas cusk and lancetfish living down to 100 fathoms and deeper have λ-max 494 and 480. The λ-max of Homarus ameriacnus, an inshore crustacean, is 515 (Kennedy and Bruno 1961) whereas the λ-max of the oceanic euphausiid is 463-468 (Kampa 1955). As previously mentioned, Euphausia pacifica has a trimodal spectral sensitivity curve with peaks at 465, 495 and 515 nm; euphausiids restricted

to shallow water have λ-max 495-500, but those free to seek greater depth have λ-max 465. Actual light measurements, made at the depth of the euphausiid layer, showed peaks of light at 494-502 nm for the shallow environment and 475-490 nm in the deeper environment. Apparently the euphausiid not only has a pigment (465 nm) adapted to the environment to which it migrates each day (200-300 m depth) in the open ocean, but it also has the added adaptive ability to reduce the concentration of the 465 nm pigment in lieu of the more effective 495 nm pigment when it is restricted to water less than 100 m in depth (Boden and Kampa 1965).

A similar situation seems to exist with the nauplius eye photopigments of P. borealis. As mentioned earlier, specimens taken in deep water (100 fathoms) off the coast of Nova Scotia had λ-max 475 nm (Eaton 1968), whereas specimens used in the present investigation, collected from shallow water (30-50 fathoms) in the Bay of Fundy, displayed λ-max's at 490 and 510 nm.

A further refinement in adaptation seems to be indicated by the distribution of λ-max's 510 and 490 between male and female shrimp. Larvae of P. borealis are planktonic for about 6 months, then they become benthic and inhabit shallow inshore waters (5-20 fathoms) until they reach maturity as males and join the adult population in deeper water.

After approximately one year as males, the shrimp go through a transitional or intersex stage at which time the male sex characteristics are lost and ovaries develop. The mature female shrimp tend to occupy the lower depth range of the species and only migrate to shallow water once a year to hatch their eggs (Haynes and Wigley 1969, Butler 1964, Allen 1959).

Males of P. borealis were found to have a λ-max at 510 nm both for the nauplius and compound eyes, compared with the λ-max 490 of the older female shrimp which had probably been living at greater depths than the males for one year or more. The λ-max shift, from 510 to 490 nm, seems to correspond with the transition from male to female. It is suggested here that this transition may be brought about by a shift in the spectral irradiance experienced by the shrimp as they move to greater depths. However, it is impossible to say whether the shift in spectral sensitivity is an adaptive response associated with the change in colour of ambient light, or whether it is somehow associated with the complex physiological change of sex reversal. A delay in spectral transition could be the reason that male shrimp, even when taken from the depth occupied by the females, still retain the λ-max 510; the delayed transition may occur coincidentally at the time of sexual metamorphosis. Larvae of P. borealis show a major λ-max of 510, consistant with their planktonic nature.

The differences between the nauplius and compound eyes of adults of P. borealis are minor and difficult to interpret. The only conclusion that might be drawn from these results (Fig. 9) is the occurrence of an earlier shift from λ-max 510 to 490 in the nauplius eye, indicated by the distribution of intersex specimens. Most of the nauplius eyes of intersex animals have λ-max 490, whereas for the compound eye results no intersex shrimp have λ-max 490. It might be concluded from this that the shift from 510 to 490 occurs earlier in the nauplius eye than in the compound eye. The difference observed between the compound and nauplius eyes of P. montagui can not be explained. An examination of this species

throughout its life cycle might reveal the presence of both pigments (490 and 510) in the compound eye at an earlier stage and a gradual loss of the 490 nm pigment with female maturity.

The application of microelectrode techniques (intracellular) to the study of the nauplius eye might be successful in revealing more about the eye's function. Only with this technique would it be possible to discover whether the 490 and 510 nm pigments are located in the same cell or in separate cells. The micro-electrode technique would also be useful in defining detailed characteristics of the intracellular O.P., and in establishing parameters so that detailed comparisons of the nauplius eye and other photoreceptor cells could be made. This investigation has opened up a physiological approach to a rather common but unstudied crustacean photoreceptor and it provides a basis of information for further investigation into the vision of planktonic and benthic crustacea.

ACKNOWLEDGEMENTS

I should like to acknowledge my appreciation and indebtedness to Dr. Carl M. Boyd of the Department of Oceanography of Dalhousie University for his valuable guidance and encouragement in the persuit of this research. My thanks also go to Mr. Glen Johnson whose time and assistance with computer programming and operation are greatly appreciated, and to Mr. Patrick Keenan whose technical skill and knowledge were of great assistance in the laboratory.

Special thanks is extended to National Sea Products for their co-operation in arranging the collection of specimens, and in particular, to Mr. Floyd Hawkins of Beaver Harbour, New Brunswick for making his boat so readily available to me for shrimp collecting.

Thanks also go to those members of my thesis committee whose constructive criticism and comment have been of much assistance in the preparation of this manuscript.

Financial support for this study was provided by a grant and scholarship from the National Research Council of Canada.

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