SPECTRAL SENSITIVITY AND RETINAL PIGMENT
MOVEMENT IN THE
CRAB LEPTOGRAPSUS VARIEGATUS (FABRICIUS)

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SUMMARY

1. The retina of Leptograpsus contains five types of movable screening pigment. The positions of these were found under various conditions of illumination in the day and at night.

2. Intracellular recordings were made of the spectral responses of retinula cells R1–7 under the same conditions, with the eye in situ.

3. The spectral absorptions of the individual screening pigments were measured by ultramicrospectrophotometry.

4. Calculations based on a simple model of screening pigment action suggest that the observed variation in spectral sensitivity with light and dark adaptation may be largely explicable in terms of the effects of these screening pigments on a rhodopsin of peak absorbance at 485 nm.

5. Light-adapted angular sensitivities are comparable to those of insects with high acuity apposition eyes.

INTRODUCTION

Retinal screening pigments in Crustacea present a bewildering variety of systems: retinae may contain from three to six pigments, and pigments may be stationary or moving, some moving according to a circadian rhythm, some because of a direct or hormonally mediated effect of light. Although closely homologous types of pigment cell can be recognized across many different species, there can be differences between species of the same genus.

Study of pigments in the crustacean eye has mostly been concentrated upon the superposition eyes of crayfish and prawns, and little is known about apposition eyes in Crustacea. Only one photopigment has been found in crabs by spectroscopic examination of eye extracts or isolated rhabdoms, but some electrophysiological and behavioural evidence (Hyatt, 1975) implies that there is more than one colour-type of retinula cell. In all crab rhabdoms that have been examined by microspectro-photometry, a visual pigment with maximum absorption ($\lambda_{\text{max}}$) of around 500 nm has been found; 493 nm in Libinia (Hays & Goldsmith, 1969), 504 nm in Callinectes...
Most published determinations of spectral sensitivity in decapod Crustacea using intracellular recordings have been made upon excised eyes and are therefore not necessarily totally reliable. Studies on dark adapted crab eyes have shown peak sensitivities at wavelengths very close to those found using ERGs (Carcinus, 493 nm, Bruno et al. 1973; Callinectes, 505 nm, Scott & Mote 1974). Leggett (1978) using the portunid crab Scylla, with eyes in situ, showed that there are sometimes considerable shifts in the spectral sensitivity of light-adapted cells, compared to a dark-adapted peak of 495 nm.

These experiments were undertaken in order to determine to what extent variations in intracellularly recorded spectral sensitivities of Leptograpsus photoreceptors could be explained by movements of the retinal shielding pigments.

**MATERIALS AND METHODS**

Grapsid rock crabs, Leptograpsus variegatus, were maintained in a tank lit by fluorescent lights on a 14:10 light:dark cycle in summer, and 12:12 in winter. Crabs were allowed to acclimatize for at least 2 days before being used.

**Electron microscopy**

For electron microscopy, eyes were fixed in 2.5% glutaraldehyde with 0.1 M sodium cacodylate, 0.14 M sucrose and 2 mM calcium chloride at pH 7.3. The crabs used were of almost identical size, with carapace widths between 2.8 and 3.0 cm. The eyes of two crabs were fixed in the light-adapted condition at midday, and the eyes of two more were fixed when dark-adapted at midnight, being dissected under a red light. They were fixed overnight at 4 °C, washed in several changes of cacodylate buffer with sucrose, post-fixed in 1% osmium in cacodylate buffer for 2 h, washed in several changes of buffer, dehydrated through an ethanol series, taken through propylene oxide and embedded in Araldite. Pale gold to silver sections were cut on glass knives, mounted on formvar-coated slot grids and stained in uranyl acetate (40 min) and Reynold's lead citrate (20 min). They were examined with a Jeol JEM 100 C electron microscope. Measurements were made from photographs. Means and standard deviations were calculated from 40-50 measurements of each type of pigment granule, and 8–10 rhabdoms in each condition.

**Pigment movements**

Crabs of 2.5-4.0 cm carapace width were used in these experiments. To reproduce the conditions under which electrophysiological recordings had been made, the animals were induced to automize their legs approximately 1 h before being placed in the appropriate light regime. Two crabs were fixed in each state investigated, by immersion in hot (70–80 °C) fixative for 1 min. The eyes were then rapidly dissected in cold fixative (2.27% glutaraldehyde in Millonig's buffer with 0.01% calcium chloride and 15% glucose). 'Day' crabs were fixed between 13:30 and 15:30 h in the following conditions: after 30 min at a window in bright sunlight but on a cool
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After dehydration through an ethanol series (as fast as possible in order to minimize loss of alcohol-soluble pigments) the eyes were taken through propylene oxide to Araldite. They were sectioned at 0.5-2 μm to provide different viewing conditions and mounted unstained under Permount. Measurements were made as a proportion of cone length or retinula cell soma length, to allow for varying crab sizes. The minimum diameter of the 'iris' formed by the dark distal pigment around the cone tip was also measured. An eyepiece graticule was used for all measurements using the light microscope.

Microspectrophotometry

The spectral absorption of the red basal pigment, the retinula cell screening pigment, the dark distal pigment and the light distal pigment was measured using a Zeiss UMSP 1. Fresh eyes were dissected in Carcinus saline (Pantin, 1972) and the retina teased apart to yield pieces containing one or two distinguishable pigment cell types. Measurements were made on squashes of these pieces in saline, with a measuring diameter of 5 μm in the object plane, from 700 to 350 nm. A section of the slide containing no tissue was used to provide baseline measurements. The red basal pigment formed large oily droplets in the saline, and some of these easily covered the whole area being measured. Clusters of granules were measured for the dark distal and retinula cell screening pigments and groups of small yellow-brown droplets for the light distal pigment. Change in extinction was measured by comparing transmission through pigment and saline at ten or twenty nm intervals. Data for each run were normalized, taking the highest extinction value of each run as unity, and the normalized data averaged and plotted. The spectral transmission of clean cornea was also measured, between 300 and 700 nm.

Electrophysiological recording

Crabs were made to autotomize their legs, and the back of the carapace fixed with quick-setting cyano-acrylate glue to a Perspex holder mounted on a magnetic stand. The eyes were positioned as they would be in an alert animal, secured with Plasticine, and immobilized by filling the eye of the carapace with 'Vertex' dental cement. The crab was placed in the recording set-up at the centre of a Cardan arm perimeter device, and the eyes wiped with damp tissue to remove any salt deposit left by drying sea-water. A chip of razor blade was used to cut a triangular hole with sides about 5 facets or 200 μm long in the dorsal cornea, and the electrode was quickly introduced vertically by a Leitz joystick micromanipulator. Haemolymph drying around the electrode provided some stability, but even so movements of the eye produced by the heart-beat were large enough to prevent stable recording in about 50% of the preparations.

The recording electrodes, or resistance 150-200 MΩ, were pulled from fibre-filled glass and contained 3 M potassium acetate. The indifferent electrode was a silver wire...
pushed into the rear leg stump. The signal was recorded through a Grass P1 amplifier and displayed on an oscilloscope and a chart recorder. All measurements, apart from some resting potentials, were made from the chart recorder. Crabs generally remained in good condition, if they were kept moist, for about a day. Recordings were made from June 1978 to January 1979.

The light source was a 150 W Xenon arc-lamp. The stimulating beam passed through a heat filter, a collimating lens, neutral density (N.D.) filters (Balzers), interference filters, a focusing lens and a shutter (Uniblitz). The 20 interference filters (Schott or Corion) ranged from 317 to 621 nm peak transmission. The beam was focused on to a 4 mm diameter quartz ligh-guide that was attached to the Cardan arm, and subtended 1.3° at the crab eye. The quantal transmission through the filters from 373 to 621 nm inclusive was adjusted to within 13% of 1.8 × 10^18 photons/cm²/s, but transmission through the UV filters 317, 345, and 358 nm was 20–40% less.

During all the experiments described here, a 20 ms flash was given at 10 s intervals, and where possible the N.D. filters were adjusted to keep the response below about 25 mV in amplitude. Brighter or more frequent flashes caused appreciable adaptation, so that the responses to consecutive stimuli were not independent.

When a cell with a stable resting potential of 50 mV or over was found, the light guide was centred on the optical axis, using flashes delivered 1/s. The experimental run was started after the response to a flash of the same intensity, delivered once every 10 s, had stopped increasing. Since cells could not be stimulated to produce their maximum response (50–80 mV in amplitude) until they had been completely dark-adapted for about 30 min, and did not fully recover for a further half-hour, the maximum response was generally not determined. White-light intensity series were interspersed between experimental runs in all experiments. All cells used were in the central, anteriorly directed part of the retina.

Spectral sensitivity was calculated from the spectral efficiencies obtained by stimulating with monochromatic flashes of the same quantal content (corrected for the small differences in actual quantal transmission of the filters). It was measured in three ‘steady states’: (a) dark-adapted for at least 30 min, (b) in the dim light (10–20 μW/cm²) of the normal room illumination (a 150 Watt pearled tungsten bulb), and (c) in the bright light (8–10 mW/cm²) of a 30 Watt tungsten microscope lamp focused on the eye from not more than 20° from the axis of the cell being tested. In cells adapted to these conditions (DA, dim LA, bright LA) a white-light intensity series was made before and after two spectral runs (ascending and descending). In transitional states of adaptation, particularly during the first few minutes of bright light adaptation, single spectral runs were alternated with intensity series.

Sensitivity to the plane of polarization of white light was measured by rotating a piece of polarizing film (Polaroid type HN38) in front of the light guide through 180° in 10° steps. The maximum and minimum responses during the polarization run were compared with a V/log I curve to find the polarization sensitivity (PS). Polarization runs were considered valid if all responses fell within the linear part of the V/log I curve (where measurement error and intrinsic variation in response have least effect), and if the size of the responses at 0 and 180° rotation of the polaroid were equal, implying that the sensitivity of the cell had not changed during the run. All PS measurements were made on dark-adapted cells.
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White light was used for angular sensitivity measurements. To provide a point source, the light guide was covered with a metal mask with an aperture that subtended 20° at the eye. After the point had been centred and an intensity series made, the point was moved in a vertical arc through the point of maximum sensitivity in 2° or 4° steps. Several runs were made, with the source moved alternately upwards and downwards. The position of some cells was determined by shining a microscope lamp on to the eye along the optical axis of the cell, and noting the position of the dark pseudopupil that could be seen facing the light.

RESULTS

(I) The screening pigments

The seven large (R1-7) and one small (R8) retinula cells of the Leptograpsus ommatidium form a fused rhabdom (Fig. 1) of the standard grapsid type (Eguchi & Waterman, 1973). The five main screening pigments are the light and dark distal pigments, the retinula cell screening pigment (‘proximal pigment’), the reflecting pigment and the red basal pigment (Figs 1, 2). Two additional coloured structures in the retina have not been investigated further – cells containing dense black pigment which wind widely separated processes about the retinula axon bundles as they cross to the lamina, and distal flecks of iridescent green observed in fresh but not sectioned material. Similar flecks in Squilla were suggested by Schonenberger (1977) to be without optical significance and related only to the superficial colour scheme of the animal.

Between the crystalline cones lie the light distal pigment cells, containing yellow-brown oily droplets (0.2 ± 0.1 μm diam.). They cover all the lateral surface of the cone that is not surrounded by the more proximal darker screening pigments, except at night, when dark adaptation causes retraction from the distal third or so of the cone. Long exposure to ‘dim’ light at night causes the pigment to extend distally; and in bright light extension takes 30 min.

Each ommatidium has two dark distal pigment cells containing dense, membrane-bound purplish granules 0.3 ± 0.07 μm in diameter. The nucleus is in the distal part of the cell. In most conditions these cells surround the cone tip, and the pigmented part of each tapers down to about half-way along the length of the retinula cells, with a fine non-pigmented extension continuing to the basement membrane. An arm of each cell, sometimes containing pigment granules, reaches between the retinula cells, one on either side of R7, the largest retinula cell. The dark distal pigment cells are largely responsible for the size of the ‘iris’ at the cone tip, but the bulk of the cells change very little except under very strong illumination, when pigment granules move into the fine strand that extends to the basement membrane. The diameter of the iris ranges from about 5-13 μm at night to 2.5-7 μm during the day. Light or dark adaptation does not cause a marked change within 30-60 min.

Within the retinula cells themselves are brown screening pigment granules, of 0.3 ± 0.1 μm diameter. These are very mobile, and may be concentrated around the rhabdom or scattered throughout the cell. In dark adapted animals at night, the screening pigment granules of the retinula cells are almost entirely below the base-
ment membrane. Under dim light at night, scattered pigment is present in the distal third of the retinula cells. After 2 min of bright light adaptation, this pigment concentrates around the rhabdom and shifts slightly distally. Some granules move up into the proximal part of the soma from below the basement membrane. Eyes fixed after 5, 15 and 30 min of bright light adaptation show more granules moving up through the basement membrane to accumulate in the distal and proximal thirds of the cell. Scattered granules appear in the central region from 15 min on, but since they do not accumulate there they are presumably moving towards the distal part of the cell, which becomes progressively darker. During the day in dim light, pigmen
Fig. 2. (A) Cross-section through the distal part of a *Leptograpsus* ommatidium. The four unpigmented lobes of retinula cell 8 are seen between the pigment-containing retinula cells 7–9. RP, Reflecting pigment; D, dark distal pigment; R, rhabdom; 7, retinula cell 7. × 2750.

(B) Longitudinal section through the proximal retina of *Leptograpsus*, showing the basement membrane (BM): R, retinula cell; RP, reflecting pigment; RB, red basal pigment; HL, haemocoel lacuna. × 5750.
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...xtends through most of the cell apart from the central region, but is more concentrated distally. Dark adaptation causes the pigment to become less dense, keeping the same general distribution but with less concentration about the rhabdom. Under strong light, the granules first concentrate distally and proximally, within 2 min. During 30 min of bright-light adaptation, more granules continue to move up into the soma from below the basement membrane. The pattern is similar to that produced by light adaptation at night but there are always more granules present. After half an hour in bright sunlight, the distribution is similar but even denser, with very few granules remaining below the basement membrane.

The reflecting pigment is contained within large cells that are usually closely apposed to the retinula cell column over most of its length. A thin strand of tissue containing reflecting pigment granules extends to the cornea. The nucleus lies about half-way down the retinula cell column. The pigment granules, 0.3 ± 0.05 μm in diameter, are a brilliant white in reflected light, but pale brown in transmitted light, and the contents are often lost in EM sections.

At night, the proximal half of each retinula cell column is enveloped by reflecting pigment cells under all conditions examined. However, a progressive change takes place in the distal half. In the dark-adapted eye the only reflecting pigment in the distal half of the eye is in fine threads that run to the cornea, leaving a clear area between retinula cells. In dim light these threads are thickened. After 15 min of strong illumination the clear area is no longer evident, and after 30 min the reflecting pigment is equally distributed between the proximal and distal halves of the retina. This is also the case during the day, under dim light or up to as much as 60 min of dark adaptation. After 2 min of strong light adaptation, the reflecting pigment begins to push up between the dark distal pigment cells. After 15 min there is more reflecting pigment between the retinula cells in the distal half of the retina than the proximal. At 30 min the extreme distal projections of reflecting pigment have been displaced by dark distal pigment. After 30 min in bright sunlight the reflecting pigment cells extend over both proximal and distal halves of the retina. However, they do not completely envelope the retinula cell columns, but present a rather tattered, fragmented appearance. The reflecting pigment cells also send processes below the basement membrane, but when light adapted in the day, these are masked by the basal red pigment.

Beneath the basement membrane the retinula cell axons run through a layer of basal red pigment cells which contain large (from 0.3 to 0.7 μm diam.) oily red droplets, densely packed. When dark adapted at night, the basal red pigment is retracted to some extent (about 20–30 μm) from the basement membrane. Under dim light at night, and dim or bright artificial light during the day, the pigment is below the basement membrane but closely apposed to it. Under bright sunlight, or bright artificial light at night, the red basal pigment cells send processes through the basement membrane, on the outside of the retinula cell column/reflecting pigment cell complex. These processes were seen to extend up to one third of the way along the retinula cells. Two of the eyes adapted to dim light at night showed small patches of red pigment extending up to 15% of the length of the retinula cells. Since some red pigment in unosmicated eyes was extracted by the dehydrating alcohols, the amount of red pigment above the basement membrane is probably under-estimated.
The retinula cell screening pigment and the light distal pigment showed a high extinction (absorbance) in the ultraviolet, decreasing fairly smoothly towards longer wavelengths (Fig. 3 A, B). The light distal pigment had a slightly lower extinction at wavelengths longer than about 500 nm. The dark distal pigment also had its highest extinction in the UV, but there was a rise in absorbance between 500 and 580 nm, peaking at 540 nm (Fig. 3 C). Extinction at the red end of the spectrum was markedly higher than for the retinula cell screening pigment or the light distal pigment. The absorbance of the red basal pigment was low in both the ultraviolet and the red, peaking in the green at about 500 nm (Fig. 3 D). The extinction curve of the reflecting pigment (not illustrated) showed a similar shape to the retinula cell screening pigment, but its reflectivity characteristics were not measured. The spectral transmission of the cornea is shown in Fig. 4.
Changes in rhabdom diameter

During the day, in the light-adapted state, there was little variation in the diameter of the rhabdom from the distal (1·67 ± 0·12 μm) to the proximal end of the rhabdom. Surrounding the rhabdom is a palisade of swollen ER cisternae of 4·5 μm outer diameter. Between the rhabdom and the palisade is a thin 'rind' of cytoplasm, c. 0·3 μm thick, from which the microvilli sprout. At night the diameter of the distal part of the rhabdom increased dramatically to 5·22 ± 0·52 μm, an increase in cross-section area by a factor of 10. Similar increases have been described in *Grapsus* by Nässel & Waterman (1979).
(II) **Electrophysiological responses**

With good impalements, recordings could be made for 30–45 min routinely and sometimes for up to 3 h. The average resting potential, measured for 42 cells, was $69.8 \pm 11.8$ mV.

**Polarization sensitivity**

Of 14 cells tested, nine were preferentially sensitive to vertically polarized light and five to horizontally polarized light. The average polarization sensitivity (PS) of 12 cells for which quantitative data were obtained was 9.0. However, the most common PS (five cells) was between 10 and 11, the distribution being skewed by two cells with very steep $V/\log I$ curves, which produced polarization sensitivities of 2.8 and 2.6, although the response modulation was large. Discarding these cells left the remaining ten with an average PS of 10, ranging from 6.3 to 14.8.

**Angular sensitivity**

The angular sensitivities of 17 cells, measured as $\Delta \rho$, the width of the angular sensitivity function at the 50% level, were tested under various conditions of light and dark adaptation. The average difference in $\Delta \rho$ between consecutive runs on the same cell in a stable adaptation state was $0.2^\circ$. All cells used were in the anteriorly directed part of the retina.

The average angular sensitivity of eight cells measured under dim light during the day was $2.5^\circ$, ranging from $1.5^\circ$ to $3.2^\circ$. $\Delta \rho$ of four of these cells were measured during adaptation to bright light. Over times ranging from 4 to 25 min, the angular sensitivities of three decreased, from $2.1^\circ$ to $1.3^\circ$, from $3.2^\circ$ to $1.9^\circ$, and from $2.4^\circ$ to $1.8^\circ$, while the fourth was unchanged, at around $3^\circ$ after 4 min.

The average angular sensitivity of six cells dark-adapted at night was $2.7^\circ$, ranging from $1.8^\circ$ to $3.7^\circ$. Two cells, initially adapted to dim light, were monitored as...
were dark-adapting. One, tested during the night, showed an increase in $\Delta \rho$ from 2.4° after 7 min DA to 3.5° after 25 min DA. The other, tested during the day, changed from 1.6° to 2.3° to 3.7° over about 15 min DA.

**Spectral sensitivity**

Thirty-three cells were measured while dark-adapted, 21 while adapted to 'dim' light, and 16 during adaptation to 'bright' light. Most cells were tested in more than one adaptation state.

![Graph showing mean log spectral sensitivities of dark-adapted cells at night ($N = 8$) and during the day ($N = 7$), normalized to the response at 520 nm. Bars show S.E.M.](image)

Fig. 5. Mean log spectral sensitivities of dark-adapted cells at night ($N = 8$) and during the day ($N = 7$), normalized to the response at 520 nm. Bars show S.E.M.

**Dark adapted.** The spectral sensitivity of dark-adapted cells was almost the same at night ($n = 8$) as in the day ($n = 7$), in spite of an increase of about 1.5 log units in relative sensitivity (measured as the difference in intensity of the standard stimulus needed to produce a constant response) at 500 nm (Fig. 5). The average $\lambda_{\text{max}}$ of these 15 cells was 484 nm (s.d. 22 nm), and the range of $\lambda_{\text{max}}$ was from about 450
to 520 nm. (The remaining 18 dark-adapted cells were measured around dusk. Their spectral sensitivities seem identical, but they are not further analysed here.)

**Dim-light-adapted.** Cells adapted to dim light had broader spectral sensitivities than dark-adapted cells. They were sometimes double-peaked, and had maxima anywhere between 422 and 537 nm. The peak response was, on average, shifted to longer wavelengths than in a dark-adapted cell, to 520 nm in the day and 500 nm at night. The averaged log spectral sensitivities of ten cells recorded during the day and four

![Graph showing mean log spectral sensitivities of cells adapted to dim light, at night (n = 4) and during the day (n = 10). Bars show S.E.M.](image)

Fig. 6. Mean log spectral sensitivities of cells adapted to dim light, at night (n = 4) and during the day (n = 10). Bars show S.E.M.

cells measured at night are shown in Fig. 6. The shapes of the two curves are not very different, although the day curve is rather flatter. The relative sensitivities are very similar, the day and night groups being less sensitive than cells dark-adapted at night by 2-4 and 2-6 log units respectively.

**Bright-light-adapted.** The general pattern of changes in spectral sensitivity during adaptation to bright light was consistent, but the precise timing and extent of the shifts varied somewhat from cell to cell. During the first 2–5 min the peak sensitivity shifted dramatically to shorter wavelengths, around 360–400 nm. The spectral sensitivity curve usually had two maxima at this stage, the smaller at 500–537 nm. The relative size of the two peaks changed steadily, the shorter-wavelength peak becoming less pronounced over 30–40 min, until the shape of the spectral sensitivity curve approached that of a cell adapted to dim light. The pattern was not as well defined in the averaged results (Fig. 7) as in individual cells (Fig. 8A, B) because of the variation in peak sensitivities and their relative sizes and rates of change from cell to cell.
With the onset of a bright adapting light, sensitivity to white light dropped by up to 4 log units. It recovered rapidly for the first few minutes and more slowly over the next half hour or so, to not far below the dim light-adapted level. It was frequently impossible to measure spectral sensitivity until 2–5 min after the beginning of the

adapting stimulus, because the source did not deliver monochromatic light of sufficient intensity. The increase in sensitivity during bright-light adaptation was difficult to interpret because the adapting light was not axial. This means that the effect of the various pigments moving into new positions was different for the adapting light and the stimulating light.

Fig. 7. Mean spectral sensitivities of retinula cells in dim light (n = 10) and after 2, 10 and 30 min adaptation to bright light (n = 4, 6 and 5 respectively).
Fig. 8. Effects of bright-light adaptation on single cells. (A) The average of three spectral runs on a dim-light-adapted cell, followed by 1 run after 2 min, 2 runs after 10 min, and 2 runs after 30 min of bright-light adaptation. (B) The average of two spectral runs made on the same cell after 5, 21 and 40 min adaptation to bright light.
Calculated effects of pigments on spectral sensitivity

Theory

The amount of light absorbed by a material is given by the relation

$$\log_e \left[ \frac{I_t}{I_i} \right] = \alpha I,$$

where $I_i$ is intensity of incident light, $I_t$ is intensity of transmitted light, $I$ is path length of the light, and $\alpha$ is an absorption (extinction) coefficient that depends on the absorptive power of the material and the wavelength of the light.

The absorbance, or optical density $D$, is given by:

$$D = \log_{10} \left( \frac{I_t}{I_i} \right),$$

from (1)

$$D = \log_{10} (e^{-\alpha I}),$$

and the absorbance at a given wavelength $\lambda$ is:

$$D(\lambda) = \log_{10} (e^{-\alpha \lambda I}).$$

The theoretical absorbance of a thin layer of rhodopsin can be found from a nomogram (Dartnell, 1953) that uses absorbance values measured for a photopigment of one $\lambda_{\text{max}}$ to generated absorbances for a photopigment of any $\lambda_{\text{max}}$.

The absorption, $A$, is the fraction of light absorbed:

$$A = \frac{I_i - I_t}{I_i},$$

and from (2)

$$A = 1 - 10^{-D},$$

and

$$A(\lambda) = 1 - 10^{-D(\lambda)}.$$

The normalized function $A(\lambda)/A(\lambda_{\text{max}})$ is the spectral sensitivity.

The theoretical effects of screening pigments on visual pigment absorption have been discussed by Goldstein & Williams (1966), and this analysis applied to some crustacean superposition eyes by Goldsmith (1978).

The functions given by Goldsmith (1978), for a rhodopsin of absorbance $D(\lambda)$, and a pigment screen of absorbance $D'(\lambda)$ are, for an overlying pigment screen:

$$A(\lambda) = 10^{-D'(\lambda)}[1 - 10^{-D(\lambda)}],$$

and for a homogeneously distributed pigment:

$$A(\lambda) = \left[ \frac{D(\lambda)}{D(\lambda) + D'(\lambda)} \right] \left[ 1 - 10^{-D(\lambda) + D'(\lambda)} \right].$$

In the case of an overlying screening pigment, the visual pigment obviously 'looks' only at the light which has already been subject to absorption by the screen. For a dispersed pigment, i.e. one that is homogeneously distributed within the rhabdom, the visual and screening pigments are treated as if they have simultaneous access to the incoming light. The effects of the screening pigments can be most easily modelled if they are treated as being in one of these two extreme positions, which may be close to the real situation in some cases – all the light distal pigment lies above the rhabdom,
for instance and much of the dark distal pigment. In some circumstances metarhodopsin may well be uniformly dispersed in the rhabdom. More often, the situation is somewhere between the two – the retinula cell screening pigment for instance, is usually concentrated just distal to the rhabdom, but it may also lie beside the rhabdom along the length of the retinula cell. While the effect of such a ‘longitudinal pupil’ in attenuating light transmission through the rhabdom is well established (Franceschini & Kirschfeld, 1976; Hardie, 1979) and can even be used as an experimental tool, there is no firm theoretical explanation for it, just as there is no definitive theory of ommatidial optics. Such pigment is treated here as if it acted according to equation (9), that is as if it has access to the incoming light at the same time as the rhodopsin. No allowance is made for mode propagation effects, or for possible differences in the absorption spectra for reflection and transmission. The result should be approximately valid for the retinula cell screening pigment, which lies very close to the rhabdom when in the light-adapted state.

The method used to measure the absorbance of the screening pigments yields the relative absorbance, $D'(\lambda)/D'(\lambda_{\text{max}})$, but not the absolute value of $D'(\lambda_{\text{max}})$, the absorbance at peak wavelength, since the thickness of the pigment being measured is not known. In the calculations, $D'(\lambda_{\text{max}})$ was therefore varied, within reasonable physiological limits, to find the best fit with the measured spectral sensitivities of the retinula cells. The results are presented as percentage spectral sensitivity, rather than on a log scale to show relative sensitivities, for several reasons: differences in the shape of the spectral responses are more apparent, and the relative sensitivities measured could have been affected by changes in angular sensitivity, since a point source was not used, and perhaps by disruption of the normal pattern of membrane turnover by the unnatural light conditions.

The peak sensitivity of rhodopsin was taken as 485 nm, from the measured spectral sensitivity of dark-adapted cells. To find the optical density of the rhabdom at this wavelength, it is necessary to know the coefficient of absorption, $\alpha$ (equation 3). This is not available for Leptograpsus, but Bruno et al. (1973) have measured the coefficient of absorption in isolated rhabdoms of the crab Carcinus as 0.6% per μm at the peak wavelength. This is considerably lower than the absorption coefficient measured in the spider crab Libinia by Hays & Goldsmith (1969) as 1.3% μm. The Carcinus coefficient is used here because Carcinus is closer to Leptograpsus phylogenetically, ecologically and morphologically than is Libinia. Taking the average length of the rhabdom as 350 μm, the axial optical density of the rhabdom is found, from equation (4), to be 0.91, and the absorption, from equation (6), is 88%.

Briggs (1961) found a metarhodopsin of $\lambda_{\text{max}}$ 495 nm in Leptograpsus eye extract. Microspectrophotometric measurements on Libinia (Hays & Goldsmith, 1969), revealed a metarhodopsin with maximum absorbance at slightly longer wavelengths than the $\lambda_{\text{max}}$ of the rhodopsin, which was 493 nm. It therefore seemed reasonable to use Briggs’ value of 495 nm as the peak absorbance of the metarhodopsin.

Self-screening by rhodopsin

The effect of self-screening on the absorption of rhodopsin is given by equation (7). Fig. 9 shows the normalized absorptions of an unscreened 485 nm pigment calculated from Dartnall’s (1953) nomogram, a 485 nm pigment of peak optical density 0.91,
corrected for self-screening, and the pooled spectral sensitivity measurements from
day and night dark-adapted cells. The experimental data are closely approached by
the theoretical curve corrected for self-screening. It can be concluded that the dark-
adapted spectral sensitivity is the result of the rhodopsin alone, and that screening
pigments do not appreciably alter spectral sensitivity in this state.

Fig. 9. The effect of self-screening on the absorption of rhodopsin. Thick line, theoretical
spectral sensitivity of a thin layer of rhodopsin of $\lambda_{max} 485$ nm, calculated from Dartnell's
nomogram; thin line, theoretical absorption of rhodopsin of $\lambda_{max} 485$ nm and optical density
0.91, showing the effects of self-screening; squares, averaged spectral sensitivity of dark-
adapted 15 cells (day and night).

Screening by metarhodopsin

The distribution of metarhodopsin within the rhabdom under conditions of con-
stant illumination depends on the rate of photoregeneration of rhodopsin from
metarhodopsin along the rhabdom, and the rate of metabolic regeneration. Both
processes are known to occur in decapods – in Homarus for example (Bruno, Barnes
& Goldsmith, 1977) – but the rate constants and the relative importance of the two
processes vary widely among arthropods, and there are no data available for grapsid
crabs. In insects, the distribution of metarhodopsin along the rhabdom is usually
strongly dependent on the wavelength of the stimulating light (Hamdorf, Paulsen &
Schwemer, 1973), but in crabs, where the peak absorbance of the rhodopsin and the
metarhodopsin are very close, this factor must be of much less importance. The
effect of metarhodopsin on absorbance was therefore calculated in the two extreme
conditions, without supposing that there was any firm justification for either. The,
actual situation presumably lies somewhere between the two. In both cases the total
optical density of rhodopsin and metarhodopsin was held constant at 0.91, while the
relative proportions were varied.

A homogeneous mixture of metarhodopsin and rhodopsin produces spectral sen-
sitivity curves with peaks shifted to slightly shorter wavelengths (c. 470 nm) than the pure rhodopsin absorbance. There was little change in the shape of the normalized spectral sensitivity curve for optical densities of metarhodopsin ranging from 0.4 to 0.9999, i.e. well beyond the physiological limits. If metarhodopsin is treated as an overlying pigment (Fig. 10) the spectral sensitivity curve becomes broader, because of relatively increased absorption at short wavelengths, and the peak is shifted to c. 440 nm, developing a shoulder at about 520 nm.

![Graph showing spectral sensitivity curves](image)

Fig. 10. Calculated effect of varying optical densities of a metarhodopsin of \( \lambda_{\text{max}} \) 495 nm overlying a rhodopsin \( \lambda_{\text{max}} \) 485 nm. Total optical density is 0.91, indicated values are metarhodopsin optical densities, N is spectral sensitivity calculated from the 485 nm nomogram.

However, if the accompanying reduction in absolute absorption is taken into account, the spectral sensitivity curves generated by rhodopsin–metarhodopsin mixtures do not resemble any obtained by recording from cells.

**Screening by retinula cell screening pigment**

As a homogeneously distributed filter, at optical densities \( (D) \) up to 3.0, the effect of this pigment is to make the spectral sensitivity slightly narrower than that of pure rhodopsin, without appreciably altering the position of the peak absorption. As an overlying screen (Fig. 11) the pigment causes the spectral sensitivity to become narrower, and the peak shifts to longer wavelengths. An increase in pigment optical density from 0.01 to 3.0 produces a shift in \( \lambda_{\text{max}} \) from 485 to 520 nm, with a decrease in absorbance of about 2 log units at 500 nm.
Screening by the dark distal pigment

As an overlying pigment, the dark distal pigment at optical densities up to 2.0 produces a slight narrowing of the spectral sensitivity curve, with negligible shift in the position of peak absorption (Fig. 12). The effect of the pigment as a homogeneously
distributed filter was not tested, since it did not correspond to any of the observed positions of this pigment.

**Screening by the red basal pigment**

Since the red basal pigment is fairly far removed from the rhabdom even when it extends distally between ommatidia, it is difficult to determine its position in the light path and hence the appropriate way to analyse its effect. Nevertheless it undergoes dramatic changes in distribution on strong light adaptation, sending distal projections one-third to one-half of the way along the ommatidia. It is quite possible that the extent of these changes was not accurately determined by the techniques used. The effect of the red pigment was modelled as an overlying screen and as a homogeneously distributed filter, assuming a rhodopsin of optical density 0.4, since some bleaching might be expected under these conditions. Treated as a homogeneously distributed pigment, with an optical density of 1.0, the red basal pigment produces a slight upwards kink in the spectral sensitivity curve at about 380 nm. As an overlying pigment, it produces a sharp peak at 380 nm, increasing in prominence with increasing optical density of the pigment. Comparison of Fig. 13 with the changes in the spectral sensitivity of individual cells during bright light adaptation (Fig. 8) shows the similarity of the effects.
Screening effects of the light distal pigment and the reflecting pigment

The shapes of the absorption curves of these pigments are very similar to that of the retinula cell screening pigment, differing only slightly in slope. Their effect has not been calculated separately, since the light distal pigment varies little in position and the reflecting pigment probably transmits a negligible amount of light.

![Graph](image)

Fig. 14. Calculated effects of a combination of varying optical densities (indicated) of retinula cell screening pigment and metarhodopsin of optical density 0.5 overlying rhodopsin of optical density 0.4.

Screening effects of combination of pigments

It is unlikely that the screening pigments in an eye such as the crab’s normally act in isolation, and given the variety of effects produced by single pigments, it should be possible to combine them to produce almost any spectral sensitivity curve. Conversely, most spectral sensitivity curves could probably be produced in several ways, so juggling combinations of pigments might seem to be a rather pointless exercise.

Nevertheless, one combination that could reflect a physiological situation is the effect of increasing optical densities of retinula cell screening pigment on a mixture of rhodopsin and metarhodopsin (Fig. 14). With a metarhodopsin optical density of 0.5, the curve produced is much broader than that of either pigment acting in isolation. It has broad maxima at around 440 and 520 nm, which change in relative height as the optical density of the retinula cell screening pigment is increased from 0.5 to 2.0.

While this combination of pigments does not necessarily reflect a physiological situation, it does provide one possible explanation for the spectral sensitivities measured in dim light. These were broader than the dark-adapted spectral sensitivities, and showed a range of peak sensitivities.
Most of the screening pigments of the *Leptograpsus* eye appear comparable to those described and sometimes chemically identified in other Crustacea. The exception is the light distal pigment, which does not seem to occur commonly in Crustacea. The yellow pigment granules described in the chromatophores of *Crangon* (Elofsson & Kauri, 1971) are not structurally similar, as they contain distinct inclusion bodies. The granules of the dark distal pigment cells and the retinula cells are similar in size and structure to those containing ommochromes in other Crustacea (Elofsson & Hallberg, 1973). The absorption spectrum of the dark distal pigment is very similar to that of ommin (Butenandt, Biekert & Linzen, 1958), which is probably the main constituent of the granules. The retinula cell pigment granules, with a higher transmission at longer wavelengths, probably contain a significant amount of the red and yellow xanthommatins. The red basal pigment droplets are structurally similar to those found in several species of mysid shrimps by Hallberg (1977), in *Oplophorus* (Land, 1976) and in the leptostracan *Nebalia* (Green, 1972). Basal red pigment is a major part of the pigment system of many crabs, including *Leptograpsus*, *Scylla*, *Ozius*, *Ocypode*, and *Mictyris* (unpublished observations). Such pigments are believed to be carotenoids, as are the red pigment-containing chromatophores of the body. Briggs (1961) found the carotenoid astaxanthin in extracts of *Leptograpsus* eyes. The white reflecting pigment of a number of brachyuran crabs has been analysed by Zyznar & Nicol (1971) to consist largely of pteridines, which fluoresce in long-wavelength ultraviolet light, and a smaller amount of purines, which quench short-wavelength ultraviolet.

The retinula cell screening pigment, the reflecting pigment, and the red basal pigment, are responsive to changes in the light level during both day and night. This might be expected, since the typical feeding and activity pattern of *Leptograpsus* is much more dependent on the tide than on the ambient light level. During the day, the animals are found in a variety of light conditions, from deep inside dark crevices to flat rocks fully exposed to the midday sun. However, since there is also a difference in the behaviour of the screening pigment complex (apart from the retinula cell screening pigment) during the night and the day, the position of the pigment is not only determined by the ambient light level, but is under some degree of control by a circadian rhythm.

The dark-adapted spectral sensitivity of *Leptograpsus* can be adequately explained as the product of an unshielded rhodopsin of $\lambda_{\text{max}}$ 485 nm. This peak sensitivity is to slightly shorter wavelengths than the 493–508 nm range that has been found in the six other crab species in which spectral sensitivity has been tested electrophysiologically.

The measured spectral sensitivities under various adaptation conditions are interpreted here as being due to the interaction of rhodopsin, meta-rhodopsin, and various screening pigments. However, other factors which might under some circumstances affect the measured spectral sensitivity must be considered.

Perhaps the most obvious of these additional factors is absorption by the cornea. The percentage transmission of the relatively thin *Leptograpsus* cornea varies by only 5% at wavelengths above 350 nm, and at the shortest wavelength used during measurement of receptor sensitivity, 317 nm, transmission is still as high as 70%.
Retinal pigment movement in the crab

Transmission of the cornea is therefore assumed to be flat over the range of wavelengths used in these experiments.

The effect of absorption by the reflecting pigment has not been considered. This pigment looks brown in transmitted light, and its absorption increases steadily towards shorter wavelengths. Kong & Goldsmith (1978) suggest that in the white-eyed crayfish the equivalent pigment can absorb light when it is within a few micrometres of the rhabdom, shifting the peak sensitivity about 30 μm towards longer wavelengths. In *Leptograpsus* the reflecting pigment is well removed from the rhabdom.

Waveguide effects (Snyder & Miller, 1972; Snyder & Pask, 1973) have not been considered, since even the day rhabdom is large enough to render them relatively unimportant, and since frequent checks were made to ensure that the stimuli were on-axis, there should have been no error due to non-axial stimulation, as described by Eguchi (1971) and Snyder & Pask (1972).

The rhabdom of R8 lies over that of R1–7, and it must be exercising some filtering effect. However, since it is something less than 5 μm long in *Leptograpsus*, this can hardly be significant.

The changes in spectral sensitivity that occur in *Leptograpsus* under the various conditions of light and dark adaptations during the day and night seem to be explicable, at least in broad outline, by the effects of pigments movement, absorption by metarhodopsin, and self-screening, assuming a rhodopsin of peak absorbance 485 nm. These factors can combine to maintain a broad, roughly constant spectral sensitivity over a wide range of ambient light intensities, although it may take about 30 min to stabilize after a sudden, large increase in illumination. The light-adapted spectral sensitivity curve is broader than the dark-adapted curve, and the peak sensitivity is shifted, on average, to longer wavelengths, although there is a wide variation between cells. The shift in λ_max is similar to, but less pronounced than, the effect produced by the 'red-brown screening pigments' in light-adapted crayfish (Kong & Goldsmith, 1978; Goldsmith, 1978a). In *Leptograpsus* it is demonstrated that the shift is probably produced by retinula cell screening pigment, and not by the dark distal pigment.

Broadening of the spectral sensitivity curve, and the maximum at shorter wavelengths, could be achieved by contributions from several other pigments. The effect of metarhodopsin acting as an overlying filter, as it would do to some extent if it was unequally distributed along the rhabdom, was modelled, but no other pigment has been examined in combination with the retinular cell screening pigment. Before such analysis could be useful, it would be necessary to know more about the metarhodopsin, and rhodopsin regeneration mechanisms in *Leptograpsus*.

From the data available for cells adapted to dim light, one cannot say whether the observed variation in spectral sensitivity is always present, or if most of the cells are in the same state at any one time. It is also quite possible that there is less variation in a completely undamaged eye.

The only pigment found to have increased transmission in the ultraviolet range is the red basal pigment. Calculation of its effects on absorption by the rhodopsin indicates a small increase in sensitivity at 380 nm when the pigment is treated as if it were homogeneously mixed with the photopigment, and a very pronounced peak in the same place if it is treated as an overlying screen. It is likely therefore to be the cause of the sharp, transient peak around 380 nm that accompanies adaptation to...
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bright light. Movement of retinula cell screening pigment could then cause the progressive attenuation of the 380 nm peak. The evidence for the involvement of the red basal pigment is purely circumstantial. It has the right absorbance characteristics, it moves into a more prominent position in the retina under the appropriate conditions, and no other possible cause presents itself. However, the position of the red basal pigment, as described here, is still rather far removed from the rhabdom, and it is hard to see, assuming conventional ideas about light paths within the eye, how the effect produced is so large. Other techniques should now be used to determine the position of pigments in these various states with more certainty.

In spite of this, it is reasonable to conclude that the optical properties of the pigments, and the response of pigment-containing cells to light, can in principle provide sufficient explanation for the observed variation in spectral sensitivity of *Leptograpsus* retinula cells R1-7. It can also be concluded that this variation cannot reasonably be supposed to provide the input necessary for colour vision. Colour vision has been demonstrated behaviourally in another brachyuran crab, *Uca*, by Hyatt (1975), and two spectral classes of retinula cells have been found electrophysiologically in the crayfish *Procambarus* (Nosaki, 1969; Waterman & Fernandez, 1970; Muller, 1973), although the anatomical localization of the short-wavelength receptor is still uncertain. If there is a blue or violet receptor in crabs, the only possible location would seem to be the small distal retinula cell, R8, which was not recorded from in this study. Evidence that R8 is the short-wavelength receptor in decapods is reviewed by S. R. Shaw and S. Stowe (in preparation).

The angular sensitivities of light-adapted crab eyes in the central region of the eye are similar to those found in many fast-flying diurnal insects, e.g. *Musca* 2.5° (Scholes, 1969), *Calliphora* R1-6, 1.5° (Hardie 1979), dragonfly 1.3-1.5° (Laughlin, 1975), bee 2.7° (Laughlin & Horridge, 1971). During the day, light adaptation produced narrowed (to less than 2°) angular sensitivities, and dark adaptation widened (to 3-4°) angular sensitivities. One cell monitored during dark adaptation at night showed an increasing acceptance angle. It seems fairly safe to conclude that the angular sensitivity changes in response to light levels during both night and day, but, at least in the central area of the retina, the changes take place over a fairly narrow range. No stable cells had an acceptance angle greater than 4° under any conditions. This may imply that *Leptograpsus* can maintain acuity of vision to a greater extent at night than say, *Scylla*, in which Leggett (1978) found a change in dark-adapted acceptance angles from 4° in the day to 10° at night. Both crabs have much smaller dark-adapted acceptance angles than the crayfish *Cherax* (Walcott, 1974), in which the acceptance angle changes from 3° when light-adapted to 24° when dark-adapted.

The wider angular sensitivities measured in dark-adapted eyes could be caused both by an increase in the acceptance angle of the rhabdom tip, and by a decrease in the optical isolation of the retinula cell columns, allowing scattered light to enter the rhabdom. Three pigments are in a position to influence the acceptance angle of the rhabdom tip: the light distal, dark distal, and retinula cell screening pigments. There is little variation in the position of the light distal pigment under any conditions. The dark distal pigment forms an aperture above the rhabdom tip that is larger at night than in the day, but there is no evidence that the size of this aperture varies in response to light intensity. The retinula cell screening pigment, particularly that part
Retinal pigment movement in the crab

Which is concentrated in the distal part of the cell, moves rapidly both radially and longitudinally in response to light. In the light-adapted state, much of this pigment lies distal to the tip of the rhabdom. There is evidence of its role in the regulation of angular sensitivity in other Crustacea (de Bruin & Crisp, 1957; Walcott, 1974) as well as in insects (Kolb & Autrum, 1972). The amount of light scattered within the retina, and the amount of leakage from the retinula cell columns, seems from the anatomy of the eye to be primarily controlled by the reflecting pigment. In the light-adapted eye this pigment forms a close sleeve around the retinula cell columns, while in a dark-adapted eye at night the pigment is largely retracted from the distal part of the retina. The nocturnal increase in rhabdom diameter might be expected to produce some increase in dark-adapted angular sensitivity. Although there were slight indications of this, insufficient cells have been measured to quantify the effect, which cannot be large, and is masked to a large extent by the variation in angular sensitivity across the retina.

The most commonly found PS values, between 10 and 11, are similar to those found by Shaw (1969) in Carcinus and Ovalipes. Theoretical analysis of fused rhabdoms by Shaw (1969) has demonstrated that a high PS is only to be expected, since selective absorption in different rhabdomeres is sufficient to counteract the effect of self-screening, however long the rhabdom. The PS in much a rhabdom should be equal to the dichroism of the photopigment in the membrane (Snyder, 1973). Part of the variation in measured values could come from distortions in the alignment of the rhabdom produced by the process of recording, although this can only decrease the PS.

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