Reports

Vision in Annelid Worms

Abstract. *In a first electrophysiological study of worm vision, electroretinograms were measured in two alciopid worms:* Torrea, *taken at the surface, and deep-sea* Vanadis. *Both forms possess a primary retina in the focal plane of the lens, and accessory retinas lying beside the lens. Such accessory retinas occur also in deep-sea fishes and cephalopods. In* Torrea *the primary retina peaks in sensitivity at 400 nanometers, the secondary retina at 560 nanometers. Both together could serve as a depth guage, since 560 nanometers attenuates much faster in seawater than 400 nanometers. The* Vanadis *eyes peaked in sensitivity at 460 to 480 nanometers, a property shared with deep-sea forms of other phyla; and appropriate, since these wavelengths penetrate seawater most deeply, and also are the wavelengths of maximum bioluminescence.*

It is often said that of the 12 major animal phyla, only three—vertebrates, molluscs, and arthropods—have evolved well-constructed, image-resolving eyes. That is not so; for a fourth phylum, the annelid worms, has developed excellent eyes. They are confined to a single family of polychaetes, the alciopids, all marine and widely distributed in the warmer waters of the Atlantic and Pacific. They are the subject of a considerable literature beginning in the 1830's, that says remarkably little, however, about the animals' habits and behavior (*1*). Few biologists have seen a whole alciopid worm, much less a live one; they have worked mainly instead with fragments of preserved specimens encountered in plankton hauls. Though these animals have been studied carefully anatomically, particularly their eyes, nothing whatever is known of their visual physiology. With some trouble they can be found in the Bay of Naples in the early spring, and that circumstance and the hospitality of the Stazione Zoologica gave us the opportunity to learn something of the visual functions in two genera, *Torrea*, a surface form, and deep-sea specimens of *Vanadis*, the two "most specialized and highly adapted forms" among the alciopids (*2*).

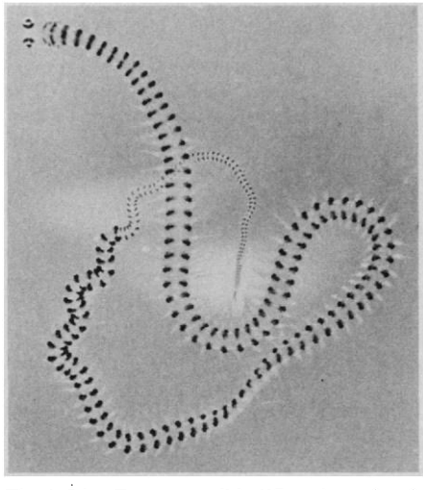


Fig. 1. Live *Torrea candida* (17 cm long, 2 to 3 mm wide) swimming in seawater. The only color is in the eyes, which are bright orange-red, the pupils glowing the same color by light reflected from inside the eye. A white, iridescent iris surrounds the pupil. The laddered appearance is owing to black podial glands, of which there is one pair in each segment of the body. Segmented brushlike setae are also visible.

Torrea (formerly *Asterope*) *candida*, Delle Chiaje, 1841 (*2*), is a long, slender worm in constant motion, propelled not only by the beating of its parapodia but through undulations running from head to tail with a period of about 1 cm (Fig. 1). Our specimens were 12 to 17 cm long, but only 1.5 to 2 mm wide. The eyes, about 0.6 to 0.9 mm in diameter, are bright red-orange, the pupils glowing about the same color with light reflected from inside, and surrounded by a colorless, iridescent iris. Trials in a darkened room with red, white, and ultraviolet light, whether steady, flashed, or shadowed, evoked no notable behavioral responses. Early in our stay we received a batch of about 150 animals; but though we tried to keep them in various ways (oxygenated, cold, in running seawater—of which the last worked best), within 4 days all had died. As they deteriorate, the worms fragment, secreting a viscous red mucus into the water.

We worked with the electroretinogram (ERG), usually to a constant amplitude of response of 10 μv. About 1 cm of the head end, which was quieter than the rest of the worm, was laid ventral side up on a Q-Tip soaked in seawater and positioned with a micromanipulator. When such a preparation proved too noisy, we anesthetized it with MS 222 (tricaine methanesulfonate, Sigma). Finally the best preparation proved to be the wholly isolated eye. A silver–silver chloride wire in the Q-Tip served as the indifferent electrode. The active electrode was a fine cotton wick connected through 0.6*M* KCl with another Ag-AgCl wire, and touching the cornea of the eye just beside the pupil.

The light source was a Sylvania 100-watt quartz-iodine lamp, the filament of which was focused on the slit of an H-10 concave-grating monochromator (Jobin-Yvon, France; J-Y Optical, Metuchen, New Jersey), with 0.5-mm slits transmitting a wave band 4 nm wide. Intensities were regulated with a pair of circular quartz neutral wedges rotating in opposite directions so as to compensate each other. The range could be extended with neutral filters (Oriel). The flash was regulated with an electronic shutter (Ilex). All lenses were quartz. This system was calibrated in place with an X-80 optometer employing a wavelength-calibrated photodiode (United Detector Technology). The ERG's were recorded with a Grass P18 d-c or P15 a-c preamplifier and a Tektronix 502 A oscilloscope, and photographed with a Dumont-Polaroid camera.

Figure 2 shows a cross section of the *Torrea* eye. The cornea is two-layered, one layer continuous with the skin, the other with the iris and retina. There are a

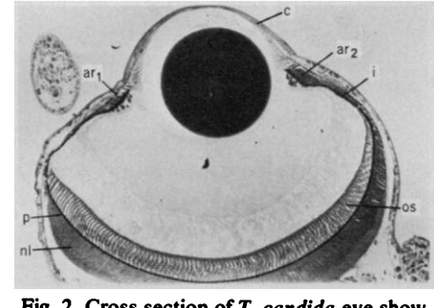


Fig. 2. Cross section of *T. candida* eye showing paired accessory retinas (ar_1, ar_2). The cornea (*c*) has an outer layer continuous with the skin and an inner layer continuous with the retinas. The main or primary retina consists of a single layer of photoreceptor and auxiliary cells stratified into three layers: outer segments (*os*), a layer of orange-red pigment (*p*) extending beyond the main retina to form the iris (*i*), and cell bodies, nuclei, and optic-nerve fibers (*nl*).

large, spherical lens and two distinct layers of humoral material filling the eyeball. An upright retina, as in the squid, coats the fundus. It contains about 10,000 photoreceptor cells. Though a single layer of cells, it has three distinct strata: a distal layer of receptor elements (outer segments), bearing laterally innumerable microvilli; a thin layer deeply pigmented with orange-red granules, continued anteriorly to form the iris; and a thick layer of cell bodies ending in optic fibers that run to the optic ganglion. To each side of the lens in Fig. 2 can be seen an accessory or secondary retina (*3*), consisting here of two small patches almost diametrically apposed, one more ventral and median, the other dorsal and lateral. Each contains a few, relatively short and stubby receptor elements, backed by pigment and cell body layers as in the main retina. A first ultrastructural study of an alciopid eye has recently appeared (*4*); and we have for several years been studying other species (*5*).

The first ERG we recorded was in the near ultraviolet at 340 nm. It was a simple, monophasic wave, cornea-negative as expected in an invertebrate eye in which the visual cells face the light. As we proceeded toward longer wavelengths, however, we were greatly surprised to see the polarity of the ERG reverse between 420 and 440 nm. Thereafter it remained cornea-positive into the red.

This reversal of polarity with wavelength is shown in Fig. 3A. It signals a fundamental change of mechanisms: not only the polarity and spectral sensitivity, but the shape and time course of the ERG are markedly altered.

This curious behavior has a simple explanation. We had a first hint of it in our next experiment, in which we obtained only the cornea-negative response. That is always present; only under certain conditions does the cornea-positive response replace it at long wavelengths. It turned out that the decisive factor is the position of the active electrode on the cornea. As Fig. 3B shows, one can evoke or lose the cornea-positive response by just moving the active electrode.

The point is that wherever the electrode is placed on the cornea, it picks up responses from the main retina, and always from the tips of the receptors; but only when the electrode is just over an accessory retina does it record its responses also, and then—since the accessory retinas face inward—from the *bases* of the receptors (see Fig. 2). The receptor response is always the same: the outer segment goes negative toward the remainder of the cell. It is only the reversed anatomical placement of the receptors relative to the electrode in the main and accessory retinas that on occasion reverses the polarity of the response.

The wavelength sensitivities of both responses are shown in Fig. 4. That of the main retina peaks at about 400 nm, that of the accessory retina at about 560 nm. The sensitivity of the main retina falls off sharply at long wavelengths, yet it is still evident at 560 nm and beyond

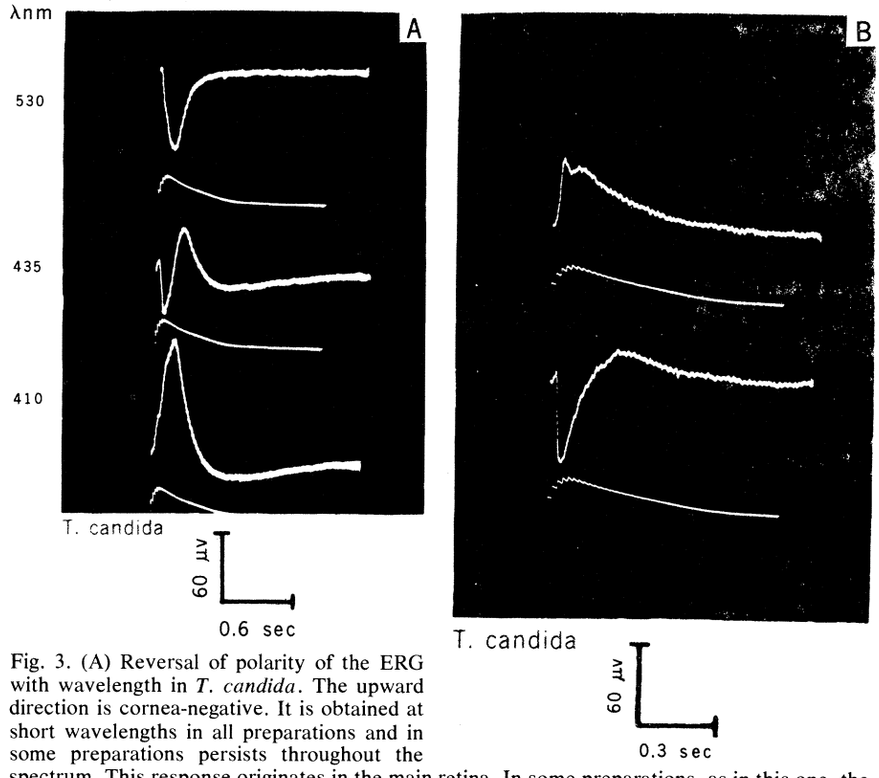


Fig. 3. (A) Reversal of polarity of the ERG with wavelength in *T. candida*. The upward direction is cornea-negative. It is obtained at short wavelengths in all preparations and in some preparations persists throughout the spectrum. This response originates in the main retina. In some preparations, as in this one, the polarity goes cornea-positive at long wavelengths, owing to responses from an accessory retina. In between, at 420 to 440 nm, the response is composite, hence diphasic. (B) Reversal of polarity of the ERG at 560 nm with position of the active electrode on the cornea. The upward, cornea-negative deflection is obtained in most positions of the electrode (upper trace); only in certain positions, not always easy to find, the polarity reverses to cornea-positive (lower trace). The cornea-negative response is from the main retina, the cornea-positive from an accessory retina. The notch in the upper record is caused by a minor intrusion of a cornea-positive response from an accessory retina, favored by stimulation at 560 nm, its sensitivity peak. Below each ERG is the light signal (d-c recording) for a 1/8-second flash.

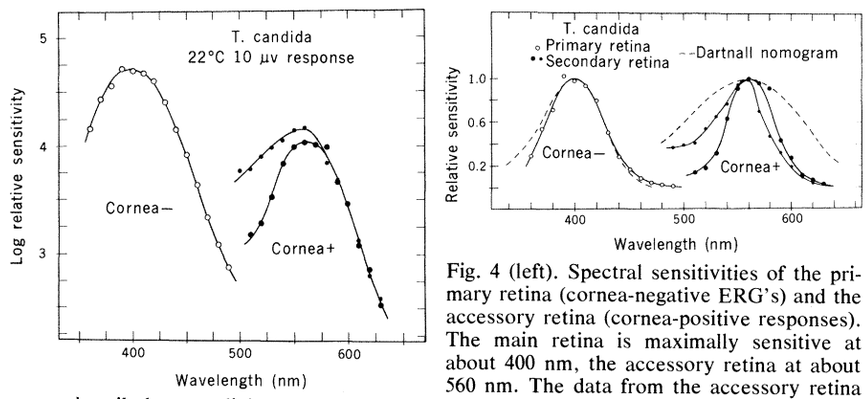


Fig. 4 (left). Spectral sensitivities of the primary retina (cornea-negative ERG's) and the accessory retina (cornea-positive responses). The main retina is maximally sensitive at about 400 nm, the accessory retina at about 560 nm. The data from the accessory retina vary, primarily because light does not strike it directly but strikes it either from behind or by reflection from the primary retina. Fig. 5 (right). Spectral sensitivities of the primary and accessory retinas, plotted linearly, and compared with the Dartnall function (based on the observation that most known visual pigments have similar shapes of absorption spectra when plotted on a frequency scale). The curve for the main retina fits the Dartnall function reasonably well, that for the accessory retina not at all, mainly because light strikes the accessory retina only indirectly, after transmission through or reflection from ocular pigments.

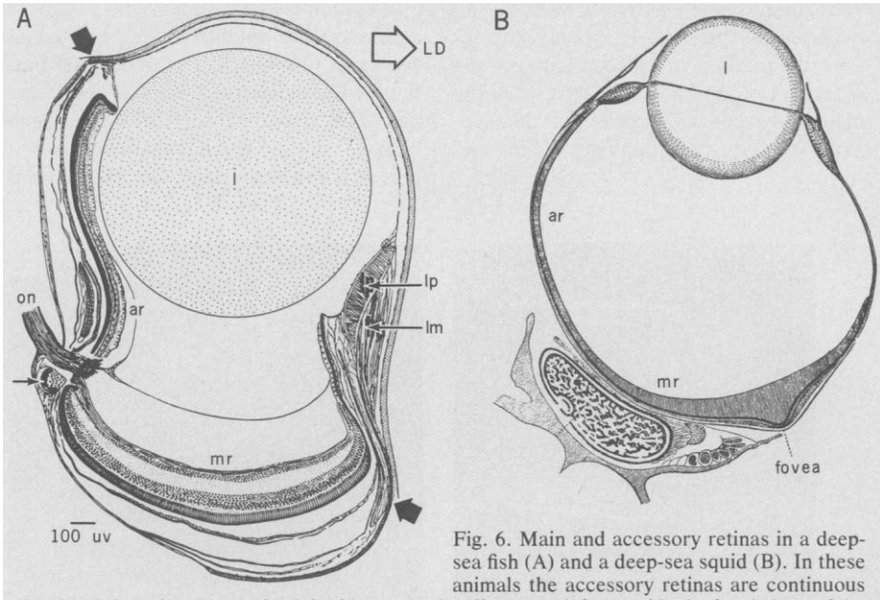


Fig. 6. Main and accessory retinas in a deep-sea fish (A) and a deep-sea squid (B). In these animals the accessory retinas are continuous with the main retina; but, as in alciopid worms, they lie or reach far outside the focal plane of the lens. (A) Section through the dorsally directed tubular eye of *Dissomma anale* Brauer, 1902 (= *Scopelarchus guentheri* Alcock, 1896). Thick arrows point to limbus corneae. Thin arrow at left points at a piece of the accessory retina located below and medianly to the optic nerve. Redrawn by Munk (1966) after Brauer (1902) (*11*). (B) Median section through eye of the squid, *Bathyteuthis* (Hoyle and Verrill) (= *Benthoteuthis* Ver.), after Chun (1903) (*12*). Chun calls the main and accessory retinas the ventral and dorsal retinas. The fovea in this animal possesses "rods" up to 0.4 to 0.5 mm long, the longest known. (*ar*, Accessory retina; *on*, optic nerve; *l*, lens; *ld*, laterad; *lm*, lens muscle; *lp*, lens pad; and *mr*, main retina.)

(Fig. 3B). The spectral sensitivity of the accessory retina is markedly narrowed and variable, owing no doubt to its peculiar setting in the eye. It seems to receive most of its light not directly but by reflection from the main retina—a process for which its spectral sensitivity is nicely adapted. We tried several times to isolate the responses of the accessory retina by adapting out the main retina with short-wavelength light; but this failed, probably because—as explained below—it did not adapt sufficiently.

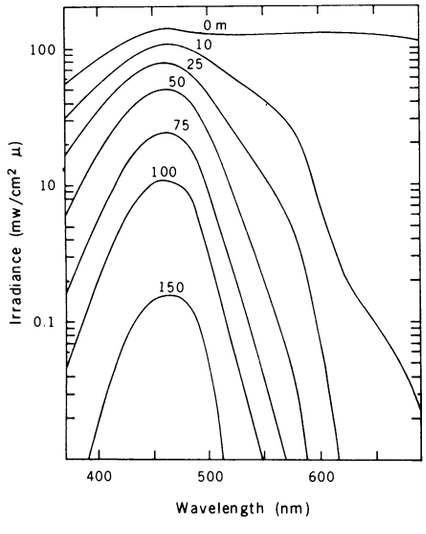
Figure 5 shows the same measurements plotted linearly and compared with curves computed from the Dartnall nomogram (*6*). Dartnall observed that many of the known visual pigments have similar shapes of absorption spectrum when plotted on a frequency scale. His nomogram is based upon frog rhodopsin and fits other visual pigments only approximately. Its main interest is the implication that one may be dealing with a closely related chemistry; and indeed all the visual pigments we have known heretofore, vertebrate and invertebrate, share much the same chemistry, all of them consisting of a visual protein, an opsin, bearing as chromophore 11-*cis* retinal (11-*cis* vitamin A aldehyde). Be it said at once that approximation to the Dartnall function offers at most a suggestion, but no assurance, of chemical relationship.

Yet nothing is known of the chemistry of vision in worms; and that makes us want to ask, however tentatively, how the spectral sensitivities in this worm relate to the Dartnall function. The answer, as shown in Fig. 5, is that the spectral sensitivity of the main retina of *Torrea* fits rather well, that of the accessory retina not at all. The latter is to be expected, for little light can reach the receptors of the accessory retina directly. Either it enters them from behind, after passing through their deep orange-red pigment; or, more likely, they are stimulated primarily by light reflected from the main retina. Either way their spectral sensitivity must depend on some composite of the absorption spectrum of their visual pigment and the transmission and reflection characteristics of the other pigments in the eye.

The ERG in *Torrea* displays a property that we encountered again in *Vanadis*, so that it may be general in alciopid eyes. If one stimulates the main retina repeatedly with flashes one or a few seconds apart, the second and sometimes the third responses rise in amplitude above the first, later flashes remaining constant at the higher level. That is, instead of the usual effect of repeated flashes lowering the response, here they raise it. For example, with flashes at 420 nm, of 1/8-second duration, and repeated 2 seconds apart, the successive amplitudes of ERG in microvolts were 94, 130, 130, 130; then, after 1 minute in the dark, again: 92, 130, 130, 130.

This phenomenon, called *facilitation*, has been observed earlier in other phyla: in such arthropods as the isopod *Ligia* (*7*), barnacles (*8*), and a spider (*9*); and in such molluscs as the octopus and squid (*10*). In the squid, the retinal facilitation was expressed also in single units of the optic nerve; a second flash evoked more spikes than the first flash (*10b*). So this phenomenon is a real component of the sensory response.

Though we made no systematic measurements of light and dark adaptation, our observations show that both phenomena occur, though they are quite restricted in range. Our measurements were by-products of using bright lights to position in preparation and then waiting in the dark for the main retina to regain maximum sensitivity. In one such case exposing the eye to very bright white



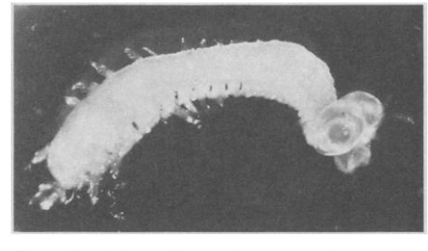


Fig. 7 (left). Transmittance of daylight through clear ocean water. From T. H. Waterman, 1974, after Lundgren and Hojerslev, 1971 (*13*). Sun elevation, 56°. Fig. 8 (above). *Vanadis* sp., probably *crystallina*. Preserved specimen taken in the Bay of Naples at a depth of about 300 m. The animal is singularly unpigmented, except for small black spots at the bases of the parapodia, and red-brown eyeballs. The pupils of the eyes glowed golden by light reflected from inside, probably mainly by the visual pigment itself.

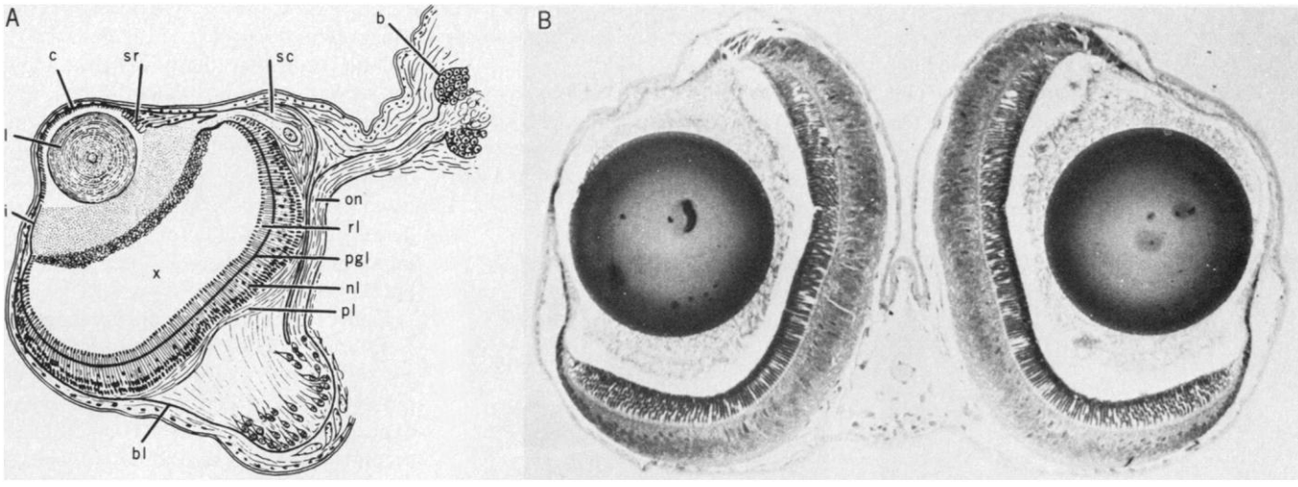


Fig. 9. (A) Cross section of eye of *V. formosa*, modified by Hermans and Eakin (*4*) after Hesse (*1*). Abbreviations are *b*, brain; *bl*, basal lamina; *c*, cornea; *i*, iris; *l*, lens; *nl*, nuclear layer; *on*, optic nerve; *pgl*, pigment layer; *pl*, plexiform layer (optic-nerve fibers); *rl*, receptor layer (outer segments); *sc*, secretory cell, usually described as a single, giant cell that secretes fluid into the space that contains the lens; *sr*, accessory ("secondary") retina; and *x*, labeled by Hermans and Eakin "shrinkage artifact?" but in our experience a true, second intraocular space filled with a markedly fibrous fluid or gel. In the section in (B) it is poorly fixed; but compare Fig. 2. (B) Cross section through the head of *Vanadis*, unfortunately cut at a level that missed the accessory retinas. Note particularly the absence of retinal screening pigment (compare Fig. 2).

light for 4 minutes raised the threshold at 420 nm only 1.35 log units; it took about 20 minutes in the dark at 23°C to come back. It is probably this narrow range of adaptation that defeated our attempts to adapt differentially the main retina so as to be able to measure the accessory retina alone.

The presence of accessory retinas in alciopid eyes offers a prime instance of the phenomenon of evolutionary convergence; for such organs occur also in the tubular eyes of deep-sea fishes (*11*) and in some deep-sea cephalopods (*12*) (Fig. 6). In both these other phyla the accessory retinas are not divided off from the main retina but form an extension up one side of the eye so as to lie in part, as in worms, beside the lens, far outside its focal plane. This convergence is all the more remarkable in that in worms and cephalopods both main and accessory retinas face the light; in fishes both are inverted. We have here, spread over three phyla, an apparently basic association between accessory retinas and deep-sea existence.

What does it mean? Earlier workers, knowing only the anatomy of these structures, argued only from their position in the eye. Hence the repeated suggestion that accessory retinas, wherever they occur, exist mainly to pick up vague signals that something is moving far off to the side—to that degree resembling the function of peripheral parts of the human retina. But why then such a wide discrepancy in wavelength sensitivity as we find in *Torrea*?

We think that the key to the function of the accessory retinas in *Torrea* lies in this wavelength discrepancy. These structures seem mainly to be looking into the eye, as though monitoring the light reflected from the fundus, a task to which their spectral sensitivity is well suited. In that position they are exposed only to long-wavelength light, a result that could be achieved otherwise only with an orange-red filter. But to what end?

The main attribute of long-wavelength light is that it attenuates with depth in the sea much more rapidly than blue, violet, or near-ultraviolet light. The ratios of energies of sunlight transmitted through clear ocean water at the sensitivity peaks of the main and accessory retinas in *Torrea*, 400 and 560 nm, respectively, are at the surface 0.59; at a depth of 10 m, 1.41; at 25 m, 3.3; at 50 m, 10.6; at 75 m, 25.2; at 100 m, 40; and at 150 m, about 70 and rising rapidly with further depth (*13*) (Fig. 7).

Hence, comparisons in these two spectral regions should offer a sensitive indicator of the worm's depth in the sea. In any but clear seawater, this scale should need recalibration, such as any animal might readily achieve through its depth migrations in any new location. We think this is probably the main point of the accessory retinas in *Torrea* and perhaps also on other alciopid eyes. It would be of great interest to test the generality of this conclusion by measuring the spectral sensitivities of the main and accessory retinas in other deep-sea forms.

Early one morning, one of us (S.R.) went with the collecting crew of the Stazione Zoologica to make plankton hauls between Capri and Ischia, in an area about 400 m deep. Of three hauls at increasing depths, only the deepest at about 300 m brought in five alciopid worms of the genus *Vanadis*. Unlike *Torrea* these were short (about 5 cm) and almost wholly unpigmented save for the eyes (Fig. 8). The eyeballs were red-brown, the pupils glowing golden by light reflected from inside, and surrounded by a white iris that at some angles reflected light as from a mirror, presumably owing to a layer of crystals deposited in the cornea.

These animals all looked alike; yet later examination of the fixed material in Cambridge showed them to include two closely related species, *Vanadis crystallina* and *V. formosa*, distinguished mainly by their mouthparts (*2*). Their most striking property was that the first seven to ten segments lacked or had only rudimentary parapodia. This condition is typical of *V. crystallina*; in *formosa*, only the first two to three segments are said to be this way. We worked on two specimens, believing that both were *crystallina*; yet marked differences in our observations suggest that both species were involved. We refer to our two experimental animals as *Vanadis* I and II.

Figure 9 shows Hesse's diagram of a section through the eye of *V. formosa* and one of our own sections which unfortunately missed the single accessory retina. The accessory retina appears on the median ventral border of the eye; in our sections which show it, the retinal elements seem few and fragile. The most striking difference between the eye of Vanadis and that of *Torrea*, clearly evi-

dent in our section, is the virtual or complete absence of the screening pigment between the outer segments and cell bodies of the visual receptors in both the main and accessory retinas in *Vanadis*. This contributed to our impression that we were working with habitually deep-water animals.

The ERG of *Vanadis* II is shown in Fig. 10A. It consists mainly of a simple monophasic cornea-negative wave with, at this sweep speed, no apparent latency. The potential rises to a peak at about 0.2 second, returns to the baseline at about 0.5 second, then overshoots slightly and is completed at about 0.7 second. In contrast, the ERG of *Vanadis* I (Fig. 10B) displays a distinct latency of about 75 msec, and there is no overshoot; also here a periodic oscillation (jags) is superimposed on the otherwise simple, monophasic response.

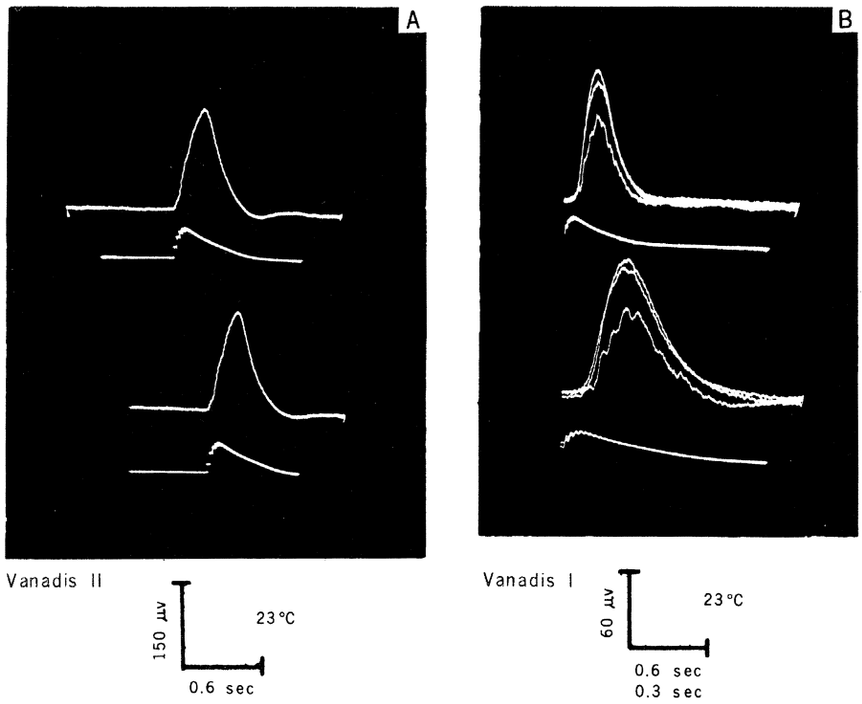


Fig. 10. (A) Electroretinograms of *Vanadis* II. The upward direction is cornea-negative. Below each ERG is the light signal. At this sweep speed, no latency is evident (dark adapted, d-c, 570 nm, 1/8 second). (B) "Facilitation" in *Vanadis* I (dark adapted, d-c, 570 nm, 1/8 second). Electroretinograms in response to three identical successive flashes, 2 seconds apart. In each set the lowermost curve is the first response. It shows a latency of about 75 msec, and the ERG displays a periodic oscillation (jags). The second and third responses are considerably higher, and the jags have smoothed out. Underneath each set of ERG's is the light signal. The sweep speed is halved in the lower set of records.

Figure 10B shows the phenomenon of facilitation which also occurred in *Torrea*, and hence may be general in alciopid eyes. When the dark-adapted eye is exposed to repeated flashes one or a few seconds apart, the second flash evokes a considerably higher response than the first, the third may be higher still, and later responses remain at the higher level. After 1 minute in the dark, the response has returned to its original height. Also the "jags" that appear in the first ERG are erased in the succeeding responses.

Figure 11A shows the spectral sensitivity of *Vanadis* I, plotted logarithmically, Fig. 11B that of *Vanadis* II. The curves differ in both shape and position. The sensitivity of *Vanadis* I peaks at about 460 nm, with a shallow shoulder below 390 nm and a broad inflection near 550 nm. The sensitivity of *Vanadis* II peaks at about 480 nm, with a single shoulder in the blue.

In Fig. 12 these measurements, now plotted linearly, are compared with curves computed from the Dartnall nomogram. Neither of the *Vanadis* spectral sensitivities fits the Dartnall function. The main band of *Vanadis* I is

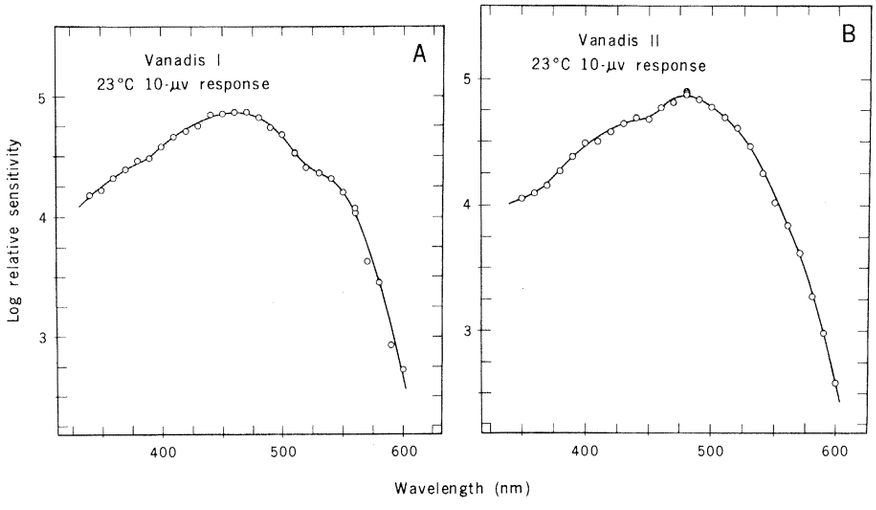


Fig. 11 (left). Spectral sensitivity of *Vanadis* I (A) and *Vanadis* II (B), plotted logarithmically. (A) The sensitivity is maximal at about 460 nm, with shoulders at about 370 and 550 nm. (B) The sensitivity is maximal at about 480 nm, with a shoulder at about 410 nm.

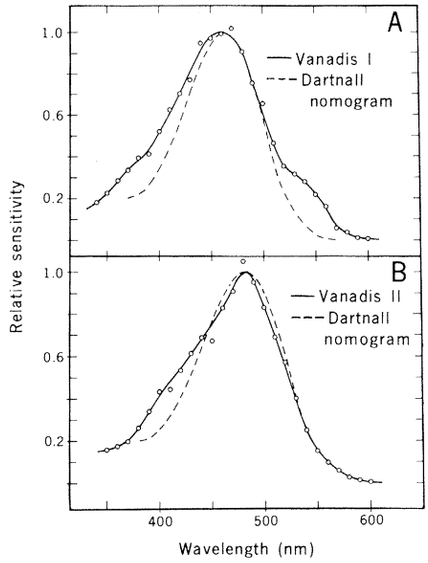


Fig. 12 (right). Comparison of spectral sensitivities, plotted linearly, of *Vanadis* I (A) and *Vanadis* II (B) with Dartnall functions peaking at the same wavelengths. The spectral sensitivity of *Vanadis* I is wider than the Dartnall function, and involves extra inflections at about 370 and 550 nm. The main sensitivity band of *Vanadis* II is narrower than the Dartnall function and seems to carry also a broad inflection centering at about 410 nm.

somewhat wider, that of *Vanadis* II a little narrower. The departures are not large enough to rule out a possible chemical relationship, yet neither do they support it.

The most striking departure from the Dartnall function is the large inflection at about 550 nm displayed by *Vanadis* I. It is tempting to suppose that this arises from the accessory retina, which in *Torrea* peaks in sensitivity near this wavelength. But why then is it not, as in *Torrea*, reversed in polarity?

In the little work we were able to do with *Vanadis*, we never observed a cornea-positive response. Yet is should be noted that even in *Torrea*, with its paired accessory retinas, it is not easy to place the active electrode over one of them so as to record the ERG through its base, hence with inverted polarity. In *Vanadis* this would be still more difficult, and in the short time at our disposal, such achievement eluded us. It seems reasonable to suppose that if the active electrode happened to be at some distance from the accessory retina, more or less across from it (see Fig. 9), it could pick up responses from the tips of the accessory retinal receptors as well as from those of the main retina, hence with the same polarity. That and its enormously greater sensitivity at long wavelengths might well permit the accessory retina to account for the inflection near 550 nm in the spectral sensitivity of *Vanadis* I.

We had also direct evidence that more than one photosensitive system functions in *Vanadis* I, in that the response on occasion changed its character markedly at different wavelengths. Thus in one experiment, whereas the response at 480 nm was a simple monophasic wave, that at 380 nm had a distinct inflection on the rising limb, as though a second, shorter-wavelength mechanism intruded. At another time a preparation that we had worked with for several hours and had treated with MS 222 suddenly began to display a very complex response to single flashes at both 380 and 580 nm; at the latter wavelength, however, this response alternated regularly with a single, very small cornea-negative blip. We have no idea what this meant, apart from its important implication that different mechanisms were at work at 380 and 580 nm. The curves for *Vanadis* I in Fig. 11A and Fig. 12A show that these wavelengths hit respectively the short- and long-wavelength inflections. It may well be that *Vanadis* I possesses three distinct photosystems, represented by the main peak at 460 nm, and the subsidiary inflections near 380 and 550 nm. We saw no comparable differences in the responses of *Vanadis* II at 380 nm and 560 nm.

We have already emphasized the extraordinary evolutionary convergence represented by the possession of accessory retinas in alciopid worms, and in deep-sea fishes and cephalopods. Our observations on *Vanadis* provide another remarkable instance of such convergence, in that our specimens taken at 300 m share with a wide range of other deep-sea organisms, including deep-sea fishes, visual sensitivity peaks in the blue, at 460 to 480 nm. These are the wavelengths of sunlight that penetrate most deeply into clear ocean water (Fig. 6). At a depth of 200 to 250 m the entire spectrum of sunlight is reduced to a band of blue light stretching from about 450 to 505 nm, and peaking in intensity at 460 to 480 nm (*13*). At still greater depths, and at night, organisms with eyes must depend for vision upon bioluminescence, particularly that of luminescent bacteria, and this, in turn, is concentrated primarily at the same wavelengths (*14*). A visual sensitivity peaking at 460 to 480 nm is therefore optimal for deep-sea life; and our deep-sea alciopids have achieved this aspect of fitness along with a wide array of deep-sea organisms belonging to other phyla that possess eyes.

Note added in proof: Since writing this report we learned of ERG measurements of spectral sensitivity in the relatively primitive cup-eye of another marine annelid worm, *Nereis* (*15*).

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References and Notes

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Laser Fluorimetry: Subpicogram Detection of Aflatoxins Using High-Pressure Liquid Chromatography

Abstract. *The use of high-pressure liquid chromatographic separation in conjunction with laser-induced fluorescence detection permits the analysis of trace fluorescent species at new limits of sensitivity. This technique was applied to the carcinogens aflatoxins B_1, B_2, G_1, and G_2, which were linearly quantitated to 7.5 × 10^{-13} gram. The procedure consists of forming more fluorescent aflatoxin derivatives, eluting the aflatoxins from a reverse-phase column, focusing the 325-nanometer output of a helium-cadmium ion laser into a suspended droplet of the eluent, and measuring the resulting fluorescence using phase-sensitive detection.*

The well-documented potency of aflatoxins, carcinogenic mold metabolites, as causative agents for tumor formation in laboratory animals gives rise to a need for extremely sensitive and selective detection methods in food products at the level of a few parts per billion (ppb) (*1–3*). We report here the development of an analytical method for detecting ultratrace amounts of fluorescent species, based on the use of high-pressure liquid chromatographic separation followed by