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# THE EYE OF THE GRASS SHRIMP *PENAEUS MONODON*

## —A Reappraisal of the Penaeid Eye[1]

J. A. COLIN NICOL* and HONG-YOUNG YAN**

*Institute of Zoology, Academia Sinica, Nankang*
*Taipei, Taiwan 115 R. O. C.*

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**J. A. Colin Nicol and Hong-Young Yan** (1981) The eye of the grass shrimp *Penaeus monodon*. A reappraisal of the penaeid eye. *Bull. Inst. Zool., Academia Sinica* **21**(1): 27-50. The eye of *Penaeus monodon* has been examined by conventional light and electron microscopy, and the eye pigments have been analyzed. The eye of a mature prawn (15 cm total length) contains over 80,000 ommatidia. There is a very wide clear zone (1 mm); change from an apposition (light-adapted) to a superposition (dark-adapted) condition is brought about by retraction of proximal screening pigment below the basilar membrane. This takes about 90 min whereas pigment expansion occurs in 4 min. In the superposition eye light rays are bent in the crystalline cones by total internal reflexion and converge upon the layer of retinular cells. There are three reflectors which contain pteridines (chiefly isoxanthopterin) in different physical form; screening pigments are ommins. Distal and proximal screening pigments act as stops in the apposition condition, restricting the light entering a facet to its retinular array, with an angle of acceptance of 1.5°. In the superposition condition, the proximal reflecting pigment scatters light into neighbouring ommatidia and produces eye glow, visible over half the diameter of the eye. The external reflector contributes to the external appearance of the eye: this shows a regular array of dark spots between which the white reflector is visible (bordering the facets), and over which it is much diminished. The internal reflector and screening pigment in the retinular cell axons oppose retrograde light leakage from the optic stalk. Some features of penaeid eyes are compared interspecifically.

The grass shrimp or tiger prawn *Penaeus monodon* Fabricius is a shallow water species of much commercial importance in Taiwan. The annual catch in 1978 was 1686 tonnes[46] and it is intensively cultured. It occurs in costal waters of less than 70 m over muddy bottoms. Essentially nocturnal, it displays a circadian rhythm of locomotor activity for which light appears to be the main synchronizer. Because of this light-regulated behaviour, it was judged that a study of the lateral eyes of the grass shrimp would be useful. Eyes of penaeid prawns have received much less attention than those of Caridea and Reptantia (the earlier literature is reviewed by Waterman[53]).

The morphology of the eyes of surface-dwelling penaeid prawns (*Metapenaeus* and *Penaeus*) has been investigated several times[41, 56]; it agrees in many respects with that of carideans[8,17,47]. The histology of the eyes of a mesopelagic penaeid *Gennadas* has also been described[35], and the optical mechanisms operating in compound eyes of higher Crustacea have been reviewed[30].

The dioptric appearatus of the ommatidium

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* Ribby Lerryn, Lostwithiel, Cornwall PL22 OPG England.
** Department of Zoology, The University of Texas at Auatin, TX 78712 U. S. A.

of a mature penaeid prawn consists of a square corneal facet, two corneagenous cells, four crystalline cone cells, a quadripartite crystalline cone and a crystalline tract. The cones are largely surrounded by distal retinal pigment cells which contain a dark distal retinal (screening) pigment. The crystalline tract (part III of the crystalline lens of Ramadan[41]) is very long and tapers distally to a fine thread[56].

The four strands of the crystalline tract are inserted within the photoreceptor unit of an ommatidium. There are seven retinular cells containing a dark proximal retinal pigment. The retinular complexes of individual ommatidia are separated from each other by reflecting or interstitial cells which contain a proximal reflecting pigment (limited to the inner two-fifths of this region of the ommatidium, see Fig. 14). These cells constitute the proximal reflector (the middle reflector of Zyznar[56]). Inside the basilar membrane, a dark pigment similar in appearance to the proximal retinal pigment occurs within the retinular cell axons. A third reflector (the proximal reflector of Zyznar[56]) lies between the layer of primary receptor axons (fasciculated zone of Debaiseux[8]) and the lamina ganglionaris; it is termed the internal reflector in this paper. Chiasmata in the dorsoventral plane connect the lamina ganglionaris to the medulla externa, and the latter to the medulla interna.

Retinal screening and reflecting pigments migrate in part or whole in decapods. Only the proximal retinal pigment makes conspicuous excursions in *Penaeus*. According to Zyznar[56], the internal reflector moves distally (about 1.7X) around the primary optic fibre bundles of the fasciculated zone during light-adaptation, while the proximal reflecting pigment retreats slightly.

The distal retinal (screening) pigment found in some prawns is capped by a narrow zone of distal reflecting pigment[25,56]. In *Penaeus setiferus* the outer ends of the distal pigment cells contain a zone, about 5 $\mu$m long, of reflecting granules. Outwardly, these cells present the appearance of a reflecting grid[56]. All three reflectors appear to be diffuse reflectors.

Penaeid prawns are believed to have reflecting superposition eyes like those of the crayfish *Astacus*, the reflectors of which are four orthogonally arranged mirrors surrounding the crystalline cone[27,30,49,51]. It is not established that the distal reflecting pigment of *Penaeus*[56] is part of or continuous with the cone mirror, as described by Vogt[49,51] in *Astacus*.

Ommochromes occur in the eyes of *Penaeus* and other decapods, where they act as screening pigments[5,14]. The ocular reflectors of *Penaeus* contain pteridines. The eyes of *Homarus* and *Astacus* contain, *inter alia*, xanthopterin and purines, viz. uric acid, xanthine and hypoxanthine[4,15,22,23,57].

## MATERIALS AND METHODS

Live *Penaeus monodon* were purchased in Taipei from wholesalers, who kept them in tanks. Preserved young (larvae and juveniles) of this prawn was obtained from private hatchery farms in southern Taiwan and the Tainan Fish Culture Station of Taiwan Fisheries Research Institute.

### Microscopy

For light microscopy, eyes were preserved in acetone, ethanol, 10% formalin plus 1% $CaCl_2$, ethanol-formalin 9:1, Baker's formol-Ca-Cd, Bouin's, Zenker's, Carnoy's fluids, and the fixatives for electron microscopy described below. Material was embedded in gelatin, paraffin wax, Peterfi's celloidin-paraffin, and celloidin. Sections were cut on rotary and freezing microtomes. They were stained with alum haematoxylin, eosin Y, Mallory's triple, Masson's triple, Heidenhain's iron haematoxylin, Alcian blue, thioinin, Feulgen and PAS (periodic acid Schiff). Formalin-fixed frozen sections were treated with oil red 0, Sudan blue and $OsO_4$ for lipids, the Schultz histochemical variant of the Liebermann-Burchardt reaction and the perchloric acid-naphthoquinone method for cholesterol[18,38].

For transmission electron microscopy, eyes were preserved with 5% glutaraldehyde in 0.05M cacodylate buffer containing 4.5% sucrose, pH

7.2, or in Sorensen's phosphate buffer, 0.07 M pH 7.0, containing 4.5% sucrose, followed by 2% $OsO_4$ in the same buffer. The material was dehydrated in alcohols, transferred to propylene oxide and embedded in Spurr's low viscosity embedding medium[7,43]. For scanning electron microscopy, eyes were preserved in 0.1 M cacodylate buffer pH 7.1, followed by 1 or 2% $OsO_4$, dehydrated in an ethanol series, fractured at room temperature or in liquid nitrogen, and critical point dried.

**Light- and dark-adaptation**

Shrimps were held in an aquarium facing a north window where the illumination reached 20 klx at midday. In the tanks the illuminance fell to about 1/3rd this value. To dark-adapt them, they were kept in darkness for 3 to 4 hours. To follow the course of light-adaptation, they were exposed to photoflood lamps, 300 W, colour temperature 3200°K, illuminance 5 klx for 1, 2, 4, 8, 16 and 32 min, and eyes were preserved. To follow the course of dark-adaptation, light-adapted shrimp were placed in darkness for 1, 2, 4, 8, 16, 30, 60, 90 and 120 min, and eyes were preserved. In another experimental series, shrimp were exposed to light of 5 klx for 1, 2 sec and 1 min, followed by 15 to 12 min in the dark (total of 16 min, light plus dark), and eyes were preserved. When the effect of different intensities was under study, the shrimp were placed at various distances from the source (photolamps or tungsten blue (daylight) lamps). Shrimp were killed by dipping heads in hot water (70°), and the eyes were preserved in Bouin's fluid.

Isolated eyes, from light- or dark-adapted shrimp, were immersed in 70% sea water. They were kept in darkness, or were illuminated as described above, and fixed in hot Bouin's fluid (70°).

The experiments were carried out between 1130 h and 1600 h. Room temperature was about 27°C.

**Chemistry**

PTERIDINES, PURINES. Whole eyes extracted in 0.5 M $NH_4OH$, 0.1 M NaOH and 0.1 M HCl. Extracts were centrifuged, 3000 rpm, 10 min, 3X and supernatants recovered.

Eyes preserved in methanol were bisected and tissues from three regions were collected in methanol, viz. an inner region containing the internal reflector and the fasciculated zone of primary receptor axons; a middle region containing retinular cells, proximal reflector and the inner parts of the crystalline tracts; and an outer region (cortex) containing the outer parts of the crystalline tracts, the crystalline cones, distal retinal pigment and distal reflector. Material from these three regions was extracted in 0.5 M $NH_4OH$ or 0.1 M NaOH 2X, centrifuged 3000 rpm, and supernatants were pooled.

Aliquots of extracts in Tris buffer, pH 8.0, were treated successively with xanthine oxidase, guanase and uricase, and absorption at 248, 272 and 290 nm, indicative of purines, was monitored. Xanthopterin was assayed by adding xanthine oxidase (1 drop per ml) and following changes of absorbance at 330 nm[40].

Extracts were chromatographed on Whatman No. 1 paper in solvent systems *n*-butanol-acetic acid-water (BAW) 4:1:1 and 3:1:1, methanol-HCl-water 7:2:1, 10% ammonia-*n*-propanol 1:2 (v/v), water and 3% $NH_4Cl$. Chromatograms were visualized under UV light.

OMMOCHROMES. Three eys, preserved in methanol, were squashed in 2 ml of methanol-2% HCl. The extract was centrifuged (3000 rpm, 10 min), and the supernatant was re-extracted with 1 m of the same solvent. Supernatants were red and non-fluorescent. They were pooled, and chromatographed on paper (vide infra). The extract was then added to a column of Sephadex LH-20, 9×0.7 cm, equilibrated with methanol. The column was successively eluted with methanol, 0.01, 0.1 and 0.5 M HCl.

Extracts were chromatographed on Whatman No. 1 paper in solvent systems 80% HCOOH-methanol-concentrated HCl 80:15:0.5, and methanol-pyridine-morpholine-water 10:40:40:70 (v/v/v or v/v/v/v).

LIPIDS. Twenty eyes were homogenized in

15 ml $CHCl_3$-$CH_3OH$ 2:1; the beri was centrifuged (3000 rpm, 15 min) and the pellet re-extracted 2× with 5 ml of the same solvent. Supernatants were pooled and treated by the method of Folch *et al.*[13]. The material was transferred to $CHCl_3$, the solvent was removed in a rotary evaporator, and the lipids were taken up in a small amount (ca 5 ml) of petroleum ether (BP 60°-80°). An aliquot was chromatographed on thin layer silica gel (Merck, 20×20 cm×0.25 mm) in solvent system petroleum ether-diethyl ether-acetic acid 80:20;1. Standards were cholesterol, cholesterol oleate, cholesterol palmitate and triolein. Visualization was by $I_2$ vapour and charring[33,44].

Cholesterol in extracts was resolved by GLC against authentic cholesterol on a column containing 3% SE-30/Chromosorb. The analysis was carried out with temperature rise from 130° to 270° at $8°C^{-1}$, the carrier gas was $N_2$, flow rate 17 ml $min^{-1}$.

Ultraviolet/visible spectrophotometers used were Hitachi Models No. 100-50 and H-204A. The gas chromatography was a Varian Aerograph Series 2800, with a flame ionization detector.

Reference standards and enzymes used were xanthopterin, isoxanthopterin, guanine, xanthine, hypoxanthine, uric acid, cholesterol, xanthine oxidase EC 1.2.3.2, guanase EC 3.5.4.3, uricase EC 1.7.3.3, from Sigma Chemical Co., and cholesterol esters from BioScience Laboratories, College Sta, Pa. 7, 8-Dihydroxanthopterin was a gift from H. S. Forrest. For a xanthommatin standard, the heads of 10 *Drosophila melanogaster* were extracted with 5 drops methanol-2% HCl. The extract was centrifuged, and the supernatant applied directly to paper[48].

## OBSERVATIONS AND RESULTS

### General morphology

Commercial size shrimp in Taiwan are about 15 cm long and weigh 20 g. Eyes normally lie 45° to the longitudinal axis in dorsal view, and are horizontal (Fig. 1). An eye is kidney-shaped, approximately hemispherical, and faces outwards (Fig. 2). Dimensions are: dorso-ventral, 5 mm; antero-posterior, 6 mm; lateral, 4.7 mm. The weight is 0.08 g. The stalk is flattened horizontally.

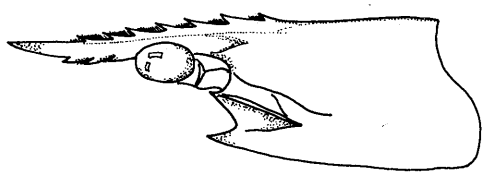


Fig. 1. Lateral view of head and eye of *Penaeus monodon.*

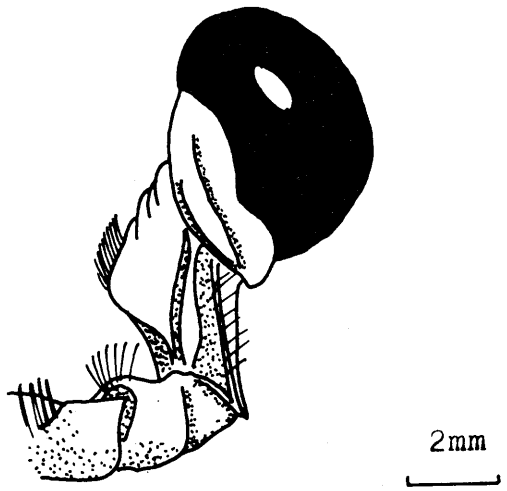



Fig. 2. Dorsal view of right eye of *P. monodon.*

Facets are square (Fig. 4), 37 μm at the lateral (outer) pole, where there are 740 $\mu m^{-2}$. They progressively decrease in size towards the periphery where they are 22 μm square and there are 2000 $\mu m^{-2}$. The eye contains about 85,000 ommatidia. At the lateral pole, the rows of facets lie 45° to an axis passing through the stalk to the anterior pole, and 45° to the horizontal plane. Proceeding towards the margin, an occasional row is lost, and regularity is regained, four rows replacing five.

### Microanatomy

The general appearance of the eye in section is shown in Fig. 3. The thickness of the cornea is 20 μm. It contains a thin cuticle and a thick stroma which shows fine striae. Cuticle and stroma show up differently under Nomarski optics, indicating dissimiliar retardations, and

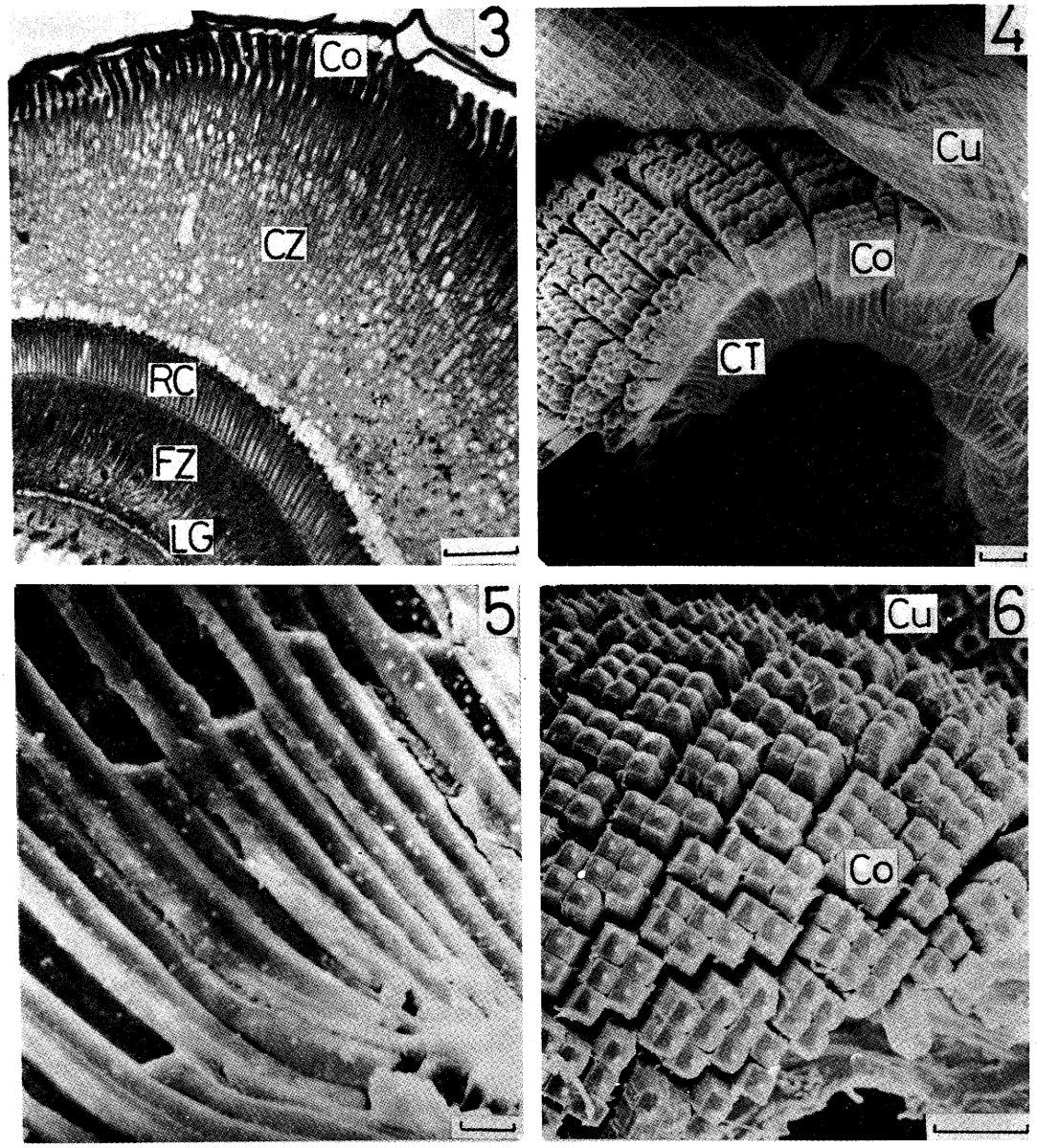



Fig. 3. Radial section of the eye of *Peraeus monodon.* Paraffin section, light micrograph. Bar=200 $\mu$m.

Fig. 4. Scanning electron micrograph (SEM) of cortex, showing cuticle, cones and crystalline tracts. Bar=50 $\mu$m.

Fig. 5. Crystalline tracts. Bar=5 $\mu$m.

Fig. 6. SEM of crystalline cones. Note nipple-like appearance of corneagenous cells, which fit into depresion of the cuticle. Bar=50 $\mu$m.

they stain differently, the cuticle orange and the stroma blue with Mallory's triple.

The two corneagenous and four cone cells occupy a zone 15 $\mu$m thick (Figs. 7, 9). Cones are square in section (Figs. 4, 6, 8). They range in length from 60 to 115 $\mu$m, from periphery to centre of the eye surface, and taper slightly (Fig. 7). The ratio of cone width to length is about 1:3.5. The cones are inhomogeneous as evidenced by differential staining affinities: the distal and proximal regions colour more intensively than the central region with Mallory and Masson trichome stains, and the distal region stains red with Best's carmine and PAS.

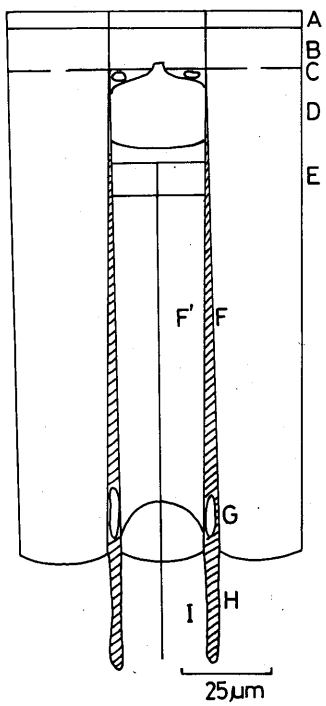


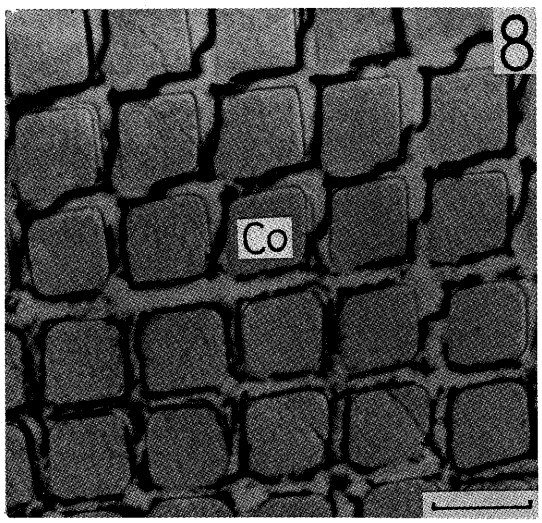

Fig. 7. Diagrammatic representation of a radial section through the centre of the cones. A, epicuticle. B, body of the cuticle. C, corneagenous cells. D, cone cells. E, distal reflector. F, distal retinal pigment cell. F', crystalline cone. G, nucleus of distal pigment cell. H, internal tail of distal pigment cell. I, beginning of a crystalline tract. Magn. ca ×1350.

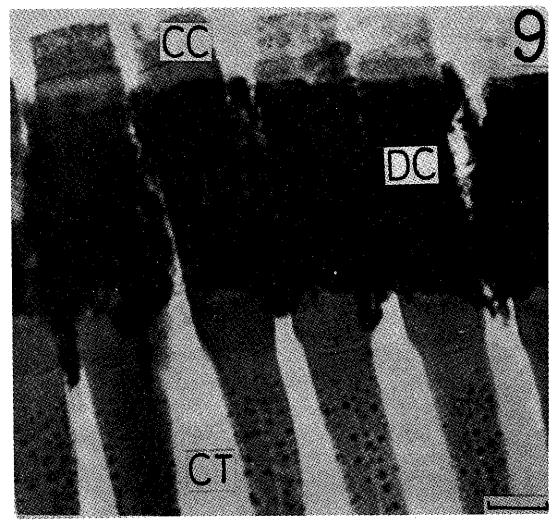
The clear zone (Figs. 3, 4, 5), containing the crystalline tracts and lying between the cones and retinular cells, is 1.15 mm centrally, slightly less posteriorly, 1.10 mm, and decreases to half that width at the anterior margin.

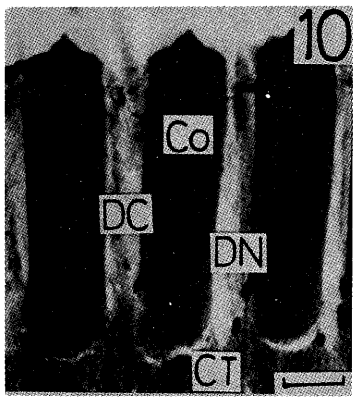
The crystalline tract (Figs. 4, 5, 9) has four concave outer surfaces closely applied to the inner surface of the cone. It is square and quadripartite distally and tapers to about 0.08 $\mu$m proximally where it passes through the retinular apparatus to become inserted upon the basilar membrane. In teased preparations and in sections the four parts of a tract frequently separate (Fig. 11); this effect is probably an artifact. For example, in paraffin sections the four segments separate progressively and come to lie about the walls of a cylinder. They become demilunes which remain connected at their edges. As they separate a cavity in the form of a Maltese cross forms in the centre of the cylinder. In epoxy sections the tracts, at first square in section, become increasingly X-shaped as they taper centrally (Fig. 12). The four parts of the tract remain associated in a flower petal-pattern arrangement until they reach the retinular layer. A tract is membrane-bound and is separated from the ground substance between the tracts by vesicular material (Fig. 12). There are periodic junctions between the four members of a tract.

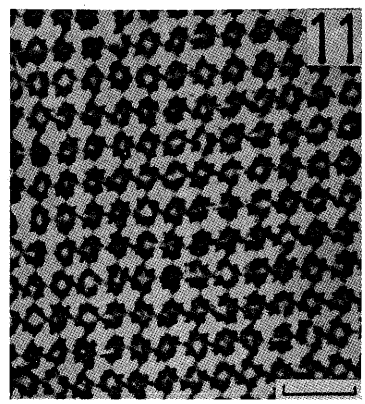
The surface of the tracts (scanning electron micrographs) exhibits a mosaic of circular and hexagonal features, about 2.5 $\mu$m in diameter (when the tract is 10 $\mu$m wide), which cover the surface. The interior of the strands of the crystalline tracts is packed with refringent spherules (Fig. 9) less than 1 $\mu$m in diameter. These are eosinophilic and stain with acid fucsin. The cytoplasm contains fine granules and fibres (Fig. 12).

The material between the crystalline tracts or inter-tract material (ITM) is seemingly homogeneous, basophilic, staining with aniline blue, Alcian blue and PAS. In both paraffin and epoxy sections it contains many vacuoles (Figs. 13, 14, 15). They are also visible in scanning electron micrographs. They occur in tandem across the clear zone between the



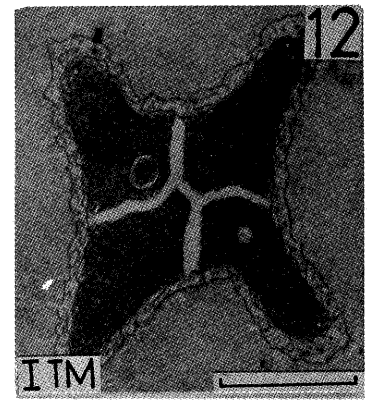




Fig. 8. Radial section through region of crystalline cones invested by screening pigment. Note the arrangement of the distal pigment cells about the cones. Bar=30 $\mu$m.

Fig. 9. Radial section through the region of the cones. Bar=25 $\mu$m.

Fig. 10. Radial section through region of the cones. Pigment removed. Bar=25 $\mu$m.

Fig. 11. Tangential section through crystalline tracts. Paraffin section. The four parts of the tracts have separated and lie a cavity. Bar=40 $\mu$m.

Fig. 12. Transmission electron micrograph of a crystalline tract. Note quadripartite arrangement and vesicles about the tract. Bar=2.5 $\mu$m.

crystalline tracts, and they contain cells with granular cytoplasm that stains red with eosin and Best's carmine. The cells are sometimes irregularly stellate in paraffin sections.

The distal pigment cell are thin; in concert they invest much of the cone surface (Figs. 7, 9). Their small flattened nuclei occur near the bases of the cones(Fig. 10). The distal retinal(screening) pigment which these cells contain is brown black in sections. It invests two-thirds of the length of the cones, being replaced by distal reflecting pigment in the outer region. Towards the bases of the cones the pigment terminates in concave arches (Fig. 7, 9) over the planar surfaces, and long extensions (20 $\mu$m) extend down at the corners between the proximal parts

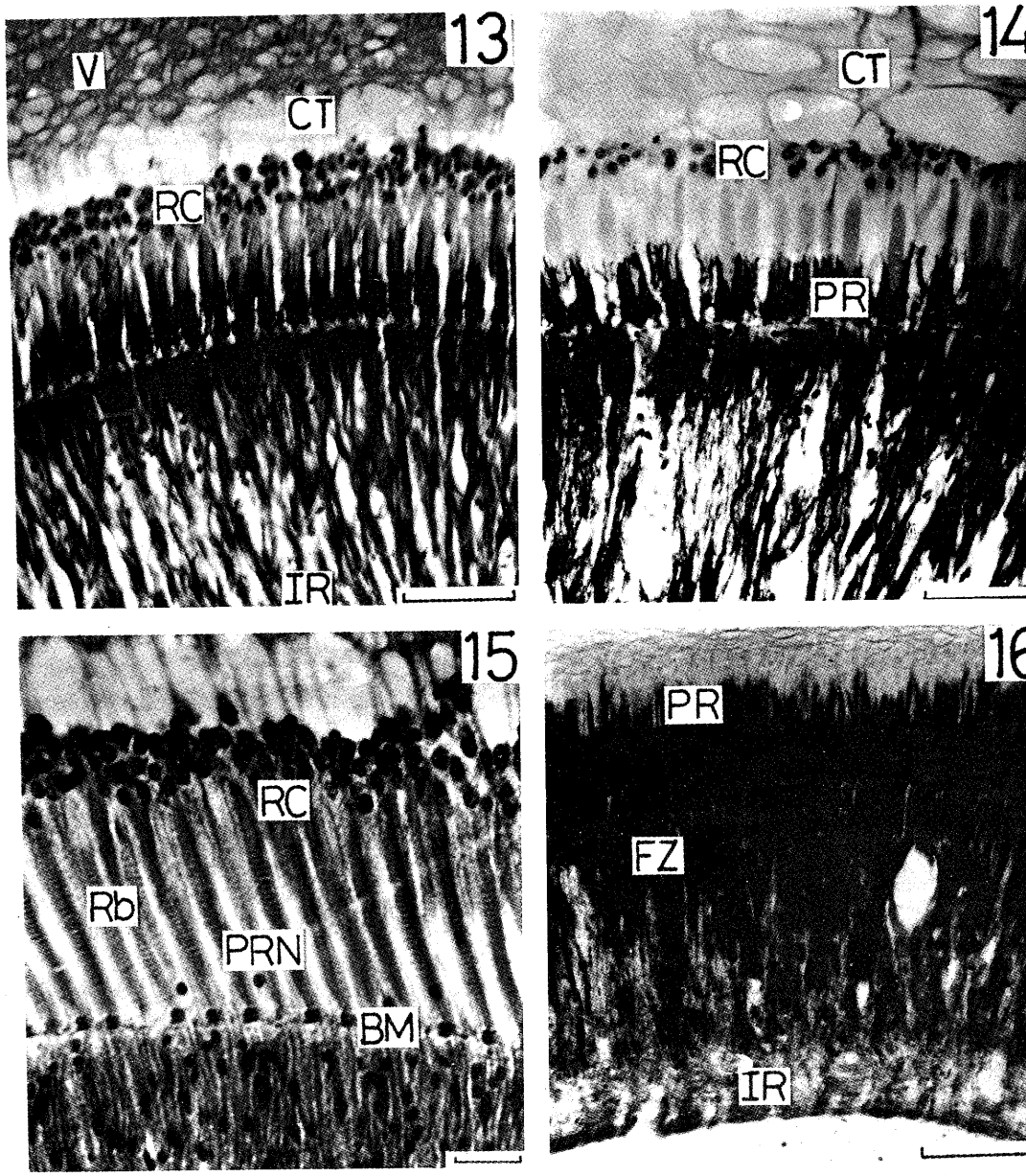
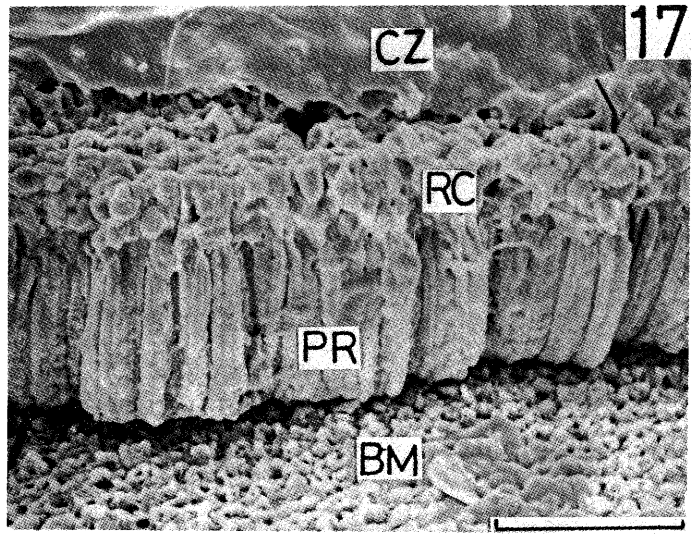
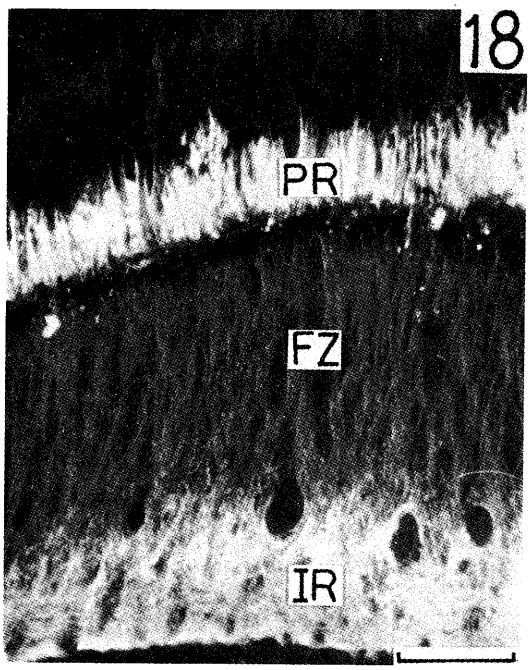



Fig. 13. to 16. Radial sections, light micrographs. Retinular cell region. 13, light-adapted eye; 14, dark-adapted eye; 15, retinal and reflecting pigment removed; 16, fasciculated zone. Magn., 13, 14, 16, Bar=100 $\mu$m; 15, Bar=50 $\mu$m.

of the cone tracts.

Aperatures at the top of the retinular cell layer (Fig. 20) of the photopic eye, as seen in scanning electron micrographs, are about 2.5 $\mu$m in diameter. The margins of these aperatures exist a series (perhaps 12) of regularly arranged triangular indentations. The rhabdoms are banded (Fig. 20), and microvilli lie orthogonal to one another in alternate layers. The bands are 0.6 $\mu$m thick.



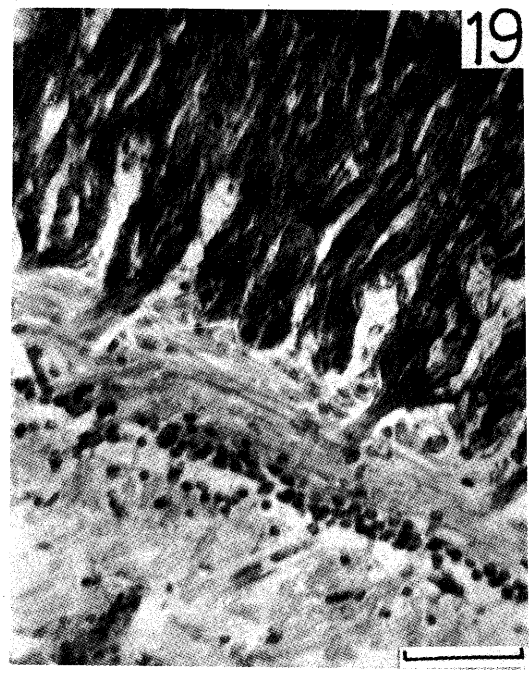




Fig. 17. SEM of retinular cell zone. Bar=50 $\mu$m.

Fig. 18. Proximal and internal reflectors. Radial section. Epi-illumination. Bar=100 $\mu$m

Fig. 19. Fasciculated zone and internal reflector. Radial section. Bar=100 $\mu$m.

The proximal retinal pigment lies within the retinular cells and their axons (Figs. 13, 14, 16). It is contained in spherical granules, 0.12 to 0.40 $\mu$m in diameter (Fig. 21). It is granular and raddish brown. Both distal and proximal screening pigments were quickly removed by methanol-2% HCl (Fig. 15).

Interstitial cells separate the retinular cell

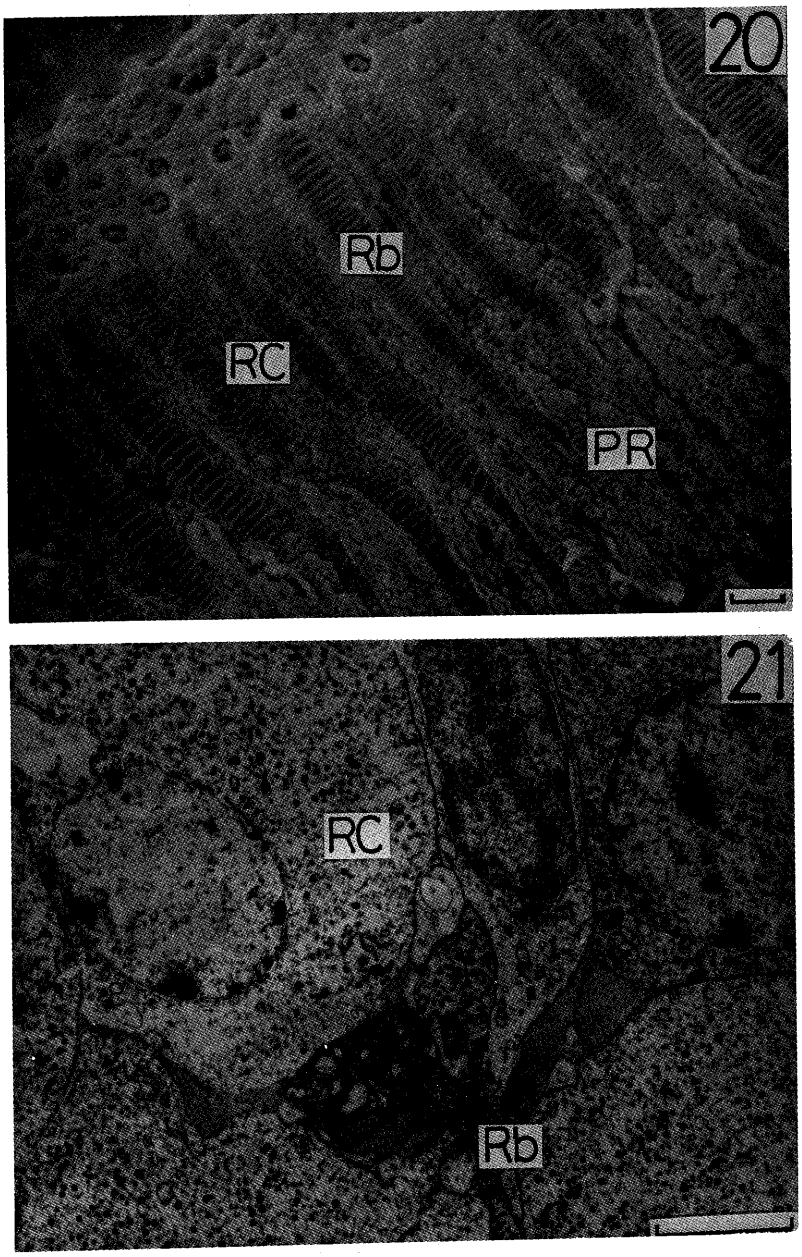



Fig. 20. SEM of retinular cell zone, showing rhabdoms. Bar=5 $\mu$m.

Fig. 21. Transmission electron micrograph of outer region of retinular cells of light-adapted eye. Tangential section. Note pigment granules in the retinular cells. Bar=5 $\mu$m.

complexes of the ommatidia (Figs. 14, 17). Their nuclei lie towards the basilar membrane (Fig. 15). The cytoplasm contains a granular reflecting pigment which extends some 30 to 60 $\mu$m above the basilar membrane.

There are numerous spindle-shaped cells between the fascicles of nerve fibres of the fasciculated zones. Basal pigment cells, reported by Struwe *et al.*[45], were not distinguished at the light microscope level in our material.

Distal and proximal reflectors are white and reflect diffusely (Fig. 18). The internal reflector (Figs. 16, 19) also is white. Hemisections of eyes preserved in methanol were examined under long wavelength (366 nm) UV light. Distal and internal reflectors fluoresced blue; the proximal reflector did not fluoresce. After the usual histological procedures, the distal and proximal reflectors remained whereas the internal reflector was absent, or present faintly. When a dissected eye, preserved in methanol, was transferred to water, blue fluorescent material could be seen streaming out of the internal reflector. All reflecting and screening pigments were removed from sections by leaving them overnight in 0.5 M $NH_4OH$ (the sections were protected by coating them with a thin film of celloidin) (Fig. 15).

Frozen and paraffin-preserved sections were examined with epifluorescence (Zeiss microscope). Under uv excitation of $\lambda$ 365 nm, the distal reflector fluoresced blue; fluorescence was faint in the internal reflector; no fluorescence was seen in the proximal reflector.

The region of the internal reflector (Figs. 16, 19) contained fascicles of fibrous material which stained with aniline blue and fast green. This material was arranged in a pattern like chain-mail.

In sections treated with lipophilic colouring agents and $OsO_4$, the rhabdoms, retinular cells and neural layers were well coloured or darkened. The ground substance between the crystalline tracts was also markedly sudanophilic. With the Schultz histochemical test for cholesterol, the ITM became bright blue-green. No result was obtained with the perchloric acid-naphthoquinone method[38].

When sections were stained with Alcian blue, the elements that stained blue were the corneagenous cells, ITM, basilar membrane, strands between the nerve bundles of the fasciculated zone, and concentric lamellae in the lamina ganglionaris. No tissue showed metachromasia after thionin[38].

**Eye glow and reflexion in the cones**

The centre of the dark-adapted eye ehhibits an orange glow from a region having a diameter about half of the eye, viz. 2.5 mm.

Pieces of cortex from eyes fixed in glutaraldehyde were examined microscopically. Viewed from the inside by transmitted light, the ommatidia appeared as bright round spots; the transmitted light was white. In frozen sections, four orthogonally arranged specular reflectors could be seen about the cones; the light reflected from them was white. These reflectors were not seen in paraffin sections, mounted in Canada balsam. In teased preparations of the cortex, from formalin-fixed material, conspicuous reflectors were visible about the cones. They were completely specular, one face appeared at a time at very oblique incidence of light from below (estimated at ca 60°C). The reflector extended throughout the length of the cone, the light was white. There was reflexion, also, from the proximal part of the crystalline tract on the same side as that from which light was being reflected from the crystalline cone. Spherules in the crystalline tract glistened. A bright curved double band was visible at the lower end of the cone. The outer surface of a cone, viewed in a physiological direction (i. e. normal to its longitudinal axis) was a bright square.

It was concluded that the cones are bounded by four orthogonally arranged mirrors, as proposed by Vogt[49] for *Astacus*, and the mirrors depend upon total internal reflection. They extend the full length of the cone from the corneagenous cells to the crystalline tract.

The outer region of the distal retinal pigment cells possessed a mat white, diffusing reflector, length about 10 $\mu$m, which contained

fine reflecting granules, Ca 1 $\mu$m in size.

To external macroscopic view, the surface of the eye shows alternating light and dark stripes, orderly arranged in meridional and latitudinal rows. At the lateral (outer pole) the stripes are spaced at intervals of 1 mm, and they converge towards the periphery. The stripes are really rows of dark spots about 0.2 mm across. Examined microscopically, the borders of the facets appear chalky white at oblique incidence, except over the dark spots. In conjunction with these observations, the cortex in radial sections was inspected. There were long stretches where the external reflectors were complete, and other stretches of 0.18 to 0.40 mm where the external reflectors were much reduced. The shortest extent of fully developed reflectors between stretches of reduced reflectors was about 0.5 mm. From these observations it is inferred that the dark spots, visible on the surface of the eye, are caused by periodic reduction of the external reflector.

In the larval and juvenile material available to us, we found that the eyes of zoea stage II possessed hexagonal facets. Post larvae, (juvenile) 18 mm long had square facets. It was noteworthy that the clear zone in the latter animals was very short, and was much increased in older juveniles, 35 mm long.

**Chemistry**

PTERIDINES, PURINES. Alkaline extracts of whole eyes and of three regions each containing one of the reflectors produced a major fraction on paper chromatograms that fluoresced blue-purple like isoxanthopterin and ran at same rate. All regions showed traces of a material corresponding to 2-amino, 4-hydroxypteridine reported for eyes of *Penaeus setiferus*. The outer region, including the distal reflector, contained a minor yellow-green fluorescent component, believed to be xanthopterin.

Eluted from papers, the major component, in 0.1 M HCl, had absorption peaks at 290 and 337 nm; in alkaline media (0.1 M NaOH, 0.5 M $NH_4OH$), the former peak disappeared (Fig. 22). These spectra corresponded to those of authentic isoxanthopterin.

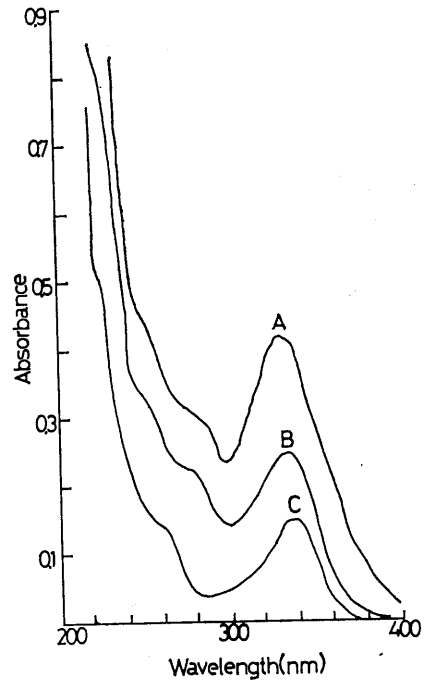



Fig. 22. Absorbance curves of extracts of the proximal reflector.
(A) internal reflector
(B) and authentic isoxanthopterin
(C) in 0.5 M $NH_4OH$.

The surface of the outer region was gently scraped and extracts were chromatographed. The chromatograms resembled those of unmolested pieces, showing isoxanthopterin and xanthopterin, the former as the major fraction.

Small pieces of tissue collected from the region of (1) cones, (2) retinular cells, and (3) internal reflector, were applied directly to paper, and the chromatogram was developed in BAW. The tissues gave rise to long, strongly fluorescent blue-purple strands with faint yellow-green leading edges, indicative of isoxanthopterin and xanthopterin, respectively.

An eye contains 0.13 mg of isoxanthopterin (1.7 mg $g^{-1}$ of eye). Xanthopterin is about 0.06× isoxanthopterin.

No quenching spots indicative of purines (guanine, hypoxanthine, xanthine, uric acid) were seen on paper and thin layer chromatograms of extracts of whole eyes and the various

regions. Addition of enzymes (guanase, xanthine oxidase and uricase) produced no changes of absorbance indicative of purines.

OMMOCHROMES. Proximal and distal retinal pigments were removed from sections of eyes by methanol-2% HCl, 5 min, whereas the corresponding reflecting pigments were intact.

In sections of isolated eyes, two colours were occasionally seen in the proximal retinal pigment: some cells were purple, others yellow brown.

On a chromatographic column (Sephadex LH-20), four bands developed, diffuse purple below, port wine, orange in the centre, and rose at the top. In the visible range they absorbed broadly between 400 and 500 to 550 nm (Fig. 23).

Eye extracts on paper showed a purple streak and spot, poorly defined, and a yellow-orange spot running at about the same rate as xanthommatin (Fig. 24).

LIPIDS. Apart from highly polar compounds at the origin, the major fraction on thin layer chromatograms of extracts of whole eyes was cholesterol. There were several minor fractions, possibly wax esters and diacyl ether. Cholesterol amounted to about 0.45 mg per eye and 5.6 mg per g wet weight. The cortex contained about 10 mg per g wet weight.

**Retinomotor activity**

In control animals in the dark, the proximal retinal pigment for the most part lay outside the basiler membrane (Fig. 14); the basal regions of the retinular cells contained a little pigment. The density of pigment was greater in the outer two-thirds of the fasciculated layer. The central ends of the axons, adjacent to the internal reflector, were darkly pigmented.

With bright illumination, pigment migraration began in 1 min, reaching the outer limit of the proximal reflector. Pigment expanded half way across the retinular cell layer in 2 min, surround the rhabdoms in 4 min, and extended throughout the retinular cells in 16 min. Some pigment remained evenly distributed throughout

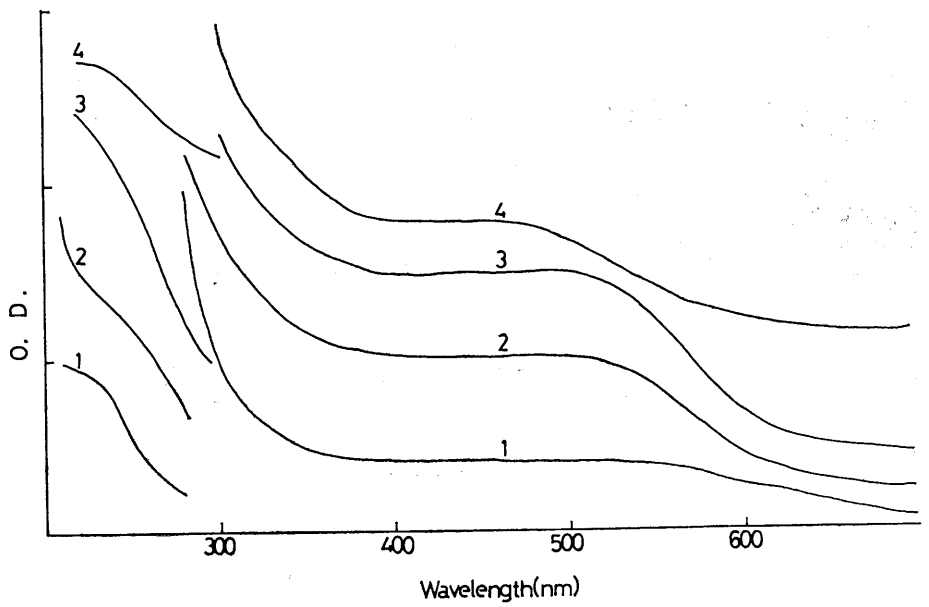



Fig. 23. Absorbance of ommochromes from the eye of *Penaeus monodon*. Curves 1 to 4, successive eluates from a column of Sephadex LH-20. 1, diffuse purple; 2, port wine; 3, orange; 4, rose. 2 to 4 Displaced upwards for convenience of presentation (densities at 700 nm shown at the right). Curves between 200 to 300 nm (left) are dilutions.

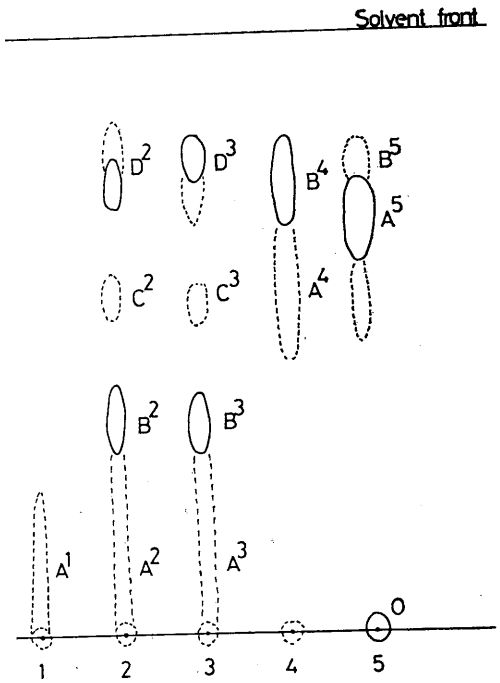



Fig. 24. Paper chromatogram (Whatman #1) of extracts of eyes of *Penaeus monodon* (in $CH_3OH$-2% HCl). Solvent: 80% HCOOH-$CH_3OH$-HCl 80:15:0.5. 1, 2, 3, Successive eluates from a column of Sephadex LH-20 (see text): A, visible purple streak; B, visible purple; C, visible faint reddish; D, visible yellow-orange. 4, Extract of *Dorsophila* eyes: A, visible faint yellow; B, visible yellow orange. 5, Extract of eyes of *P. monodon*. O, purple fluorescent; $A^5$, blue-purple fluorescent; $B^5$, yellow-green fluorescent.

the fasciculated layer except for the inner ends of the axons which were densely pigmented (Fig. 13).

After exposures of 1 and 5 sec to bright light, followed by 16 min darkness, the pigment was retracted. Following a 1 min exposure (1 min light, 15 min darkness), the proximal pigment was expanded.

After 30 min light exposure, the proximal screening pigment was retracted at 0.5 lx, partially expanded at 1.5 to 4 lx, and largely extended at 30 lx. The threshold for full expansion lies below 30 lx.

The proximal screening pigment of light-adapted eyes was extended at $\frac{1}{2}$ h in darkness, partially withdrawn (about half) at 1 h, and fully withdrawn after $1\frac{1}{2}$ h in darkness (Fig. 14).

The proximal screening pigment was retarded in shrimp kept in continuous darkness for 18 h until 1200 h. It was fully extended in shrimp retained in the light until 2300 h.

No significant change was observed with position of distal screening pigment and proximal reflecting material in scotopic and photopic eyes.

In isolated eyes from dark-adapted shrimp, the pigment was contracted when eyes were kept in darkness for 30 min, and expanded when eyes were exposed to light for 30 min. When isolated eyes of light-adapted shrimp were kept in light or darkness for 1 h and 2 h, the pigment was expanded.

## DISCUSSION

*Penaeus mondon* has a superposition-adaptation type of eye. Under photopic conditions screening and reflecting pigments shift it to the apposition type whereby a narrow cone of light passes from a facet to its underlying group of photoreceptors. Under scotopic conditions the eye becomes a superposition one by retraction of pigment, and light from many facets is directed upon a large retinular area. Only the proximal retinal pigment migrates in shallow water penaeid prawns[21,55,56], and in *P. mondon* its position depends upon ambient illumination. In the fully light-adapted eye the pigment extends throughout the length of the retinular cells, and in the dark-adapted eye it retreats below the basilar membrane. The distal retinal pigment is static.

Other decapods in which pigment migration is limited to the retinular cells are the Norway lobster *Nephrops* and the swimming crab *Callinectes*[1,32]. Both proximal and distal screening pigments migrate in the common lobster *Homarus*, and these pigments plus the proximal reflcting pigment in the caridean prawns *Pandalus*, *Palaemon* and *Palaemonetes*[9],

[19,25,34].

During light-adaptation the rhabdoms become enveloped by retinal pigment in 4 min and the pigment is distributed throughout the length of the retinular cells in 16 min. These times are like those required for outward migration of proximal retinal pigment in caridean prawns, viz. 4 to 6 min, with full expansion in 10 to 30 min in *Leander* (*Palaemon*) and *Pandalus*[9]. Retreat of the pigment is slow, requiring about 90 min in *Penaeus monodon.*

A brief exposure to light (1 min, 5 klx) is sufficient to cause full expansion of the proximal retinal pigment. The degree to which the pigment migrates outwards depends upon the strength of illuminance between 0.5 and 30 lx. In the crayfish *Procambarus* the process initiating pigment migration is cumulative and is dependent upon quantal flux × time of exposure; the threshold lies between 0.1 and 1 $\mu$m cm$^{-2}$, the same order of magnitude as for *Penaeus*[37].

Migration of the distal retinal pigment of caridean prawns is mediated by the neuroendocrine system, for which an extensive literature exists[12,21,25,26]. The manner in which the position of the proximal retinal pigment is controlled has been controversial[25]. The pigment expands under illumination in the isolated eye of *Penaeus monodon.* This preparation contains glandular and ganglionic elements in addition to retinular cells, and the evidence is equivocal. Recent studies have shown that the sensitivity spectrum of pigment migration matches the spectrum sensitivity of the electroretinogram in *Procambrus*[37], and the position of the pigment is different within the cells of a single ommatidium after selective adaptation with plane polarized light in *Callinectes*[32]. This vidence reinforces inferences from earlier studies that migration of the proximal retinal pigment is under control of the retinular cell in which it occurs. The response is a direct effect; there is no persistent circadian rhythm.

**Screening and reflecting pigments**

The retinal screening pigments of the fasciculated zone, retinular cells and the distal retinal pigment cells are ommochromes, specifically ommins[5,14,15]. Three fractions separated on Sephadex contained a purple component, and two contained a yellow component that chromatographically resembled xanthommatin (Fig. 24).

There is strong evidence that all three reflectors, distal, proximal and internal, contain isoxanthopterin, which seems to be the chief component of the reflecting material. The eye contains about 0.2 mg of isoxanthopterin. There is a lesser amount of xanthopterin, the ratio of xanthopterin to isoxanthopterin is about 1: 6.5, it may be incorporated in the distal reflector, and there are traces of 2-amino-4-hydroxypteridine. In *P. setiferus*, xanthopterin was found only in the distal reflector, 2-amino-4-hydroxypteridine in distal and internal reflectors[57]. It appears that the pteridines in the three reflectors are not in the same physicochemical state, because they differ in solubilities and fluorescence. The quenched pteridine may be conjugated with protein[4].

A pteridine, viz. 7, 8-dihydroxanthopterin, has been discovered in retinal tapeta lucida of fishes[58]. The pteridine is in the form of short cylinders, reflexion is diffuse. So far as we know, pteridines have not found in multilayer specular reflectors.

Five components, believed to be in the ocular reflectors, have been found in *Homarus*, viz. xanthopterin, and unidentified substance, and the purines, uric acid, xanthine and hypoxanthine[4,22,23,24,57].

**Lipids**

Lipids, as might be expected, are especially concentrated in the nervous tissue but also occur in the ground substance between the crystalline tracts. The high level of cholesterol in the eye (3.5% dry weight) has caused comment[56]. In our analysis we found about 5.6 mg g$^{-1}$ wet weight. For comparison, levels in vertebrate brain are about 2% (wet weight)[6].

Cholesterol and cholesterol esters occur in ocular reflectors of some vertebrates, e. g. the opossum[39]. A possible role of this kind in the shrimp eye was considered, and pieces of

eye from several regions were examined by TLC: they were extracts of whole eye, internal reflector plus fasciculated zone, retinular cells, cones plus crystalline tracts. All regions showed some cholesterol, especially the nervous tissue and the cortex. In the latter cholesterol was localized in ITM, and amounted to about 10 mg $g^{-1}$ wet weight of cortex.

**Light reflexion**

In general, the grass shrimp has a superposition eye like those described in the crayfish *Astacus* and the caridean prawns *Oplophorus* and *Palaemonetes*[27,28,29,30,49,50,51]. Crystalline cones are enveloped by four orthogonally arranged specular reflectors. In the crayfish there are two reflecting mechanisms: specular reflexion from a multilayer of thin films enveloping the cone distally, and total internal reflexion from the sides of the cones. The latter has a higher refractive index than the surrounding cytoplasm (1.41 vs 1.34)[30,51], permitting total internal reflexion. Together, distal reflector and cone-pigment cell interface from a system of four radial plane mirrors enveloping the cone in an orthogonal pattern. Rays, incident at small angles to the ommatidial axis, are reflected once, more oblique rays are reflected twice[51]. This type of reflecting system has also been found in caridean shrimp: in the deep-sea *Oplophorus*, reflexion is produced by a multilayered mirror; in the shallow water *Palaemonetes* there is total internal reflexion[27,28,29,30].

In *Penaeus* total internal reflexion occurs along the entire length of the cone, including the region beneath the distal reflector. To avoid confusion, the distal reflector is designated the distal diffuse reflector, the specular reflecting surface of the cone, the cone reflecting envelope. The length of the cone is 3.5× the width (Fig. 7), whereas in the crayfish and the caridean prawn, it is about 2× the width. Land[30] has found that the latter proportion is optimal for reflexion to occur twice but no more within a cone.

The distal retinal pigment of some prawns is capped by a narrow zone of distal reflecting pigment[25,26]. In *Penaeus* the distal diffuse reflector can be seen easily in surface view and in sections. If forms a zone, about 10 $\mu$m long, containing minute reflecting granules. Outwardly those cells form a reflecting grid outlining the facet margins; periodically, the reflector is diminished, corresponding to the dark spots, visible externally in ordered arrays. The reflector is not birefringent and appears mat white externally. The entire burden of reflexion from the region of the cones was thrown upon this reflector by Zyznar[56]; this was before the discovery of orthogonal mirrors which produce the reflecting type superposition eye. The distal diffuse reflector extends the iris stop of the distal retinal pigment outwards and blocks passage of oblique rays 16° to the ommatidial axis.

Acceptance of light rays by the cone reflecting envelope corresponds to the longitudinal extent of the cone screen (distal diffuse reflector plus distal retinal pigment).

The proximal reflector surrounds the proximal one-third of the retinular cells and reflects diffusely. It is also a screen, optically isolating the proximal portion of the ommatidium. The position of this reflector is such that, in the scotopic superposition eye, it permits a rhabdom to receive light from many surrounding ommatidal units. It also reflects light diffusely into its own rhabdom and into some surrounding rhabdoms. The theoretical solid angle of reflexion is 180°; the area affected in terms of photon-capture depends, however, upon the extent to which light passing obliquely through rhabdoms is absorbed. If the absorbance of a rhabdom be 0.2 (*Nephrops norvegicus*)[31], 90% of reflected light could be absorbed in passing laterally through 5 rhabdoms. This reflector is white and reflects well across the visible spectrum.

Light scattered inwards by the distal reflector exceeds the critical angle of the cones, and none goes to the retinular cells.

Eye glow of the scotopic penaeid eye is seen over about half its diameter. From tracing of ray paths (Fig. 26), eyeshine would not be expected beyond a third of the eye diameter (the effective pupil). This follows if light is

reflected outwards over the same path by which it enters. Assume, however, that light can be scattered from the proximal reflector across the receptor groups of six ommatidia, and this light can be reflected out of the eye. Then, the diameter of eye glow would be about half that of the eye, which is the virtual coudition. The diameter of eye glow in *Oplophorus* is about half that of the eye; cone reflexion in this animal, effected by thin films, permits rays to be reflected at a greater angle than in *Penaeus*[27]. Furthermore, Land[30] has noted that the total effective pupil of the scotopic eye of *Palaemonetes* is about one third of the eye diameter. The cone mechanism of this prawn is total internal reflexion, the cone tracts are relatively short, about twice the length of the cones, the region of focus for parallel rays is small, and there is small possibility of spread of light beyond incoming paths.

In *Astacus* and *Oplophorus* the retinular layer forms a spherical surface of diameter slightly more than half that of the eye, and the cone length is about twice the width. In the scotopic condition, light rays reflected from many cone envelopes are brought to an approximate focus on the retinular surface (illustrated in Fig. 2 of Land[27]).

For the superposition eye of *Astacus*, it has been estimated that more than half the light accepted by an ommatidium enters the eye through adjacent ommatida[42]. In these animals the ratio of cone length to crystalline tract length is about 1:1.

Crystalline tracts in *Penaeus*, on the other hand, are extra-ordinarily long, the ratio of cone length to tract length is about 1: 10 (Fig. 25). When ray paths for parallel light reflected from the cones are traced (Fig. 26), it is seen that they cross and are spread over a rather area of the retinular surface. (When the diameter of the zone of acceptance at the surface of the eye is 1.5 mm, the diameter of the retitinular region receiving the rays is 0.7 mm; the ratio of areas is 4.5: 1).

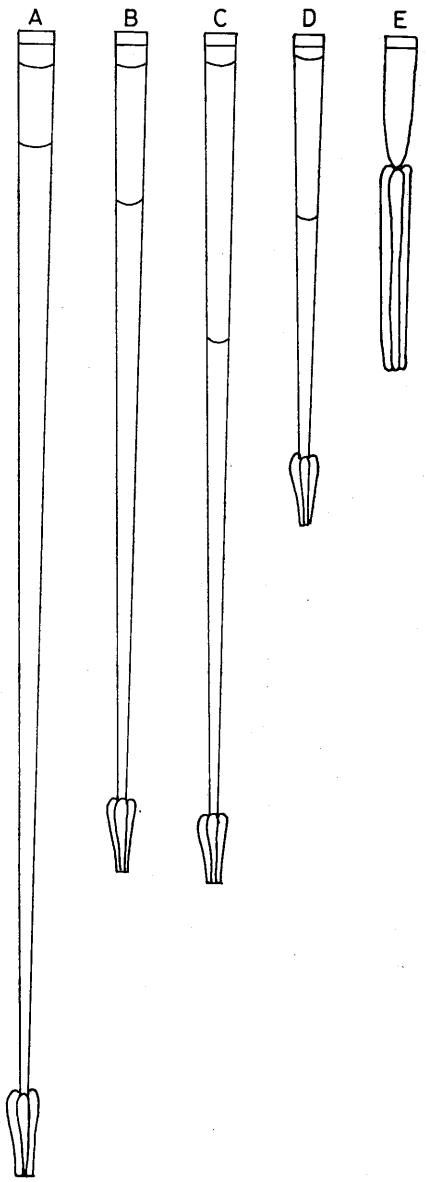



Fig. 25. Diagrammatic representation of patterns in the ommatidia of some penaeids. A, *Penaeus monodon*. B, *Aristaemorpha foliacea*. C, *Aristeus alcocki*. D, *Solenocera hextii*. E. *Gennadas* sp. (B, C, D, after Ramadan, 1952; E. after Meyer-Rochow and Walsh, 1977.)

**Crystalline tracts**

The large eye of *Penaeus*, with its many small facets, appears designed to provide high visual acuity in the photopic state. Because

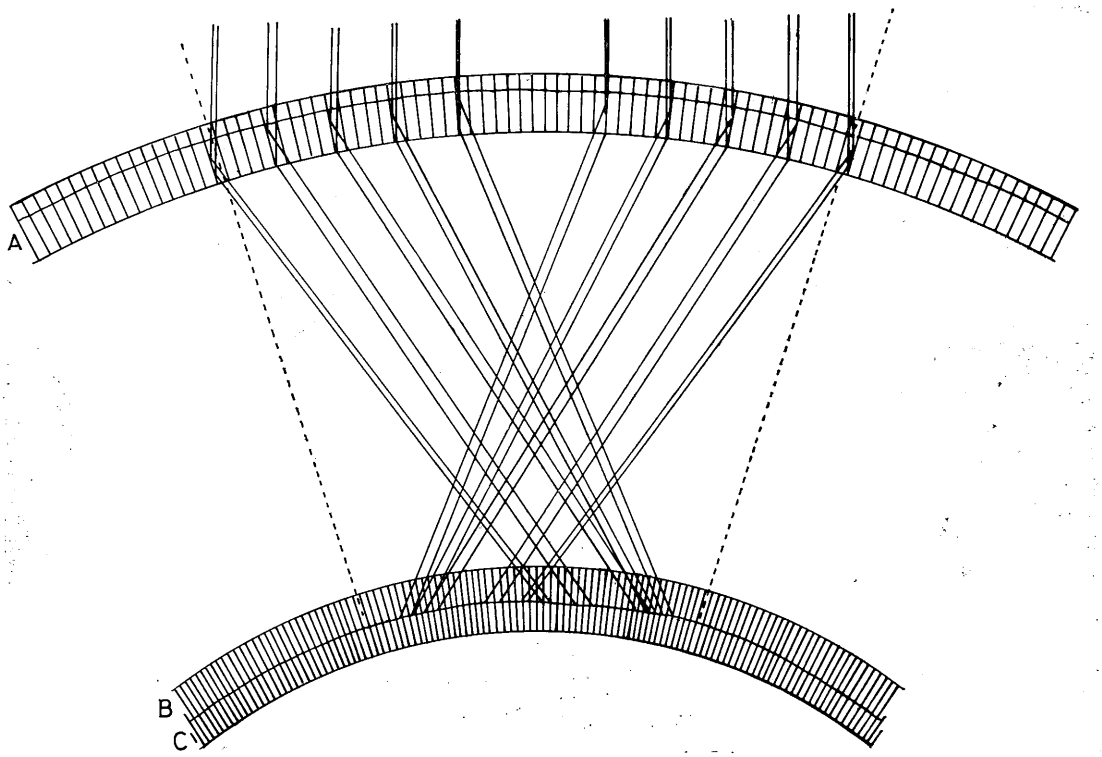



Fig. 26. Diagram of ray paths for parallel incident light in the scotopic (superposition) eye of *P. monodon*. A, Crystalline cones, limits of distal pigments. B, Outer region of retinular cells. C, Proximal reflecting pigment.

the cone is narrow and the tract long, the acceptance angle of a photopic ommatidium is small, slightly less than 2°; the aperature is f/36.

The variation in length of crystalline tracts among penaeids is remarkable, and some examples culled from the literature are illustrated in Fig. 25. At one extreme are the extremely long tracts of *Penaeus*, *Metapenaeus* and *Penaeopsis* (Subfamily Penaeinae), according to Ramadan[41]. Ommatidia of Eusicyonia (Eusicyoninae) are said to be exactly like those of the *Penaeiae*. *Aristaeomorpha* (Aristaeinae) has very long crystalline tracts. Short tracts are found in *Solenocera* and *Hymenopenaeus* (Solenocerinae). In *Gennadas* (Aristaeinae) a clear zone is absent and the rhabdom has become enormously enlarged at its expense[35,41]. The crystalline tract is notably shorter in small specimens of *Hymenopenaeus* and *Aristaeus*. Cone lengths also vary interspecifically to a great degree: cone length to breath is 2.8 in *Hymenopenaeus propinquus* and 10 in *Aristaeus alcocki*[41].

Two Reptantia whose eyes generally resemble those of *Penaeus* are *Homarus* and *Panulirus*. They have very long crystalline tracts, about 7× the length of the cones[1,34].

Among the Penaeidae, square facets occur in the Penaeinae, Eusicyoninae, Solenocerinae, and in *Aristea* and *Aristaeomorpha* among the Aristaeinae (series *Aristea*)[16,41]. Hexagonal facets occur in *Benthesicymus*, *Gennadas* and *Amalopenaeus* among the Aristaenae (series Benthesicymae)[16,41]. In another report[35], the facets of *Gennadas* are described as square with rounded corners. According to Land[28], square facets are found in reflecting eyes, hexagonal

facets in refracting eyes.

The second zoea of *Penaeus monodon* has hexagonal facets which give way to square facets in postlarvae. Presumably, those eyes are hexagonal facets are reflecting eyes. It may be that the hexagonal facet in pelagic shrimp is associated with an apposition type eye.

The second zoea of *Penaeus monodon* has hexagonal facets, whereas the post larvae has square facets. The clear zone is very short in small post larvae and widens as the animal matures. Presumably, the eyes of zoeae and early postlarvae are functioning as apposition eyes. The larval eye of *Panulirus* is of the apposition type, whereas that of the adult has a typical clear zone[34]. Larval eyes of *Palaemonetes* have hexagonal facets of apposition structure, replaced by square facets as the animals mature[11,30].

The circles of light transmitted by the cones viewed internally in the normal direction of propagation (they are not seen in the reverse direction) have been illustrated in *P. setiferus*[56]. This effect must be produced by the tails of the distal retinal pigment cells, i. e. the dark pigment processes which extend about 20 $\mu$m centrally at the four corners of the crystalline cones and the cone tracts (Fig. 7). In the reflecting mechanism of the penaeid eye, the concave-shaped lower margins of the retinal pigment, coupled with extensions, cut off light-propagation to an increasing degree from centre to edge of the side of a square; a cone of transmitted light is the result.

The nature of the material between the crystalline tracts (intertract material, ITM) has received little attention. It is sometimes referred to as fluid. The highly vacuolated appearance of the ITM has been noted. The penaeid eye is very difficult to fix and section: Bouin-fixation and paraffin embedding severely distort the microstructure. We saw vacuoles in teased, formalin fixed material in frozen, fractured, and epoxy sections, as well. They show in the scanning electron micrograph of the eye of *Penaeus setiferus* of Waterman and Pooley[54]. We found a multitude of small nuclei in the ITM. Because of distortion in preparation and fortuity of encountering satisfactory planes of view, it is difficult to obtain a definitive picture of the organization of this material. It appears to consist of a matrix containing numerous cells, which may be regularly arranged about the crystalline tracts. The cells contain eosinophilic granules and are peculiarly susceptible to distortion, possibly resulting in vacuoles. The ITM is finely granular and contains polysaccharides, cholesterol is also localized in this region. The ITM is a transparent medium for light transmission in the superposition eye. It is not obvious what maintains the globular shape of the eye. It could be turgor pressure, perhaps produced by the cells of the ITM acting against the resistant cuticle, in a manner analagous to the eyes humours of the vertebrate eye.

**Reflexion, absorption**

Fluorescence, which could affect vision, is absent from the proximal reflecting pigment cells. This pigment, being mat white, reflects diffusely across the visible spectrum; it also forms a screen about the proximal region of the retinular cells (Figs. 14, 17, 18). The screening pigments of *Penaeus* absorb short wavelengths, between 400 and 550 nm, decreasing to 600 nm (Fig. 23). Those of *Crangon*, in sections and squashes, have incresed absorption between 400–450 nm and 530–570 nm[45]. Maximum absorption of the photosensitive pigment in the eye of *P. duorarum* is at 516 nm[10], in which region ommochromes can have a screening effect. By absorption and reflexion, the ommochromes can also affect the spectrum sensitivity of the reflector apparatus. The peak of spectrum sensitivity for pigment migration in *Procambarus* is shifted bathochromically by some 20 nm from that of the spectrum maximum of the ERG, probably owing to the filtering action of the screening pigments themselves[37]. Reflexion of long wavelengths by the pigmented spherules of the retinular cells is a distinct possibility. If the ommochromes have a refractive index significantly higher than the surrounding cytoplasm, their size and packing

density would favour backscattering. Reflexion in the tapetum probably occurs by this mechanism.

Grass shrimp *Penaeus monodon* and some other species of this genus are more active in the scotopic phase[3,36]. It is unlikely, however, that the scotopic eye has much resolving power. Resolution by scotopic *Leander* (optomotor response) is 13.5° vs 4.6° by the photopic animal[9]. Comparable values for scotopic and photopic crayfish *Cherax* are 24°—8° and 4°—2°. The eye of the western rock lobster *Panulirus longipes*, which is more like that of *Penaeus*, has a resolving power of 1°-2° in the light-adapted state, but in the dark-adapted state all visual acuity is lost[34]. A determination of the visual acuity of the eye of the grass shrimp in relation to eye structure and photoconditions would be useful.

**Abbreviations used on all figures.** CC, cone cell. Co, crystalline cones. CT, crystalline tract. Cu, cuticle. DN, nucleus of distal retinal pigment cell. CZ, clear zone. DC, distal pigment cells. RC, retinular cells. RCN, retinular cell nuclei. Rb, rhabdoms. BM, basilar membrane. FZ, fasciculated zone. PR, proximal reflecting layer. V, vacuoles in clear zone. PRN, nuclei of proximal reflecting cells. IR, internal reflector. BV, blood vessel. LG, lamina ganglionaris.

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# 草蝦眼球的研究

## —斑節蝦屬蝦類眼球的再討論

J. A. COLIN NICOL 嚴宏洋

以光學顯微鏡法，電子顯微鏡法（穿透式、掃瞄式）對草蝦的眼球構造加以研究。眼球所含色素也以化學方法加以分析。

成蝦（全長 15 cm）的每一個眼球含有 80,000 個以上的小眼。眼球的透明區相當寬 (1 mm)。由並列成像（明適眼）改變成重疊成像（暗適眼）情況，係經由基部濾光色素，收縮至基底膜下而達成。此過程約需時 90 分鐘，但相反的過程却僅約時 4 分鐘。

在暗適眼，光線會在晶錐體裏以內部全反射方式，集中投射在小網膜細胞上。共有三層反射體，分別含有不同物理形式的蝶呤類化合物（主要爲異黃蝶呤）。濾光色素是 Ommins。

在明適眼，頂部濾光色素及基部濾光色素的作用如同光圈，可限制光線經由小眼面進入N網膜，其角度約爲 1.5°。在暗適眼時，基部濾光色素可將光線散射至毗鄰的小眼，從而產生眼矩。眼矩可自外部目視，而其大小超過眼球直徑一半以上。外部反射體卽是構成眼球外觀的主因，顯示出有一系列排列整齊的黑點，而其間的白色反射體，可以目視觀察。

其他斑節蝦屬蝦類的眼球構造，也一併加以比較。