

THE REFRACTIVE INDEX GRADIENT IN THE CRYSTALLINE CONES OF THE EYES OF A EUPHAUSIID CRUSTACEAN

BY M. F. LAND AND F. A. BURTON

*School of Biological Sciences, University of Sussex,
Brighton, BN1 9QG, England*

(Received 19 April 1979)

The eyes of the euphausiids, a group of malacostracan Crustacea, show a remarkable similarity to the superposition (Exner, 1891) eyes of moths. In both groups the eye has an outer layer of crystalline cones, a wide clear zone, and a layer of rhabdomes beneath it, whose radius of curvature is about half that of the eye itself (Fig. 2*a*). We demonstrated recently that image formation in these eyes is indeed performed by a refracting superposition mechanism (Land, Burton & Meyer-Rochow, 1979), as supposed by Chun (1896). That is to say, light is bent by the crystalline cones in such a way that a ray reaching the outer surface of a cone, and making an angle α with the cone axis, is bent in the cone through an angle 2α (Fig. 1). This is the condition necessary if all parallel rays are to be brought to a focus in the rhabdome layer, where an erect image is produced (see Horridge, 1975). This mechanism, shared by moths, fireflies and some other beetles, is not the same as that of the decapod shrimps and prawns, where essentially the same trick is performed not by refracting cones, but by mirrors (Vogt, 1977; Land, 1978). Kampa (1965) proposed that the euphausiid eye was basically of the apposition type, with the cones funnelling light down light guides into the rhabdomes. However, the cones send light, as noted above, and the light-guides are not present (Meyer-Rochow & Walsh, 1979).

Exner was faced with the problem of how the crystalline cones manage to bend light in this way. In terms of conventional optics they each appear to behave as a two-lens telescope, with a magnification of -1 . However, the distal and proximal surfaces of the cones cannot, by themselves, provide sufficient ray-bending. This is particularly true in the euphausiids, because, unlike the terrestrial insects, the distal surface of the cornea contributes almost nothing to the refractive power of the cone, and furthermore the distal surface of the cone itself is practically flat. Exner's solution was that the cone is constructed as a 'lens cylinder', that is, a cylinder whose refractive index alters from centre to periphery, being highest in the centre. Light rays are bent first towards the axis and then away from it again (Fig. 1); an inverted image is produced halfway down the cylinder (as in the telescope equivalent); and the final ray path is in a direction opposite to the direction of the incident rays (as with a mirror).

The exact form that the refractive-index gradient in such a lens cylinder should

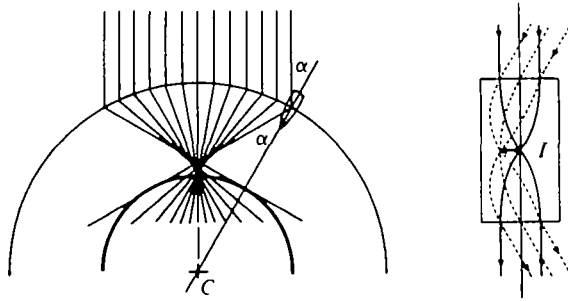


Fig. 1. Left: image formation in a superposition eye. An image is formed half a radius out from the centre of the eye (C) when each crystalline cone bends light through twice the angle of incidence (α) at the distal surface. Right: ray paths through a lens cylinder (after Exner).

have was not worked out until 1954, by Fletcher, Murphy & Young. They showed that the refractive index (n) at a distance (r) from the axis of the lens cylinder should be given by:

$$n = n_0 \operatorname{sech}(\pi r/2F),$$

where n_0 is the refractive index on the axis of the cylinder, and $2F$ is twice the focal length of the device, which in the case of a flat-ended cylinder will simply be equal to its actual length (the first focus being in the middle). It has now been shown that this relationship holds approximately in insects (Seitz, 1969; Hausen, 1973; Horridge, Giddings & Stange, 1972; Meyer-Rochow, 1975). These studies all employed interference microscopy, which seems to be the only accurate method of measuring the refractive indices of very small objects. In this paper we show that the crystalline cones of euphausiids have a lens-cylinder-like refractive-index profile very similar to that in the various insects so far studied, and in striking agreement with the predictions of Fletcher *et al.* (1954).

Observations were made on *Meganctiphanes norvegica*, a large spherical-eyed euphausiid obtained from the Millport marine laboratory of Glasgow University. They were fixed in 5% formaldehyde in sea water but were otherwise untreated (fixation alone does not affect the optical properties of the crystalline cones; Land, Burton & Meyer-Rochow, 1979). The cones were either examined intact (Fig. 2*b*) or sectioned transversely at $11 \mu\text{m}$ using a freezing microtome (Fig. 2*c, d*). The microscope was a Vickers M41, equipped with shearing interference optics. The instrument has two beams derived from a single source. One passes through the specimen and the other through the suspending medium (sea water, $n = 1.334$). When the beams are recombined the differences in optical path length between specimen and medium become visible as interference colours, or in monochromatic light ($\lambda = 542 \text{ nm}$) as a series of 'contour lines' where each dark line indicates a path difference that is a whole multiple of the wavelength.

Fig. 2(*b*) is an intact cone, and it can be seen that at its widest, distal part the path difference is 9 wavelengths. The path difference (p.d.) is given by

$$\text{p.d.} = m\lambda = t(n_s - n_m),$$

where m is the observed number of wavelengths (λ), t is the actual specimen thick

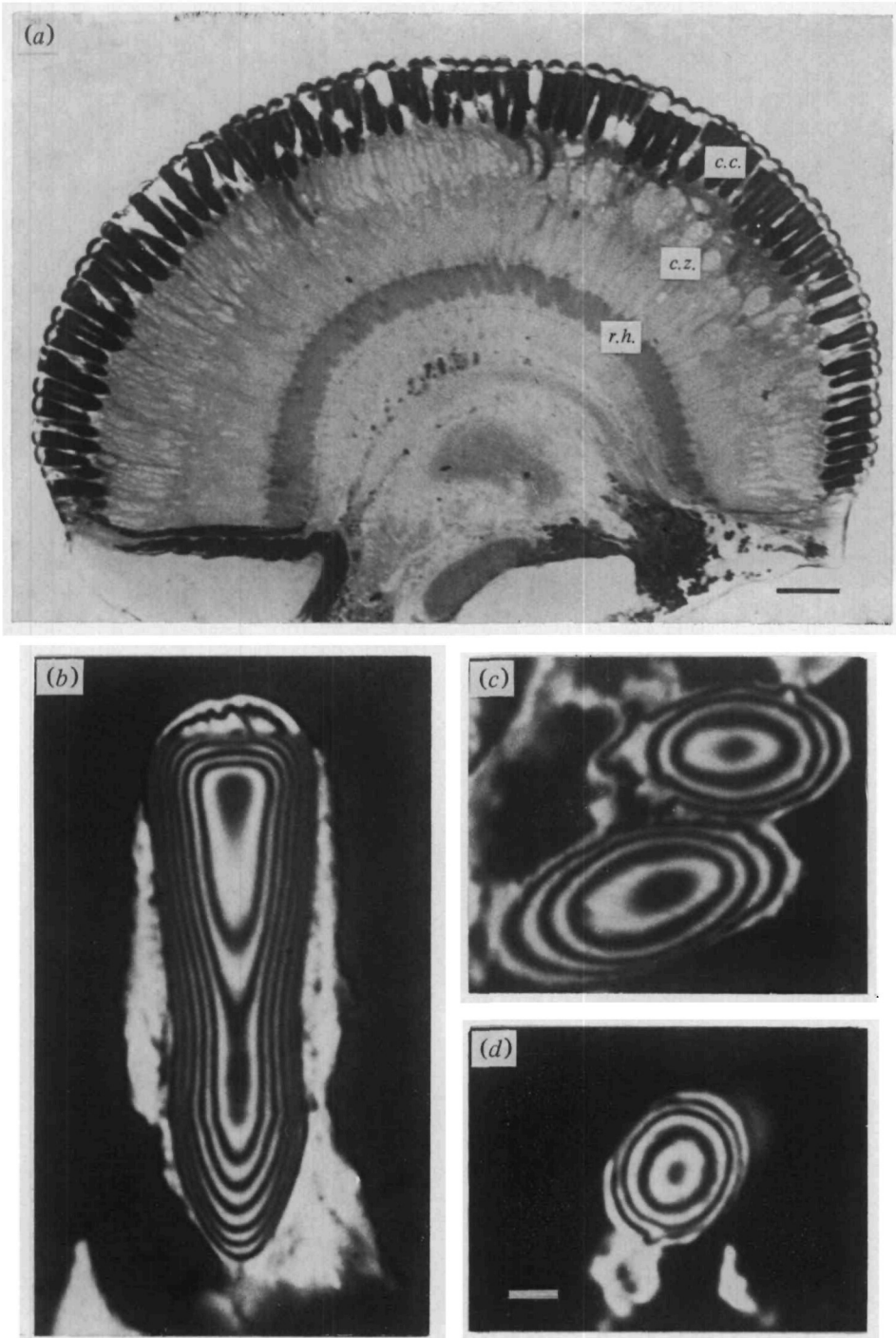


Fig. 2. (a) 1 μm epoxy resin section through the centre of the eye of *Meganyctiphanes norvegica* showing its geometrical identity to Fig. 1. *c.c.*, Crystalline cones; *c.z.*, clear zone; *r.h.*, rhabdoms. (b) Interference micrograph ($10\times$ shearing objective) of an intact crystalline cone showing a total path difference of nine wavelengths. (c) 10.9 μm sections (slightly oblique) through the distal regions of two cones. (d) Similar section through the central region of one cone. In both (c) and (d) the maximum path difference is 4 wavelengths. Scale bars: 100 μm on (a), 10 μm on (d) which also applies to (b) and (c).

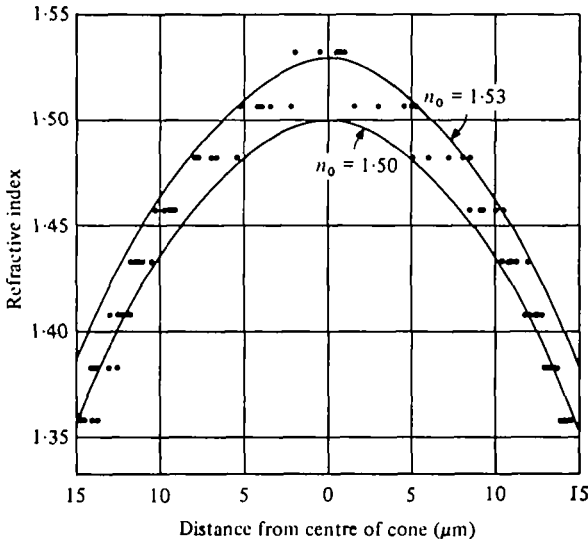


Fig. 3. Measured refractive index gradient in the distal region of five $30\ \mu\text{m}$ wide euphausiid cones. The solid curves are solutions of the Fletcher, Murphy and Young equation for two values of the central refractive index (n_0).

ness ($34\ \mu\text{m}$ max), n_s the refractive index of the specimen and n_m of the medium (1.334). Rearranging the expression gives:

$$n_s = m\lambda/t + n_m.$$

If the crystalline cone were homogeneous (which it is not) a path difference of 9 wavelengths ($4.88\ \mu\text{m}$) would imply a refractive index of 1.477. On the other hand an inhomogeneous cone with the gradient given by the first equation would give the same total path difference if it had a central refractive index (n_0) of 1.53, falling to 1.38 at the periphery. It is worth mentioning that very little can be learned about the internal structure of the crystalline cones from intact preparations, because the pattern of interference fringes is almost identical for a homogeneous and non-homogeneous structure. The pattern is almost completely dominated by differences in thickness (t) of the cylindrical structure, and only at the very edge where observation is difficult would one expect to find measurable differences between the two solutions.

This can be overcome by making parallel-sided sections (Fig. 2c, d). Here, with t constant, the fringe pattern is simply a refractive index contour map. In these sections, whose actual thickness was $10.9\ \mu\text{m}$ as measured on sections mounted edge-on, 4 dark fringes are visible, the 4th being a large dot in the centre. Thus the maximum path difference is $2.17\ \mu\text{m}$, and the central refractive index is 1.533. The distribution of refractive index across the cone is found by noting the positions of the light and dark rings, calculating the refractive indices and plotting these out against the distance from the centre of the section. This has been done in Fig. 3, for the distal regions of five cones whose diameters ranged from 28 to $32\ \mu\text{m}$. The two solid curves are solutions of the Fletcher, Murphy and Young equation for $n_0 = 1.53$ and 1.50, and a double focal length ($2F$) of $105\ \mu\text{m}$, the average length

of cones in this eye. It can be seen that the theoretical and actual refractive index profiles resemble each other remarkably closely, except at the very edge, where the measurements suggest a somewhat steeper slope than that predicted. For the central region, the best fit is obtained with a value of n_0 closer to 1.52 than 1.53, which probably indicates that the central fringe visible in the photographs is fractionally less than four complete wavelengths.

These observations, coupled with those on the optics of the intact eye (Land, Burton & Meyer-Rochow, 1979) indicate that the marine euphausiids have evolved an optical system that is similar to that of nocturnal insects not only in overall design, but also in the fine details of its construction.

This work was supported by the S.R.C. We would like to thank Jochen Zeil for critically reading the manuscript.

REFERENCES

- CHUN, C. (1896). Atlantis. *Biologische Studien über pelagische Organismen* (Zoologica, Stuttgart) **7**, 1-260.
- EXNER, S. (1891). *Die Physiologie der facettierten Augen von Krebsen und Insecten*. Leipzig und Wien: Deuticke.
- FLETCHER, A., MURPHY, T. & YOUNG, A. (1954). Solutions of two optical problems. *Proc. R. Soc. Lond. A* **223**, 216-225.
- HAUSEN, K. (1973). Die Brechungsindices im Kristallkegel der Mehlmotte *Ephestia kühniella*. *J. comp. Physiol.* **82**, 365-378.
- HORRIDGE, G. A. (1975). Optical mechanisms of clear-zone eyes. In *The Compound Eye and Vision of Insects* (ed. G. A. Horridge), pp. 255-298. Oxford: Clarendon.
- HORRIDGE, G. A., GIDDINGS, C. & STANGE, G. (1972). The superposition eye of skipper butterflies. *Proc. R. Soc. Lond. B* **182**, 457-495.
- KAMPA, E. M. (1965). The euphausiid eye - a re-evaluation. *Vision Res.* **5**, 475-481.
- LAND, M. F. (1978). Animal eyes with mirror optics. *Scient. Am.* **239** (6), 126-134.
- LAND, M. F., BURTON, F. A. & MEYER-ROCHOW, V. B. (1979). The optical geometry of euphausiid eyes. *J. comp. Physiol.* **130**, 49-62.
- MEYER-ROCHOW, V. B. (1975). The dioptric system in beetle compound eyes. In *The Compound Eye and Vision of Insects* (ed. G. A. Horridge), pp. 299-313. Oxford: Clarendon.
- MEYER-ROCHOW, V. B. & WALSH, S. (1978). The eyes of mesopelagic crustaceans. III. *Thysanopoda tricuspidata* (Euphausiacea). *Cell Tiss. Res.* **195**, 59-79.
- SEITZ, G. (1969). Untersuchungen am dioptrischen Apparat des Leuchtkäferauges. *Z. vergl. Physiol.* **62**, 61-74.
- VOGT, K. (1977). Ray path and reflection mechanisms in crayfish eyes. *Z. Naturforsch.* **32c**, 466-468.

RESEARCH FACILITIES AT THE NAPLES ZOOLOGICAL STATION

The Naples Zoological Station has arrangements with the Royal Society for British marine biologists to work there for periods of a few weeks on topics of their own choice, making use of the facilities available there for international use. Typical subjects of research by UK scientists in recent years have been studies of the nervous system of cephalopods, the use of bacteria as pollution indicators, algal symbiosis in molluscs and the biochemistry of feeding mechanisms in lower marine invertebrates. Applications for table-time may be made through the Royal Society, the Physiological Society or one of the ten contributing UK universities (details from the Executive Secretary of the Royal Society). The Royal Society will also consider applications for financial support through its European Science Exchange Programme or from its Browne Fund for marine research.

NZS/DJHG/AF

May 1979