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Comparative Studies of Crustacean Spectral Sensitivity

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Summary. Spectral sensitivity of the lateral eyes of the isopod *Porcellio scaber* (wood louse) and the decapods *Callinectes sapidus* (blue crab), *Palaemonetes paludosus* (Everglades prawn), *Orconectes virilis*, and *O. immunis* (crayfish) have been measured between 300 and 660 nm by determining the reciprocal number of photons required to evoke a constant size retinal action potential.

Porcellio is maximally sensitive at 515 nm and *Callinectes* at 505 nm. Both species have a single pigment system, as spectral sensitivity is unchanged by red light adaptation.

Palaemonetes appears to have a dichromatic color vision. Sensitivity of the dark-adapted eye is dominated by a receptor maximally sensitive at 550—555 nm, but red or yellow adaptation discloses a uv pigment with λ_{max} at about 380 nm. Present evidence suggests the 555 and 380 nm pigments are located in different receptor cells.

Orconectes has peak sensitivity at 565 nm, but under red light adaptation and close to the electroretinographic threshold a second sensitivity maximum appears at 425 nm. As in the prawn, these peaks seem to indicate the presence of a two-receptor color vision system.

The corneas of *Orconectes*, *Callinectes*, and *Homarus* (lobster) are relatively thick, and microspectrophotometric measurements show near ultraviolet absorption as well as the protein peak at 280 nm. By contrast, *Palaemonetes* and *Musca* (housefly), species with near ultraviolet receptors, have thinner corneas which are transparent through the near ultraviolet. The crystalline cone of *Palaemonetes* likewise shows no near ultraviolet absorption but a strong protein band at 280 nm.

The scarcity of ultraviolet receptors in the compound eyes of crustacea, in contrast to their common occurrence in insects, is thought to be related to the relative absence of ultraviolet wavelengths in most aquatic environments.

Introduction

One of the distinctive features of insect eyes is their sensitivity to near ultraviolet wavelengths. In contrast to the eyes of vertebrates, where in general the yellowish lens filters out wavelengths shorter than about 390 nm and renders the β-band — the secondary near ultraviolet absorption band of rhodopsin — inoperative in visual excitation (WALD, 1952; KENNEDY and MILKMAN, 1956), the eyes of insects seem designed to exploit the shortest wavelengths present in the environment. The cornea is transparent to 300 nm (GOLDSMITH and RUCK, 1958; BERNHARD, MILLER, and MØLLER, 1965; GOLDSMITH and FERNANDEZ, 1968), and in the cases which have been examined in detail, either the photoreceptors have sensitivity maxima at 340—350 nm (BURKHARDT, 1962),

or specific uv receptors are present (WALTHER and DODT, 1959; GOLDSMITH, 1961b; HASSELMANN, 1962; AUTRUM, 1965). Moreover, at a behavioral level, near ultraviolet light frequently is the most effective region of the spectrum in triggering phototaxes (cf. GOLDSMITH, 1961a) or the light-compass reaction of honeybees (VON FRISCH, 1954).

This study was begun because we were curious about the distribution of ultraviolet receptors in other arthropods, particularly crustacea. Until very recently, surprisingly little work has been done on the spectral sensitivity of crustacean eyes in any region of the spectrum (STIEVE, 1960; KENNEDY and BRUNO, 1961; WALD, 1968). We here report on several species, chosen in part for convenience and in part because they represent different taxonomic groups and live in varied environments (Table 1).

Table 1

Order (suborder: section)	Genus and species	Common name	Habits
Isopoda	*Porcellio scaber*	wood louse	terrestrial
Decapoda (Natantia)	*Paleomonetes paludosus*	Everglades prawn	fresh water (Florida Everglades)
Decapoda (Reptantia: Brachyura)	*Callinectes sapidus*	blue crab	marine and brackish water
Decapoda (Reptantia: Macrura)	*Orconectes virilis* *O. immunis*	northern crayfish	fresh water

(In a preliminary abstract of this work (GOLDSMITH and FERNANDEZ, 1966) the species of *Orconectes* are incorrectly identified.)

The experimental approach was simple: spectral sensitivity was measured by determining the relative quantum flux required to evoke retinal action potentials of constant size from the eyes of immobilized, unanesthetized animals. As the maximum sensitivity of the dark-adapted eye invariably lay in the blue-green or green region of the spectrum, the technique of selective adaptation was employed to expose additional pigments maximally sensitive in the blue, violet, or ultraviolet.

The results of this work suggest that ultraviolet receptors are more common among insects than among other arthropods. Moreover, microspectrophotometry of individual facets of arthropod corneas suggests that only those species with ultraviolet receptors have corneas completely clear of near ultraviolet-absorbing material. Finally, the results indicate that in some species of crustacea vision is monochromatic, whereas others have the sensory apparatus for at least a dichromatic color vision, an inference in harmony with the recent study of WALD (1968).

Methods

Experimental Animals

Porcellio was collected locally; *Callinectes* was purchased at a local fish market; *Palaemonetes paludosus* was collected from the Florida Everglades; and *Orconectes virilis* and *O. immunis* were obtained through a biological supply house in western Massachusetts.

For recording, *Porcellio* was immobilized in a soft wax ("Tackiwax", Cenco) with one eye placed at the focus of the stimulating beam. The decapods were wrapped in several layers of wet gauze and secured by rubber bands to a perforated plastic plate. Plate and animal were partially immersed in water or sea water as appropriate. The eyes of *Palaemonetes* and *Orconectes* were further immobilized by wads of wet cotton wedged between the eye stalk and the rostrum; the eye stalk of *Callinectes* was held rigid with dental cement.

Stimulus and Adapting Lights

The stimulating source was a 150 watt xenon lamp (Hanovia D-901C) operated at 7.5 amps, dc. This light was passed through a Bausch and Lomb monochromator with a 52 mm square grating, 1200 lines per mm, and with slits adjusted for a 3.3 nm half band width. At wavelengths longer than 560 nm, the second order spectrum was blocked with a Corning 3—72 filter. The adapting light was a tungsten microscope lamp fitted with a Zeiss RG2 filter, which passes wavelengths longer than 630 nm. For a few experiments on *Palaemonetes*, this red-orange filter was replaced by an interference filter with peak transmission at 575 nm, but the experimental results were the same. Appropriate lenses concentrated the light beam on the experimental animal so that the entire eye was bathed in light. The adapting beam arrived at an angle of about 10° to the stimulating beam.

The energy output of the stimulating source was periodically measured at 20 nm intervals with a calibrated thermopile (Eppley) fitted with a quartz window and connected to a Keithley No. 149 amplifier and a pen recorder. During the course of an experiment the intensity of the stimulus beam was controlled with a pair of optical wedges made of inconel deposited on quartz. The wedges had been calibrated previously.

Recording

In all experiments, the electrodes were silver:silver chloride. In the case of *Porcellio*, one electrode contacted the illuminated eye through a physiological saline-filled pipette with a tip diameter of about 30 μ placed through a pilot hole in the cornea. The reference electrode made contact through a saline-soaked wick on the opposite side of the head and was shielded from the stimulus beam by a mask of aluminum foil. In experiments on *Callinectes*, *Palaemonetes*, and *Orconectes*, both electrodes contacted the animal through cotton wicks, one placed on the illuminated eye and the other on the opposite side of the head. In most experiments the amplifier was a Tektronix 122 operated at a band pass of 0.2—1,000 Hz, although in some experiments D.C. recording was done. Responses were displayed on an oscilloscope and photographed.

Experimental Procedure

Animals were dark-adapted for a minimum of 1 hour before an experiment began. Test flashes were usually one-fifth second duration and were repeated at intervals no shorter than a minute to insure that the test flashes themselves did

not disturb the state of adaptation. Spectral sensitivity was measured by determining the relative quantum flux required to elicit a standard response of about 250 μv. Responses yielding small segments of the response-energy function on either side of the criterion were recorded at each wavelength, and the energies required to produce a 250 μv negativity determined graphically after the film was measured. In some experiments 50 μv criteria were used.

Microspectrophotometry

Absorption of individual corneal facets was measured with a dual beam recording microspectrophotometer slightly modified from the design of LIEBMAN and ENTINE (1964). Light from a 500 watt deuterium lamp was passed through a Bausch and Lomb grating monochromator to illuminate a pair of apertures. The apertures were demagnified and imaged in the specimen plane with a 10× quartz ocular and Zeiss 32× neofluar objective (n.a. 0.4). Pieces of cornea were placed in slightly diluted glycerin (refractive index 1.455) between quartz coverslips and positioned so that the sample beam passed through a single facet. The collecting optics focused the light onto the cathode of a photomultiplier tube (EMI 9558 QA). The reference and sample beams were separated in time by a rotating sector disk, and the output of the photomultiplier in response to the reference light was amplified and fed back to control the voltage on the dynodes and so maintain the reference output constant with wavelength.

Results

Porcellio scaber

The spectral sensitivity of the dark-adapted wood louse *Porcellio scaber* is shown by the filled circles in Fig. 1. The main peak lies at about 515 nm with a secondary maximum near 350 nm. This curve is similar to a vertebrate rhodopsin with an α absorption band in the blue-green and a minor β-band in the near ultraviolet. *Porcellio* seems to have but a single visual pigment, for when the spectral sensitivity curve is measured in the presence of a red adapting light, its shape is unaltered (open circles). The effect of red light adaptation is to depress the sensitivity equally throughout the spectrum. The curves in Fig. 1 are based on criterion responses of 250 μv, but the result is independent of the response level.

Callinectes sapidus

Fig. 2 shows the spectral sensitivity of the blue crab *Callinectes*. As in *Porcellio* there appears to be but a single visual pigment, but in this species the measurements were made at more wavelengths. Sensitivity of the dark-adapted eye is greatest at about 505 nm (filled circles). On the short wavelength side of the curve there is a broad shoulder which falls abruptly below 360 nm. Red light adapts the eye without changing the shape of the spectral sensitivity function (open circles). As with *Porcellio*, spectral sensitivity does not depend on the intensity

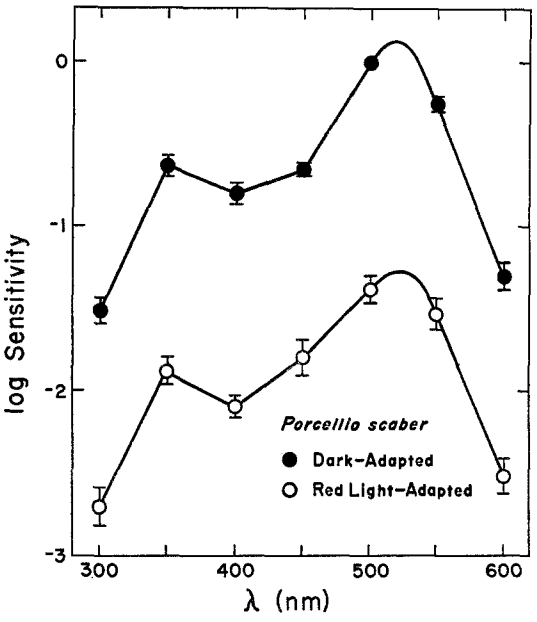

Fig. 1. Spectral sensitivity of the isopod *Porcellio scaber*. Filled circles are average results from 10 dark-adapted animals; open circles are average results from 6 animals adapted to red light. Vertical bars represent standard errors. Ordinate is the log of the reciprocal of the relative number of photons for a 250 μv response

of illumination; i.e., it is independent the size of the criterion response, at least up to a few mv.

Fig. 3 is an arithmetic plot of the two curves in Fig. 2, scaled to bring out the constancy of shape with adaptation. This plot should reflect the absorption spectrum of the visual pigment, except to the extent that it may be distorted by selective filtering by the dioptric apparatus and accessory pigments (see below). As shown by the crosses in Fig. 3, the shape of the spectrum is similar to a vertebrate rhodopsin.

Palaemonetes paludosus

This fresh water prawn yielded somewhat more complicated results than did the previous two species. As shown in Fig. 4 (filled circles), the dark-adapted eyes are maximally sensitive at 550—555 nm, and there is a shoulder at about 380 nm. Unlike the previous two examples, however, red light adaptation alters the shape of the spectral sensitivity function. Sensitivity at long wavelengths is depressed relatively more than at short wavelengths, with the result that the near ultraviolet shoulder is converted into a distinct peak with λ_{max} at 375—380 nm.

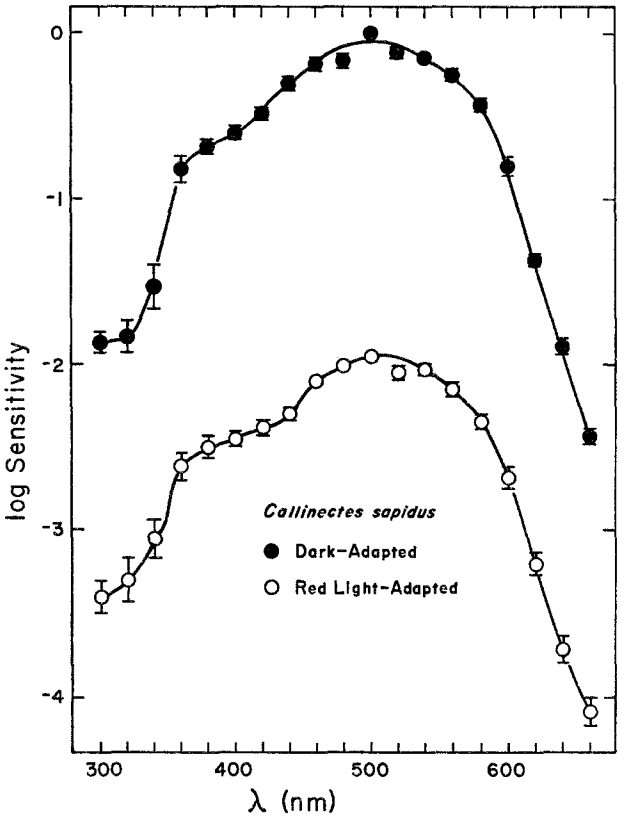

Fig. 2. Spectral sensitivity of the blue crab, *Callinectes sapidus*. Filled circles are average results from 14 dark-adapted animals; open circles are average results from 10 red-adapted crabs. Standard errors are indicated. Ordinate is the log of the reciprocal of the relative quantum flux for a constant response of 250 μv

Thus the eye contains two pigment systems, one in the green and one in the near ultraviolet. The former seems the more abundant, as it dominates the sensitivity of the dark-adapted eye. The closely related marine prawn *P. vulgaris* is reported to behave in similar fashion to *P. paludosus* (WALD and SELDIN, 1968). This result is reminiscent of the compound eye of the worker honeybee, where a 535 nm system is conspicuous in the dark-adapted state and a 340 nm receptor is disclosed on adaptation to long wavelengths (GOLDSMITH, 1960). In the bee these pigments are known to be present in different retinular cells (AUTRUM and VON ZWEHL, 1964).

The response-energy functions of *Palaemonetes* are approximately parallel at different wavelengths, both in the dark- and red-adapted

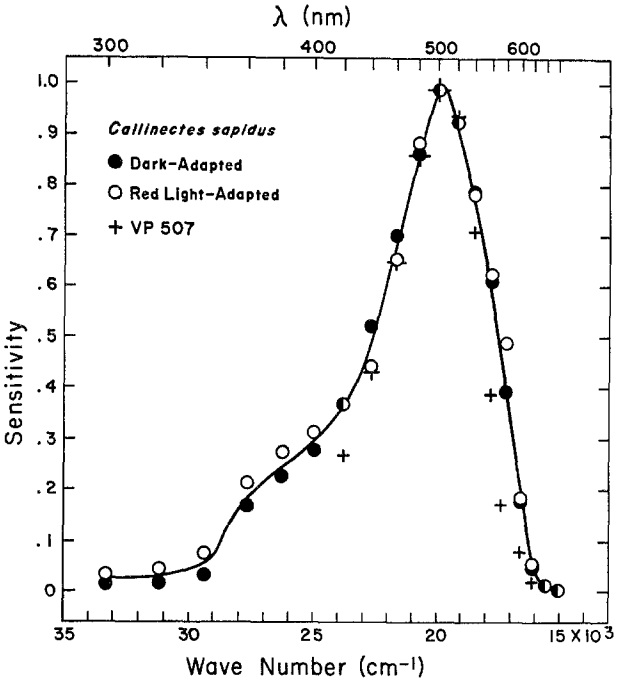

Fig. 3. Spectral sensitivity of the blue crab, *Callinectes sapidus*. The points represent the two curves of Fig. 2 replotted on an arithmetic ordinate and normalized for comparison. The crosses represent the absorption of a hypothetical vertebrate rhodopsin with λ_{max} at 507 nm taken from DARTNALL'S (1953) nomogram

eye. Thus the two pigment systems are operating in the same intensity range, as would be expected in a system for color vision.

Two additional observations suggest that the 555 and 380 nm pigments are in different cells. Fig. 5 shows that the electrical responses of the eye cannot be exactly matched at 560 and 380 nm, either in the dark- or red-adapted animal. At longer wavelengths the rise time of the response is faster, and the graded transient — which appears as an on effect at intermediate and high levels of stimulation — rises out of the plateau at relatively lower energies. This finding means that 380 and 560 nm lights do not affect the eye in identical fashion, which would not be expected if the eye contained a homogeneous population of receptors, each with a mixture of two pigments[1]. The difficulty is that

[1] The assumption here is that a cell containing two visual pigments responds with a depolarizing potential when light is absorbed, and there is nothing in the response that permits one to tell which pigment species contributed to the excitation. This assumption might not be valid, however, if the two pigments were selectively packaged in regions of the cell which had different membrane properties.

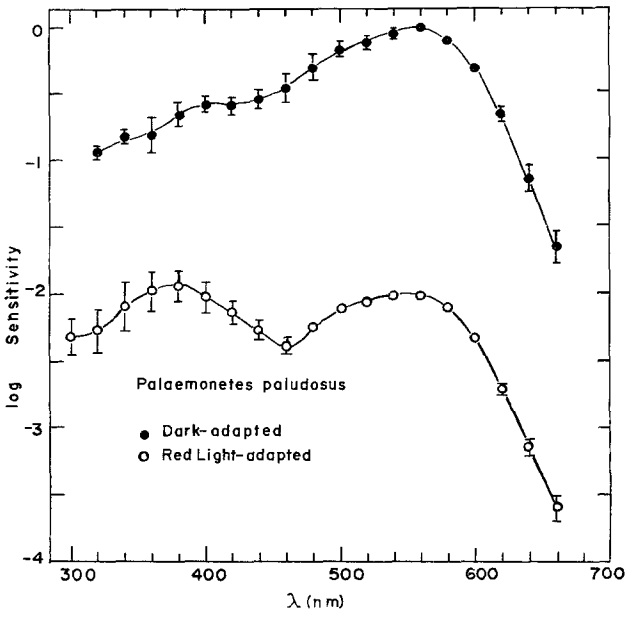

Fig. 4. Spectral sensitivity of the prawn, *Palaemonetes paludosus*, when dark-adapted (filled circles) and when adapted to red light (open circles). Vertical bars are standard errors; the upper curve is based on five animals, the lower on four. Ordinate is the log reciprocal of the relative number of photons for a 250 μv response

wavelength-specific differences in the mass response do not necessarily indicate the presence of color receptors (GOLDSMITH, 1965). In flies, because of wavelength-selective leakage of the sleeves of accessory pigment, different wavelengths can produce different spatial patterns of excitation. As a consequence, even when equal-size responses are obtained from the receptor layer, red light evokes larger contributions from the optic ganglion than does green. On the other hand, there are probably two critical differences between *Palaemonetes* and flies: the screening pigment of the prawn's eye is more nearly neutral, and the retinal action potential comes closer to being a pure receptor response. Nevertheless, the precedent of the fly's eye bespeaks the need for more direct evidence on the localization of the two visual pigments.

The second observation is more to the point. In microspectrophotometric measurements of individual rhabdoms of *Palaemonetes*, we have seen a 555 nm pigment but as yet not one at 380 nm (GOLDSMITH, DIZON and FERNANDEZ, 1968). On the basis of present evidence, those rhabdoms with the 555 nm pigment seem to lack the 380 nm pigment,

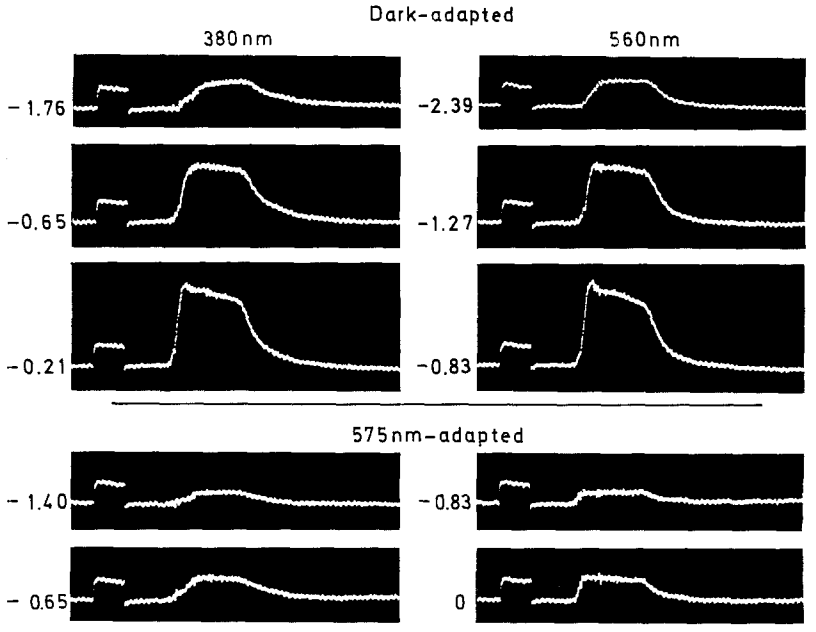

Fig. 5. This figure shows small but consistent differences in the shape of the retinal action potential at wavelengths near the absorption maxima of the two pigment systems of *Palaemonetes paludosus*. The differences are particularly evident when the long wavelength pigment is selectively adapted, in this case with light from a 575 nm interference filter. The square calibration pulse preceding each response is 50 μv $\times$ 100 msec. Amplifier high-pass filter was set for 0.2 cycles per sec. Negativity of the illuminated eye is represented by an upward deflection. The number to the left of each frame is the log of the relative quantum flux. In addition to showing a slower rise time of the low-level responses at 380 nm, this experiment also illustrates the effect of selective adaptation on spectral sensitivity. For the three pairs of responses in the dark-adapted eye, the animal was an average of 0.62 log units more sensitive to 560 nm than to 380 nm. Under the influence of the yellow adapting light, however, the eye was an average of 0.61 log units more sensitive to 380 nm

and rhabdoms with a 380 nm pigment are apparently far fewer in number.

Orconectes virilis and O. immunis

Essentially identical results were obtained from these two species, and so they will be discussed together.

In 1961 KENNEDY and BRUNO reported the spectral sensitivity of the southern crayfish *Procambarus clarkii* to be maximal at about 570 nm and unchanged by selective adaptation with red light. Subsequently WALD (1968) found similar sensitivity maxima in both *Procambarus clarkii* and *Orconectes virilis*, but with the very important

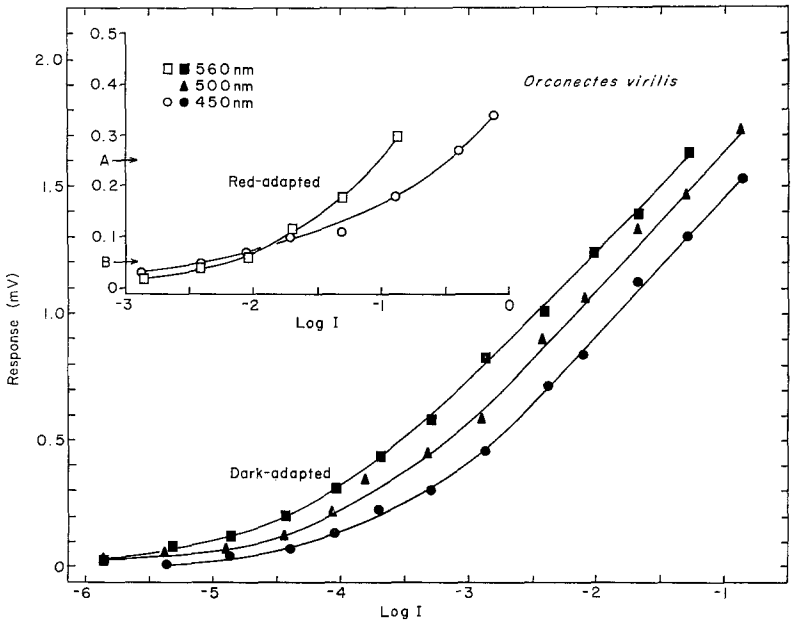

Fig. 6. Response-energy curve for the retinal action potential of *Orconectes*. In the dark-adapted eye the curves at different wavelengths are parallel (filled circles), but under red light adaptation this tidy relationship breaks down (open circles, inset). See the text for further details

difference that red light adaptation uncovered a violet receptor at 450 nm in *Procambarus* and 435 nm in *Orconectes*. Our initial results agreed with those of KENNEDY and BRUNO, and for a time we were puzzled by WALD'S finding of a violet receptor. The discrepancy vanished, however, when we were able to identify the essential difference between KENNEDY and BRUNO'S and WALD'S experimental procedures. The first authors chose criterion responses on the steep part of the response-energy function, whereas WALD worked with 50 μv responses, close to the threshold of the retinal action potential. The latter choice was providential.

Fig. 6 shows response-energy curves for a single animal at several different wavelengths. For the dark-adapted eye, the curves have the same shape; their position with respect to the energy axis depends solely on the relative sensitivity at that wavelength. The open circles in the inset show the lower portions of the response-energy functions for 560 and 450 nm when the eye was exposed to a steady red background light. The curve for 560 nm has the same shape as it did in the dark-adapted eye but is shifted to the right by about three log units. Above

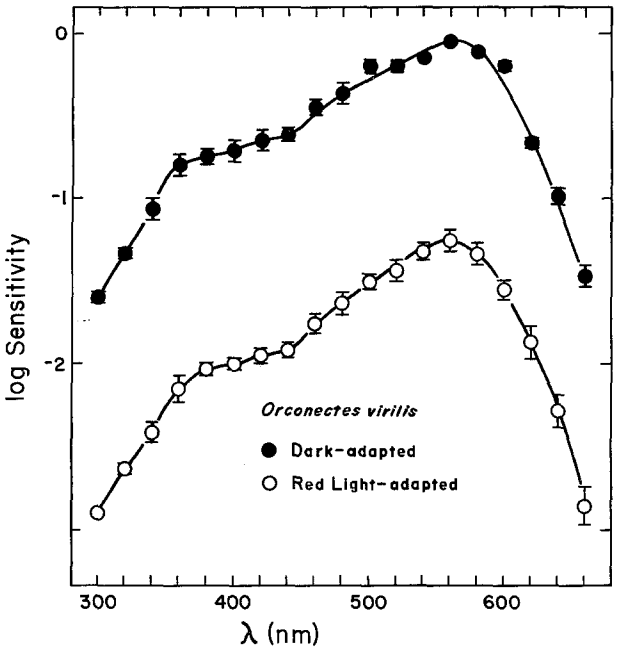

Fig. 7. Spectral sensitivity of the northern crayfish, *Orconectes virilis*, for criterion responses removed from threshold. Filled circles are average results from 13 dark-adapted animals; open circles are average results from 7 animals adapted to red light. Vertical bars represent standard errors. Ordinate is the log of the reciprocal of the relative number of photons for a 250 μv response

about 0.25 mv the curve for 450 nm bears the same relation to the 560 nm curve as in the dark-adapted animal; however, in the region of smaller responses the slope is shallower than before. Consequently the curves for 560 and 450 nm cross, and the relative sensitivity of the eye to these two wavelengths depends entirely on the criterion response one selects. For example, at a response of 250 μv (A), the eye was 0.55 log units more sensitive to green light; but at 50 μv (B), it was 0.2 log units more sensitive to violet.

The dark-adapted eye of *Orconectes* has maximum sensitivity at 565 nm (Fig. 7). At shorter wavelengths the curve falls to a broad shoulder at 360—440 nm, and below 360 nm it falls more steeply again. This result is independent of the magnitude of the criterion response, at least over an energy range approaching 5 log units above the threshold of the retinal action potential. Furthermore, over most of the dynamic range of the retinal action potential, the shape of the spectral sensitivity function is unaltered by adaptation to red light. The open circles in Fig. 7 show the average results on seven animals, based on a criterion

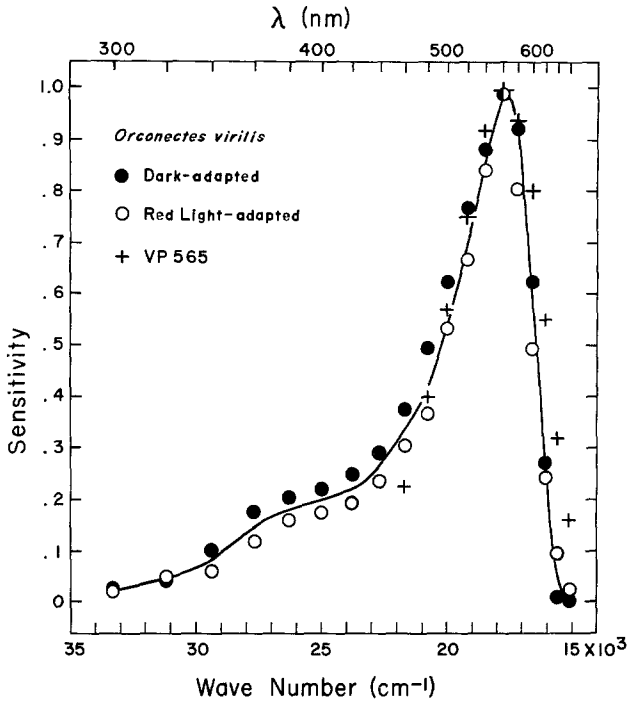

Fig. 8. Spectral sensitivity of the crayfish, *Orconectes virilis*. The points represent the two curves of Fig. 6 replotted on an arithmetic ordinate and normalized for comparison. The crosses represent the absorption of a hypothetical vertebrate rhodopsin with λ_{max} at 565 nm taken from DARTNALL's (1953) nomogram

response of 250 μv. The arithmetic plot in Fig. 8 shows more clearly the constancy of the spectral sensitivity curve; except in the ultraviolet region, this curve should describe the absorption spectrum of the visual pigment with good accuracy.

If the eye is adapted to red light and the criterion responses are close to threshold, the eye is most sensitive at about 425 nm and only a secondary peak appears at 565 nm. Fig. 9 shows results when the criterion response was 50 μv. The spectral sensitivity of the dark-adapted eye is little different from the curve in Fig. 7, but red light adaptation now unveils the presence of a second visual pigment. There must be relatively little of this pigment present, as even under intense red light adaptation the receptors which contain it seem to saturate at about 1.5 log units above the threshold of the retinal action potential, and at higher energies the responses are mediated by the 565 nm pigment.

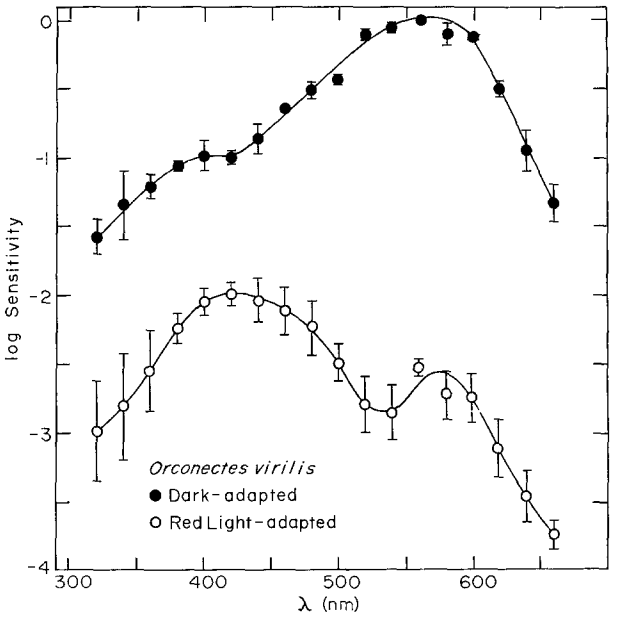

Fig. 9. Spectral sensitivity of the crayfish, *Orconectes virilis*, for criterion responses which are close to threshold. Filled circles are average results from 3 dark-adapted animals; open circles, adapted to red light. Vertical bars represent standard errors. Ordinate is the log of the reciprocal of the relative number of photons for a 50 μv response

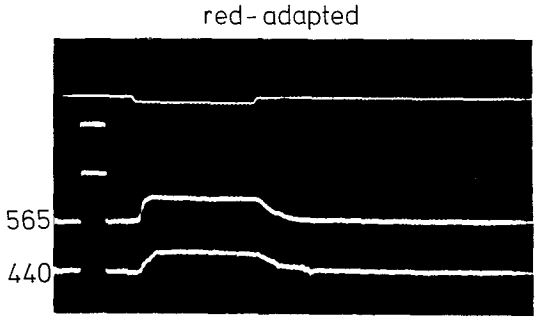

Fig. 10. Two responses from a crayfish *(Orconectes virilis)* eye during red light adaptation (λ's > 620 nm) showing differences in rise times at 565 and 440 nm. Calibration pulses are 0.5 mv $\times$ 100 msec. D.C. recording. Stimulus duration (upper trace) was approximately 1.2 sec. Upward movement of the trace signifies negativity of the illuminated cornea. See the text

The 425 and 565 nm pigments are most likely in different receptor cells, for the same two arguments that were offered for *Palaemonetes*

are met again here. Differences in the shapes of the responses to violet and yellow lights were observed, particularly in eyes adapted to long wavelengths[2]. The differences were usually not as marked as in *Palaemonetes* and could not always be seen in the dark-adapted eye. It is difficult to generalize about them, but an example is shown in Fig. 10. We have also measured the absorption of single rhabdoms of *Orconectes* (WATERMAN, FERNANDEZ and GOLDSMITH, 1968 and work in progress). The 565 nm pigment has been seen, but not yet the 425 nm pigment. We therefore believe the pigments lie in different cells.

Absorption of the Dioptric Elements

Spectral sensitivity curves such as those described above will not reflect the absorption spectra of the visual pigments if there are wavelength-selective filters between the external surface of the cornea and the rhabdom[3]. The dioptric structures are clear in the visible region of the spectrum, but their effect in the ultraviolet cannot be ascertained without measurement. Consequently the absorption of individual corneal facets was determined in several species. Small pieces of fresh cornea were mounted in a glycerin with refractive index 1.455, placed between quartz coverslips, and examined by microspectrophotometry. The results are shown in Fig. 11.

All corneas examined showed an absorption band in the vicinity of 280 nm, doubtless due to the tryptophan and tyrosine residues of the protein component of the cuticle. Two species with significant ultraviolet sensitivity, *Palaemonetes* and an insect *Musca*, have relatively thin corneas with little absorption at wavelengths longer than 300 nm. Blue crab, lobster, and crayfish, all of which lack ultraviolet receptors, have thicker corneas with distinct shoulders of absorption in the near ultraviolet. If the pieces of cornea are viewed from the cut surface so that light passes through several facets, this additional absorbing substance gives the cuticle a distinct yellowish cast. The rise in absorption at about 350 nm is probably what causes the abrupt fall in sensitivity at about the same wavelength in both *Orconectes* and *Callinectes*. The main point, however, is that only those species which have ultraviolet receptors have kept their corneas free of material absorbing in the near ultraviolet.

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[2] WALD (1968) has reported wavelength differences in responses to much shorter flashes of light in both *Orconectes* and *Procambarus*.

[3] As was pointed out above for *Palaemonetes*, the accessory pigments between the ommatidia appear dark and relatively neutral in color. Thus unlike the housefly and its near relatives (BURKHARDT, 1962; GOLDSMITH, 1965; LANGER, 1967), there is probably little distortion of spectral sensitivity caused by differential leakage of stray light.

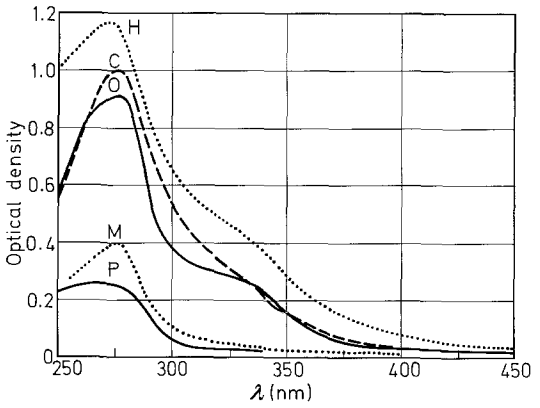

Fig. 11. Average corneal absorption of individual facets from several species of arthropod. Each curve is based on about 8 individual measurements. P *Palaemonetes vulgaris;* M *Musca domestica;* O *Orconectes virilis;* C *Callinectes sapidus;* H *Homarus americanus*

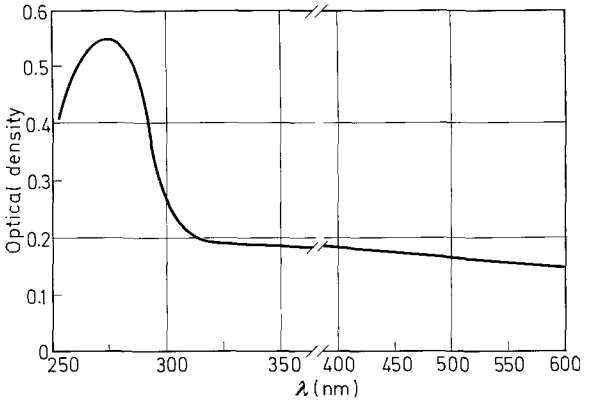

Fig. 12. Absorption of a single crystalline cone of *Palaemonetes vulgaris* measured with lateral illumination. Density along the major axis of the cone — the path followed by light reaching the receptors *in vivo* — is at least twice that recorded here. See the text for further discussion

Table 2 shows that the difference in protein absorption, in the crustacea at least, is due principally to differences in corneal thickness, for the absorbance per micron is similar in each case.

The absorption of the crystalline cone has been measured in *Palaemonetes vulgaris*. Single cones detached from other cells were observed in squashes of eyes and were examined with lateral illumination. Suspended in artificial sea water between cover slips at room temperature, fresh cones had a finely granular appearance under bright field

Table 2

		Corneal thickness (μ)	O.D. at λ_{max}	O.D./μ
Homarus americanus	lobster	60	1.17	0.019
Callinectes sapidus	blue crab	43	1.0	0.023
Orconectes virilis	northern crayfish	45	0.91	0.020
Palaemonetes vulgaris	prawn	10	0.27	0.027
Musca domestica	house fly	10	0.40	0.040

Measurements of corneal thickness are from histologically fixed preparations.

illumination. Within a few minutes they began to round up and internal changes were evident, as though vacuoles were forming. Consequently, absorption measurements were made as soon after preparing the mount as possible. All cones showed a 280 nm absorption band characteristic of protein, as well as a relatively neutral "absorption" which in all likelihood was due to light scattering (Fig. 12). The possibility exists that *in vivo* the cones are more homogeneous and scatter less. Some cones (not shown) had a prominent shoulder of absorption through most of the near ultraviolet, but whether this was caused by greater light scattering, surface adsorption of ultraviolet absorbing material, or an additional internal constituent was not determined. Nevertheless, Fig. 12 shows that at least some of the crystalline cones of *Palaemonetes* are virtually as transparent to near ultraviolet wavelengths as to blue, green and red.

Discussion

Ecological Considerations

The absorption of pure water is minimal in the blue, rising in the yellow and more steeply in the red. Available measurements in the near ultraviolet are not in good agreement. According to the data of SAWYER, cited in SVERDRUP, JOHNSON and FLEMING (1942), absorption rises sharply at wavelengths shorter than 400—420 nm. The measurements of JAMES and BIRGE (1938) do not extend as far, but they show little increase in absorption in the near ultraviolet. At 365 nm the extinction coefficient per meter is about 0.037, or only one seventh the value reported by SAWYER. As HUTCHINSON (1957) suggests, the difference most likely stems from the fact that JAMES and BIRGE used purer water.

Light is attenuated in natural waters in two ways: absorption and scattering by dissolved and suspended contaminants[4]. Like pure water, the clearest lake and oceanic waters show maximum transmission in the

[4] For further details and original data, see the discussions by HUTCHINSON (1957) and SVERDRUP, JOHNSON and FLEMING (1942).

blue at around 470—480 nm. Even the clearest lake waters, however, show some ultraviolet absorption by dissolved material, and this absorption becomes more pronounced and extends further into the visible region of the spectrum the more obviously colored is the lake water. Presumably the same situation prevails in marine environments, with the result that penetration of near ultraviolet light into natural bodies of water is, in general, limited. The occurrence of dissolved pigmentation and suspended particulate material have the further effect of displacing the wavelengths of maximum transmission from the blue toward the yellow. Thus the shallower coastal waters of the ocean show their greatest transmission at 530 nm or longer, and the most deeply colored lakes may have the peak shifted into the red.

Ultraviolet receptors might therefore be expected to be of limited use to animals inhabiting aquatic environments. The present study indicates that among the crustacea they are the exception rather than the rule, and it may prove to be of significance that the ultraviolet receptor of *Palaemonetes* (380 nm) lies at distinctly longer wavelengths than those of insects (340—350 nm)[5].

One can readily summarize the present state of knowledge of the species variation in spectral sensitivity among Decapod crustacea, but it is less easy to discern any overriding causal factors. In addition to *Callinectes*, the marine crabs *Eupagarus* (Stieve, 1960), *Libinia*, and *Carcinus* (Wald, 1968) also have the maximum sensitivity of the dark-adapted eye in the neighborhood of 500 nm. The visual systems of the latter two species are complex, however, and in *Carcinus* at least two visual pigments appear to be contributing. Thus some crabs may have color vision, whereas others may not. The lobster, *Homarus*, another marine form, is maximally sensitive at 525 nm and seems to have but one visual pigment (Kennedy and Bruno, 1961; Wald, 1968).

The crayfish *Orconectes* and *Procambarus* (Kennedy and Bruno, 1961; Wald, 1968) as well as the fresh water prawn *Palaemonetes paludosus* have their maximum sensitivity at considerably longer wavelengths, 555—570 nm. This is not a hard and fast difference between species inhabiting fresh and salt water — and indeed it is difficult to see any factor in the environment that should make it so — for there

[5] It is probably unwise to attempt to generalize from the very limited data available. Electrophysiological evidence for ultraviolet maxima at 340—350 nm now exists for five orders of insects: Hymenoptera, Diptera, Blattaria, Lepidoptera, and Coleoptera (see the references cited in the introduction). The one other example of an ultraviolet receptor in a noninsect species, however, is the median eye of *Limulus* which has a receptor with maximum sensitivity at 360 nm, intermediate between insects and *Palaemonetes* (Wald and Krainin, 1963; Chapman and Lall, 1967). It is tempting to speculate that the median eye of *Limulus* is used when the animals appear on the beaches.

is relatively little difference between the spectral sensitivity of *P. paludosus* and the closely related marine species *P. vulgaris* (WALD and SELDIN, 1968). We suspect that as more comparative information is obtained, the sensitivity maxima of crustacea will be found over a wide range of wavelengths, with perhaps only a general tendency for groups inhabiting shallow and turbid water to exhibit greatest sensitivity near the yellow region of the spectrum.

Visual Pigments

No spectrophotometric studies of the visual pigment of *Porcellio* have been made. Digitonin extracts of *Callinectes*, made in the fashion that has been successful with macrurans (WALD and HUBBARD, 1957; WALD, 1967) have contained a rhodopsin-like pigment with λ_{max} at about 480 nm, but the yields were very small (unpublished experiments of the authors). If this is the absorption of the native visual pigment, some unidentified factor is pushing the spectral sensitivity about 20 or 25 nm to longer wavelengths.

As shown by microspectrophotometry, *Palaemonetes vulgaris* rhabdoms have two light-sensitive pigments, at 555 and 496 nm (GOLDSMITH, DIZON and FERNANDEZ, 1967, 1968). The 555 nm pigment appears to be the visual pigment responsible for the long wavelength peak in the spectral sensitivity function of *P. paludosus* (Fig. 4). The 380 nm pigment has not been seen by spectrophotometric techniques, most likely because there is less of it. No physiological function for the 496 photopigment has been identified.

The crayfish *Orconectes* holds a parallel relationship to *Palaemonetes*. Digitonin extracts have two pigments, absorbing maximally at 562 and 510 nm (WALD, 1967), and microspectrophotometric examination shows 568 and 515 nm pigments to be present in single rhabdoms (WATERMAN, FERNANDEZ and GOLDSMITH, 1968). The pigment for the violet receptor, like the uv receptor of *Palaemonetes*, has not been measured directly. Likewise, the significance of the 515 nm pigment, as well as of the 496 nm pigment of *Palaemonetes*, is presently still an enigma. The 568 nm pigment, however, accounts well for the spectral sensitivity maximum of the dark-adapred eye (WATERMAN, FERNANDEZ and GOLDSMITH, 1968).

Color Vision

We regard the presence of two sensitivity maxima in both *Palaemonetes* and *Orconectes* as evidence for two visual pigments, for their relative contributions to the retinal action potential can be adjusted by adapting the eye to colored light. As described in the results, the pigments also

appear to be in different receptors. Finally, as was also argued above, it seems to us unlikely that the observations that lead us to these conclusions are artifacts produced by leakage of the sleeves of accessory pigment as in the fly (e.g. GOLDSMITH, 1965). There is therefore presumptive evidence that *Palaemonetes* and *Orconectes* have color vision, a conclusion indirectly supported by behavioral experiments on other species of crustacea (VON BUDDENBROCK and FRIEDRICH, 1933; HORRIDGE, 1967).

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