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The visual pigments of crabs

I. Spectral characteristics

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Summary. 1. The visual pigments of 27 species of crabs from a variety of habitats were investigated by microspectrophotometry of the isolated rhabdomeric photoreceptors. The rhodopsins ranged in λ_{max} from 473 to 515 nm (Tables 1 and 2). No evidence was found for the presence of more than a single rhodopsin in retinular cells 1–7.

2. All rhodopsins produced thermally stable metarhodopsins on irradiation with long-wavelength light. The metarhodopsins of hermit crabs (Anomura: section Paguridea) all absorbed hypsochromically to their rhodopsins. Brachyuran metarhodopsins, with the exception of that of *Cancer irroratus*, absorbed at the same spectral position as the rhodopsin or bathochromically to it.

3. The absorption spectra of all rhodopsins but one could be fit closely by the Dartnall nomogram. Since prior studies have located only retinal in the eyes of crabs, retinal may serve universally as a chromophore in crab visual pigments

Introduction

Animal visual systems presumably have been modified in the course of their evolution for high performance in the tasks for which they are most required. This evolutionary optimization extends to the operation of the visual pigments, including the spectral location at which each absorbs light. Their maximum absorption may be matched to the wavelengths of light having greatest photon flux in the animal's environment, to provide the greatest possible sensitivity (Sensitivity Hypothesis, reviewed by Lythgoe 1972). Alternatively, the absorption maximum may be placed to optimize contrast between viewed objects and the background spacelight (Contrast Hypothesis, reviewed by Lythgoe 1979). Other evolutionary constraints may act as well; for example, animals using species-specific colors for intraspecific communication may have special visual pigments for that task (Levine et al. 1980).

Underwater light has highly modified angular and spectral distributions, both of which vary with depth over the course of a day (McFarland 1986). Visual systems of aquatic animals may thus be obligated to perform just one or a few tasks with high efficiency. Only the visual pigments of fishes have been studied in sufficient detail to address the issue of sensory optimization. In general, deep-water species, or species with activity maxima at twilight or night, have visual pigments providing high sensitivity (Lythgoe 1972; Munz and McFarland 1973; Hobson et al. 1981; Crescitelli et al. 1985). Shallow-water, diurnal fishes often combine a matched scotopic visual system with an offset, contrast-sensitive photopic system frequently capable of hue discrimination (McFarland and Munz 1975; Loew and Lythgoe 1978; Levine et al. 1980).

The estuarine and marine invertebrates occupy an even greater diversity of habitats than the fishes, but their visual pigments are much less well characterized. Existing data suggest that invertebrates match their visual pigment absorption maxima to the photic environment, thus conforming to the Sensitivity Hypothesis (Goldsmith 1972; Cronin 1986). These data, however, are compiled from a great variety of invertebrate taxa, gathered using different techniques in many laboratories. It is risky to generalize from them.

We therefore undertook a study of a single invertebrate group occupying diverse habitats: the crabs (Arthropoda, class Crustacea). In our survey, we examined the visual pigments of 27 species,

Abbreviation: MSP microspectrophotometry

including 3 anomuran families and 8 brachyuran families, living in environments from fully terrestrial, through estuarine and coastal habitats, to deep marine waters. We report our findings in a pair of papers, of which this, the first, describes the properties of crab visual pigments. The second paper (Forward et al. 1987) describes the photic characteristics of the associated environments and discusses the evolutionary adaptations of the visual pigments of the crab species inhabiting them.

Materials and methods

Animals and experimental preparation. A total of 31 species was surveyed, of which 27 produced usable visual pigment data. Most species studied were collected in their natural habitats near Beaufort NC, USA. Following their collection, aquatic species were maintained either in running seawater tables at the Duke University Marine Laboratory or in marine aquaria at the University of Maryland Baltimore County and fed fresh or frozen crustacean meat until used. In most cases, animals were used within a week of collection. Terrestrial species were kept in terraria and fed fruits, rodent food, and leafy vegetables. Animals were dark-adapted at least overnight, but more commonly for several days, before use. Eyes were then removed under dim red light and gently macerated in 2.5% glutaraldehyde in pH 7.5 artificial seawater or pH 7.5 marine crustacean Ringer's solution (recipes in Cavenaugh 1956). Following fixation for at least 15 min at 0 °C, the suspension of rhabdoms was briefly centrifuged and resuspended in the artificial seawater or Ringer's solution at 0 °C for use. The eyes of a few species were so small that centrifugation and resuspension were impractical; in these cases the rhabdoms were left in the fixative (see Results). Individual rhabdoms were located and scanned after placing a drop of the suspension between coverslips sealed with a ring of silicone grease.

Microspectrophotometry (MSP). A single-beam instrument controlled by a microcomputer was used for MSP. The wavelength calibration of the microspectrophotometer was checked periodically using the emission lines of a mercury lamp; it never required resetting throughout the term of this investigation. The equipment and general procedure used have been described by Cronin (1985). Briefly, a linearly polarized scanning beam (dimensions 1.2×6.0 μm or 1.2×2.0 μm, depending on sample diameter) was placed whenever possible within a single band of microvilli with its polarization axis parallel to the microvillar axes. Scans were made from 400 to 700 nm, with measurements taken at 1-nm steps. Each dark-adapted rhabdom was scanned two times in succession to check for stability. If the 2 scans were identical (as they almost always were), the second one was saved as the direct absorption spectrum of the dark-adapted photoreceptor. The rhabdom was then exposed to 15 s of bright red light using the substage illuminator (Corning CS2-61 long-pass filter, 50% transmission at 619 nm), a sufficient exposure to produce a photosteady-state mixture of rhodopsin (R) and metarhodopsin (M). The absorption spectrum of the mixture was measured, after which the rhabdom was photobleached by exposure to at least 5 min of bright white light. A final absorption spectrum was then obtained. During the bright-light treatments, the field diaphragm of the substage illuminator was closed down to produce a spot of about 10 μm diameter at the level of the rhabdom, minimizing local heating of the preparation.

Note that for this procedure to succeed, both R and M must be thermally stable, but must bleach when exposed to bright light. These properties are characteristic of crustacean visual pigments following fixation in aldehydes (Hays and Goldsmith 1969; Bruno et al. 1977; Goldsmith 1978; Cronin 1985). Rescans of physically stable preparations after any treatment (dark-adaptation, red-light exposure, or bright white photobleaches) revealed no measurable absorption change within the time limits during which a photoreceptor was under study. However, because of the photobleaching effect of bright light, it is possible that a small amount ($<3\%$) of the total photopigment was removed by the red-light exposures used to create the $R+M$ mixtures (see Cronin 1985). No attempt was made to correct for this.

Mathematical analysis. For each photoreceptor, the R absorption spectrum was taken as the difference between the initial, dark-adapted spectrum and the final, photobleached spectrum, while the mixture's spectrum was the difference between the red-treated and final scans. Results from 6 to 20 rhabdoms, usually from 2 or more individual animals, were obtained for each species (see Table 1). Any long-term shift in baseline absorbance during the measurements was removed by subtracting from the curve the average absorbance from 651 to 700 nm, where visual pigments have near-zero absorption (the correction was usually much less than 0.005 absorbance unit).

The individual results from each rhabdom, as well as the species average spectra for both the R and the mixture, were fit with a Dartnall (1953) nomogram, since all values of λ_{max} fell within the band where this function applies (Ebrey and Honig 1977). The fitting of each spectrum to the nomogram template was done using a least-squares routine. The value of λ_{max} was chosen by first normalizing the corrected R data to the average of the 5 experimental values from 2 nm below to 2 nm above each tested wavelength, from 460 to 530 nm. We then computed the sum of squares of differences between the normalized data and the nomogram curve at 1-nm intervals from 20 nm below to 80 nm above the test wavelength. The analysis was biased towards the long-wavelength limbs of the test spectra because these were generally more similar to the test nomograms than the short-wavelength limbs, probably because of the presence of a short-wavelength absorbing photoproduct (see Discussion). The test peak wavelength producing the smallest sum was chosen as the best fit for the spectrum being tested. In the cases of the $R+M$ mixtures, the absorbance of a mixture of 2 visual pigments having nomogram spectra does not have the precise shape of a Dartnall nomogram (see Cronin 1985). Nevertheless, we always were able to fit our mixture curves with high precision either because the mixture was dominated by metarhodopsin (anomuran pigments) or because the rhodopsin and metarhodopsin had nearly identical spectral positions (brachyurans).

We also estimated the λ_{max} of M of each species. When a visual pigment system containing stable endpoints R and M has been brought to a photosteady state mixture by a saturating exposure of light to wavelength λ, the fraction of M in the mixture, $F_M(\lambda)$, is (Hochstein et al. 1978; see also Cronin and Goldsmith 1982; Stavenga and Schwemer 1984):

$$F_M(\lambda) = \alpha_R(\lambda) \cdot \phi / [\alpha_R(\lambda) \cdot \phi + \alpha_M(\lambda)], \quad (1)$$

where α is the molecular absorbance at λ and ϕ is the ratio of the quantum efficiency for the photoconversion $R \rightarrow M$ to that of the photoconversion $M \rightarrow R$. Defining $a(\lambda)$ as the absorbance of the mixture of R and M, divided by path length and pigment concentration (see Cronin and Goldsmith 1982),

$$a(\lambda) = \alpha_R(\lambda) \cdot F_R(\lambda) + \alpha_M(\lambda) \cdot F_M(\lambda), \quad (2)$$

Rearranging, and leaving out (λ) for simplicity

$$\alpha_M = (a - \alpha_R \cdot F_R)/F_M. \quad (3)$$

Substituting (3) in (1),

$$F_M = \alpha_R \cdot \phi / [\alpha_R \cdot \phi + (a - \alpha_R \cdot F_R)/F_M]. \quad (4)$$

After simplifying and dividing through by F_M, we obtain

$$1 = \alpha_R \cdot \phi / (\alpha_R \cdot \phi \cdot F_M + a - \alpha_R \cdot F_R). \quad (5)$$

Since $F_R + F_M = 1$, F_M may be replaced by $(1 - F_R)$:

$$1 = \alpha_R \cdot \phi / (\alpha_R \cdot \phi - \alpha_R \cdot \phi \cdot F_R + a - \alpha_R \cdot F_R) \quad (6)$$

which may be rearranged to

$$\alpha_R \cdot \phi - \alpha_R \cdot \phi \cdot F_R + a - \alpha_R \cdot F_R = \alpha_R \cdot \phi. \quad (7)$$

Finally, this expression may be simplified and solved for F_R:

$$F_R = a/(\alpha_R \cdot \phi + \alpha_R) = a/[\alpha_R \cdot (1 + \phi)). \quad (8)$$

In words, the fraction of rhodopsin in any photosteady state mixture is equal to the ratio of the absorbances at the saturating wavelength of the mixture and the rhodopsin, divided by $(1 + \phi)$. Once F_R in the mixture is known, the metarhodopsin absorption spectrum is easily obtained by subtracting the contribution of rhodopsin from the spectrum of the mixture and dividing the result by F_M.

Although relationship (8) holds strictly for a single wavelength, it may be applied to broadband saturating irradiation such as we used if the quantity ϕ is constant within the band and the relative amounts of light absorbed in the band by R and the mixture are known. ϕ is wavelength-independent in the main absorption band of visual pigments (Dartnall 1972); we used the value for crayfish photopigment, $\phi = 1.41$ (Cronin and Goldsmith 1982) in our calculations. This value was measured using crayfish maintained under conditions that maximize ocular retinal:dehydroretinal ratios (Suzuki et al. 1984) and is consistent with other known arthropod values for ϕ (Cronin and Goldsmith 1982). We were also able to use the special characteristics of visual pigment absorption spectra to avoid complications introduced by not knowing precisely how much light R and the mixture absorbed.

At long wavelengths, photosensitivity, and thus rhodopsin absorption, falls approximately exponentially with wavelength over at least 4 orders of magnitude. This has been demonstrated both psychophysically (for human scotopic thresholds, Jameson 1972) and electrophysiologically (for spectral sensitivity of rod outer segments, Baylor et al. 1979). Using Dartnall's (1953) original data we verified that the long-wavelength tails of nomogram spectra also fall exponentially for all values of λ_{max}. We therefore fit exponential tails to our template spectra for wavelengths greater than 1.18 times the peak, the point at which the Dartnall (1953) curve for frog rhodopsin begins its exponential decline. To compare R and mixture absorption bands in the spectral region of light transmitted by the CS2-61 filter, each template curve was scaled to its relative peak, as determined by the prior least-squares fitting procedure. The total areas under the curves from 600 to 700 nm were then computed, from which the absorption ratio was obtained. We included in this area wavelengths somewhat shorter than the 50% transmission point of the filter (619 nm) because of the proportionately greater actinic effect at wavelengths where absorption is stronger. Not knowing the precise spectral distribution of the saturating light was not in practice a problem since visual pigment spectra are almost parallel in this region. For example, the greatest variation in F_R with wavelength of analysis for any species was found in *Calappa flammea*. Here, if the spectral ratio analysis included absorption between 590 and 700 nm, the computed $F_R = 0.755$. If the analysis instead included wavelengths from 610 to 700 nm, $F_R = 0.780$. These led to identical λ_{max} values for M, and the computed extinction ratios differed by only 5.3%.

Results

Overview

We worked with 31 species of crabs in this project, obtaining usable data from 27 of them (Table 1). All species had apposition eyes, so their isolated rhabdoms tended to be long relative to their diameter. Whole eye diameters varied by an order of

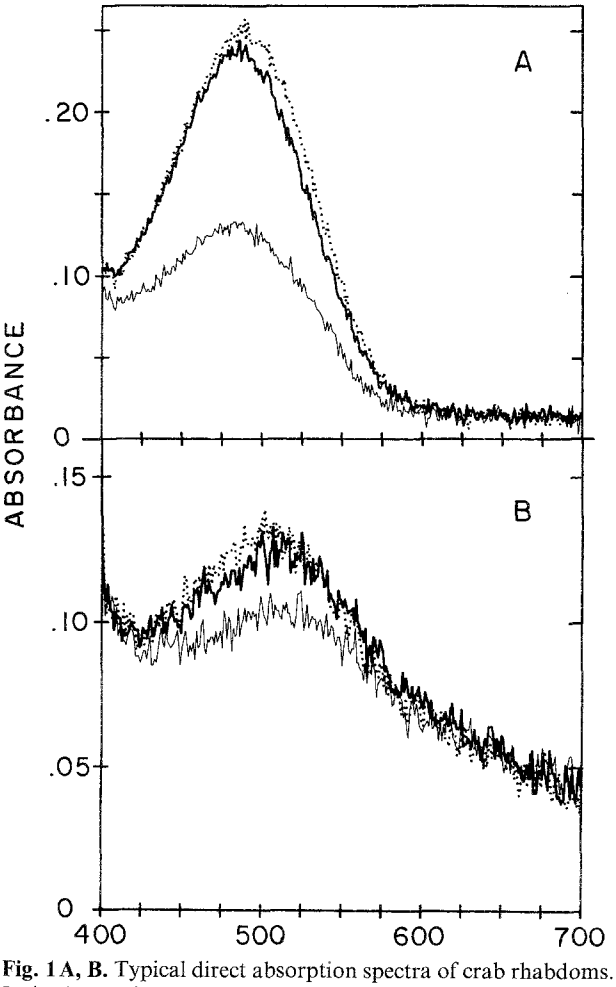

Fig. 1 A, B. Typical direct absorption spectra of crab rhabdoms. In both panels, trace 1 (dark) is the absorbance of the dark-adapted rhabdom, trace 2 (dotted) the absorbance following saturating red-light irradiation, and trace 3 (light) the absorbance following photobleaching with bright white light. The difference spectrum for rhodopsin photobleaching is equal to trace 1 minus trace 3, and the difference spectrum for photobleaching of the red-light treated $R+M$ mixture is trace 2 minus trace 3. **A** An example of a clean rhabdom, having no external granules of screening pigment, from *Portunus spinimanus*. **B** Scans of a slender rhabdom, having an irregular coating of screening pigment, from *Coenobita clypeatus*

Table 1. List of study species and statistics of overall results

Section Family Species	Date studied	Number of rhabdoms (crabs) sampled	Pigment granules	Mean λ_{max} of R (±s.d.)	Mean λ_{max} of $R+M$ mixture (±s.d.)	Mean extinction ratio ε_{max}mixture/$\varepsilon_{max}R$ (±s.d.)
Anomura: Paguridea						
Diogenidae						
Clibanarius vittatus	7/85	13 (2)	++	509.6 (±8.2)	481.2 (±6.3)	1.21 (±0.23)
Dardanus fucosus	9/86	14 (1)	–	510.8 (±3.8)	491.6 (±6.1)	1.36 (±0.07)
Petrochirus diogenes	9/86	16 (1)	–	508.2 (±2.6)	491.7 (±5.7)	1.31 (±0.06)
Coenobitidae						
Coenobita clypeatus	7/86	13 (2)	++	506.2 (±14.3)	491.8 (±6.4)	1.27 (±0.11)
Coenobita rugosa	12/85	13 (4)	++	489.5 (±11.0)	489.3 (±6.4)	1.11 (±0.18)
Paguridae						
Pagurus annulipes	7/86	6 (3)	–	492.7 (±10.9)	491.0 (±9.9)	0.93 (±0.16)
Pagurus longicarpus	11/85	15 (3)	–	513.9 (±6.9)	491.6 (±5.9)	0.87 (±0.14)
Pagurus pollicaris	11/85	16 (1)	–	514.3 (±3.9)	493.3 (±5.7)	1.00 (±0.08)
Anomura: Galatheidea						
Porcellanidae						
Polyonyx gibbesi	7/86	[a] (2)	–	–	–	–
Anomura: Hippidea						
Hippidae						
Emerita talpoida	6/86	[b](2)	+++	–	–	–
Brachyura: Oxystomata						
Calappidae						
Calappa flammea	6/86	13 (2)	+	485.2 (±4.7)	490.9 (±6.4)	1.10 (±0.07)
Hepatus epheliticus	4/86, 7/86	17 (2)	–	486.6 (±4.6)	490.8 (±5.5)	1.05 (±0.10)
Brachyura: Oxyrhyncha						
Majidae						
Libinia dubia	6/86, 7/86	20 (2)	+	488.7 (±2.2)	493.2 (±5.1)	1.07 (±0.05)
Brachyura: Cancridea						
Cancridae						
Cancer irroratus	4/86	14 (1)	+	495.7 (±3.1)	490.7 (±6.1)	0.99 (±0.04)
Brachyura: Brachyrhyncha						
Portunidae						
Arenaeus cribrarius	7/86	11 (1)	+	497.5 (±3.5)	499.0 (±7.1)	1.11 (±0.14)
Callinectes ornatus	8/85	15 (2)	–	498.5 (±5.5)	498.7 (±6.0)	1.13 (±0.12)
Callinectes sapidus	7/86	12 (2)	–	502.5 (±2.2)	502.8 (±6.8)	1.16 (±0.08)
Ovipales stephensoni	7/86	10 (1)	+	502.4 (±6.9)	505.2 (±7.5)	1.21 (±0.16)
Portunus spinimanus	9/85	15 (1)	–	482.1 (±6.0)	492.4 (±5.9)	1.30 (±0.17)
Xanthidae						
Eurypanopeus depressus	11/85, 12/85	20 (4)	–	490.1 (±3.0)	494.6 (±5.1)	1.02 (±0.07)
Menippe mercenaria	8/84	10 (1)	–	494.8 (±2.7)	498 (±7.4)	1.11 (±0.11)
Panopeus herbstii	7/84	10 (2)	–	493.0 (±2.1)	493.7 (±7.4)	0.96 (±0.04)
Panopeus obesus	8/84	10 (2)	–	493.5 (±1.9)	494.6 (±7.4)	0.96 (±0.06)
Pilumnus sayi	6/86, 7/86	15 (2)	+	489.1 (±3.3)	492.3 (±5.9)	1.02 (±0.08)
Rhithropanopeus harrisii	10/86	12 (2)	+	495.2 (±2.1)	498.4 (±6.7)	1.01 (±0.05)
Geryonidae						
Geryon quinquedens	8/86	15 (2)	++	472.9 (±12.3)	473.5 (±5.8)	1.01 (±0.09)

Table 1. (continued)

Section Family Species	Date studied	Number of rhabdoms (crabs) sampled	Pigment granules	Mean λ_{max} of R ($\pm$s.d.)	Mean λ_{max} of $R+M$ mixture ($\pm$s.d.)	Mean extinction ratio ε_{max}mixture/$\varepsilon_{max}R$ ($\pm$s.d.)
Grapsidae						
Sesarma cinereum	7/85	12 (2)	+ +	491.1 (±13.8)	493.6 (±6.7)	1.09 (±0.23)
Sesarma reticulatum	11/85	18 (2)	+ +	492.4 (±5.9)	492.6 (±5.4)	1.03 (±0.15)
Ocypodidae						
Ocypode quadrata	6/84	[b] (1)	+ + +	–	–	–
Uca pugilator	8/85	[b] (2)	+ + +	–	–	–
Gecarcinidae						
Gecarcinus lateralis	9/86	13 (2)	+ +	485.2 (±4.0)	490.6 (±6.4)	1.01 (±0.13)

Species are arranged taxonomically (Williams 1984). All species examined are included, even if we were unable to obtain usable data from some of them. 'Pigment granules' refers to the presence of pigment granules on isolated dark-adapted photoreceptors: –, sparse or absent; +, a few granules; + +, many granules; + + +, dense coating of granules; s.d.: standard deviation

[a] No photoreceptors were isolated in this species

[b] Heavy coatings of screening pigment granules prevented spectral measurements of visual pigment absorption

magnitude (from 550 μm in *Pagurus annulipes* to about 5 mm in *Geryon quinquedens*). Rhabdoms varied much less in size; in these species their dimensions were roughly 8 × 40 μm and 15 × 250 μm, respectively.

In the best preparations, typical of those obtained from about half of the study species, the direct absorption spectrum of the dark-adapted rhabdom resembled a pure rhodopsin spectrum (Fig. 1 A, curve 1). In these cases the change in absorption after red irradiation was easily seen (Fig. 1 A, curve 2), and upon bleaching a substantial drop in absorption was apparent (Fig. 1, curve 3). Other species (particularly those from bright-light habitats), had disorganized rhabdomal membranes, which often were lined with small adhering screening pigment granules. In the worst cases these rhabdoms also had relatively short optical pathlengths and minimal bleaching changes, which led to data of substantially reduced quality (Fig. 1 B). Nevertheless, in only 3 species (*Emerita talpoida, Ocypode quadrata,* and *Uca pugilator*) were visual pigment spectra unobtainable due to screening effects, and in 1 additional species (*Polyonyx gibbesi*) no isolated photoreceptors could be identified (see Table 1).

Overall, the results neatly sorted themselves by taxonomic groupings, so that is the most convenient way to present them. The taxonomic scheme generally follows that in the monograph of Williams (1984). Results of the analyses of individual photoreceptors, grouped by species, are given in Table 1. Analyses of the averaged absorption curves for each species are summarized in Table 2. The 2 sets of analyses provide almost identical results, but we believe that the averaged curves best describe the visual pigments of each species.

Infraorder Anomura

This group includes a number of very loosely related crablike taxa, all of which are distinct from the 'true' (brachyuran) crabs. Species of 2 major anomuran sections provided material that could not be analyzed (Galatheidea, represented by *Polyonyx gibbesi*, and Hippidea, represented by *Emerita talpoida*), but analyzable photoreceptors were obtained from members of 3 hermit crab families in the section Paguridea. Properties of the visual pigments of each of these families were distinctive, while all hermit crab visual pigment systems differed from those of brachyuran crabs by having metarhodopsins with hypsochromic absorption relative to the rhodopsins.

Family Diogenidae

Three species of diogenid hermit crabs were collected. All included fairly large individuals, and they ranged in habitat from intertidal waters (*Clibanarius vittatus*, the common striped hermit crab) to well offshore (*Dardanus fucosus*). Their isolated photoreceptors varied in morphology: *C. vittatus* had thin and stringy rhabdoms, *D. fucosus* rhabdoms were broad and spindle-shaped, while those of *Petrochirus diogenes* were very long and had

Table 2. Characteristic properties of crab visual pigments determined from averaged data (Figs. 2–9)

Family Species	λ_{max} of R (nm)	λ_{max} of $R+M$ mixture (nm)	Extinction ratio: ε_{max} mixture/ε_{max} R	F_R	λ_{max} of M (nm)	Extinction ratio: $\varepsilon_{max}M/\varepsilon_{max}R$
Anomura: Paguridea						
Diogenidae						
Clibanarius vittatus	510	481	1.244	0.042	481	1.262
Dardanus fucosus	511	492	1.373	0.119	490	1.434
Petrochirus diogenes	508	492	1.325	0.145	490	1.391
Coenobitidae						
Coenobita clypeatus	508	497	1.311	0.221	495	1.409
Coenobita rugosa	491	489	1.161	0.402	487	1.273
Paguridae						
Pagurus annulipes	495	493	0.935	0.326	491	0.906
Pagurus longicarpus	515	492	0.857	0.055	490	0.855
Pagurus pollicaris	515	493	0.991	0.069	491	0.997
Brachyura						
Calappidae						
Calappa flammea	486	492	1.068	0.768	509	1.435
Hepatus epheliticus	487	491	1.024	0.613	498	1.085
Majidae						
Libinia dubia	489	493	1.064	0.633	501	1.199
Cancridae						
Cancer irroratus	496	491	0.985	0.263	489	0.985
Portunidae						
Arenaeus cribrarius	498	498	1.088	0.451	498	1.160
Callinectes ornatus	501	500	1.131	0.432	498	1.231
Callinectes sapidus	503	503	1.148	0.476	503	1.283
Ovalipes stephensoni	505	506	1.233	0.554	506	1.526
Portunus spinimanus	483	493	1.266	[a]	[a]	[a]
Xanthidae						
Eurypanopeus depressus	490	494	1.031	0.611	502	1.103
Menippe mercenaria	494	497	1.085	0.584	497	1.217
Panopeus herbstii	493	494	0.955	0.433	494	0.923
Panopeus obesus	493	495	0.969	0.479	495	0.946
Pilumnus sayi	489	492	1.003	0.546	492	1.017
Rhithropanopeus harrisii	495	499	1.008	0.589	507	1.037
Geryonidae						
Geryon quinquedens	473	473	1.007	0.418	473	1.012
Grapsidae						
Sesarma cinereum	492	497	1.023	0.657	505	1.115
Sesarma reticulatum	493	496	1.017	0.548	496	1.049
Gecarcinidae						
Gecarcinus lateralis	487	491	1.035	0.619	498	1.115

Metarhodopsin data were computed using the method described in the text, and are to be regarded as estimates

[a] For *Portunus spinimanus*, the computed F_R exceeded 1.0, so metarhodopsin parameters could not be computed; see Results

R rhodopsin; M metarhodopsin

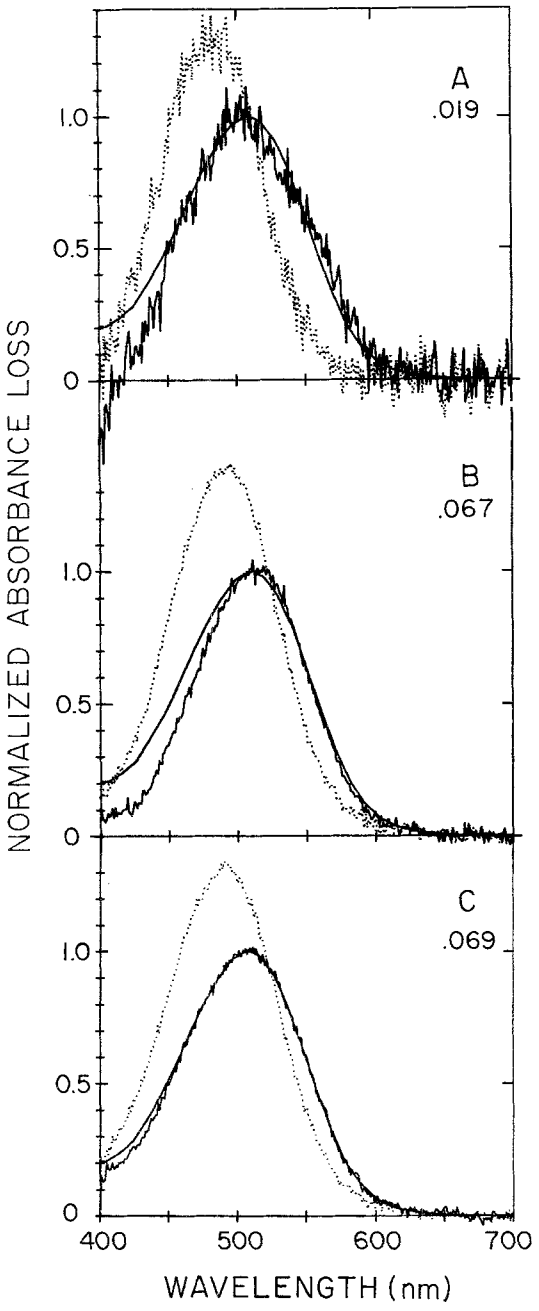

Fig. 2A–C. Visual pigments of hermit crabs, family Diogenidae. In this and all subsequent figures, the dark trace is the difference spectrum for photobleaching of rhodopsin, the dotted trace is the difference spectrum for photobleaching of the rhodopsin-metarhodopsin photosteady state mixture produced by saturating red irradiation, and the smooth curve is the Dartnall nomogram plot that best fits the rhodopsin bleach spectrum. All curves are normalized to the average absorbance loss for the rhodopsin photobleach, taken to be the absorbance of the Dartnall nomogram maximum, which is indicated in the upper right corner of each panel. For each species the number of rhabdoms averaged, the wavelength at the maximum of the fitted nomogram template spectrum, and the sum of squares of the differences between the nomogram template and the normalized rhodopsin photobleach curve are given in parentheses. The number given in the upper right corner of each panel is the average rhodopsin photobleach absorbance loss, in units of absorbance. **A** *Clibanarius vittatus* (13 rhabdoms, 510 nm, 0.552). **B** *Dardanus fucosus* (14 rhabdoms, 511 nm, 0.072). **C** *Petrochirus diogenes* (16 rhabdoms, 509 nm, 0.016)

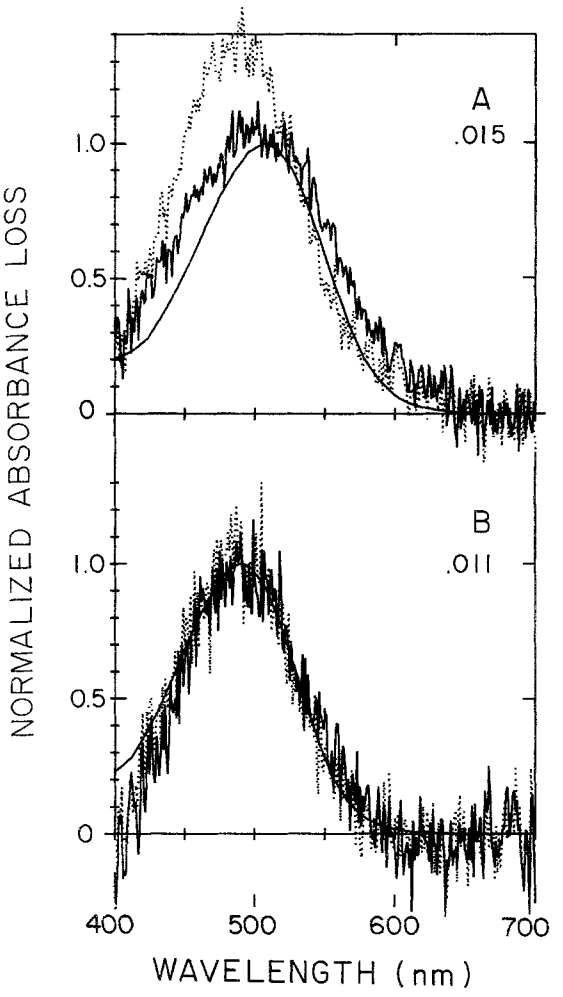

Fig. 3A, B. Visual pigments of hermit crabs, family Coenobitidae. **A** *Coenobita clypeatus* (13 rhabdoms, 508 nm) This species alone could not have its rhodopsin absorption fit to a template nomogram spectrum, because of the anomalous breadth of the spectrum. The 508-nm nomogram curve was fit by eye. **B** *Coenobita rugosa* (13 rhabdoms, 491 nm, 1.017)

a bulbous proximal region. Nevertheless, the rhodopsins of the 3 species were essentially identical and had λ_{max} near 510 nm. Formation of the metarhodopsins produced hypsochromic spectral shifts of 20–30 nm and a sharp rise in maximum absorption (Fig. 2, Tables 1 and 2).

Family Coenobitidae

Coenobitids are land hermit crabs. One of our study species (*Coenobita clypeatus*) is the familiar one sold by pet shops. *C. rugosa* was collected in

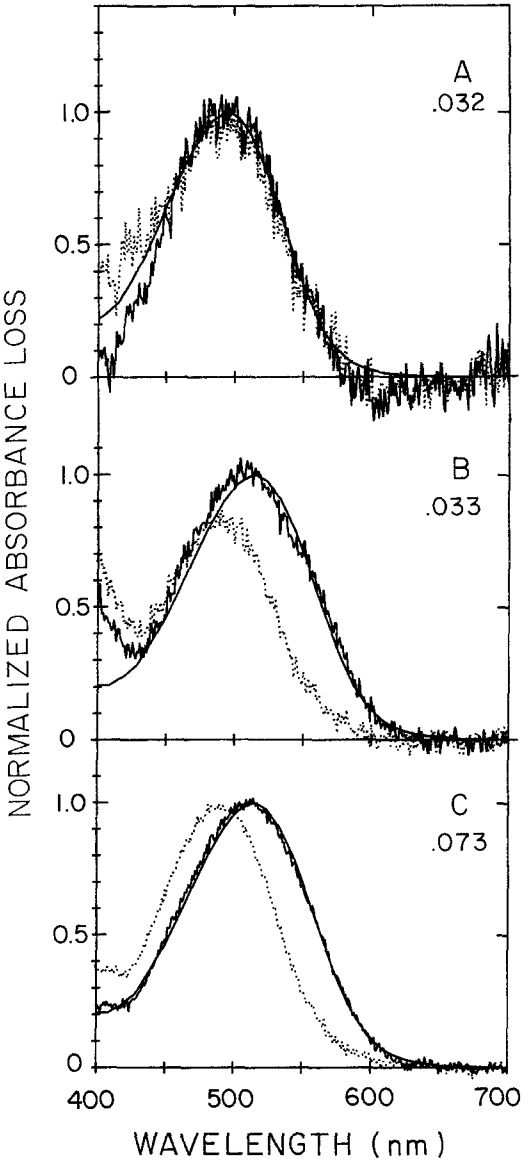

Fig. 4A–C. Visual pigments of hermit crabs, family Paguridae. **A** *Pagurus annulipes* (6 rhabdoms, 495 nm, 0.301). **B** *Pagurus longicarpus* (15 rhabdoms, 515 nm, 0.198). **C** *Pagurus pollicaris* (16 rhabdoms, 616 nm, 0.037)

Costa Rica. Both species had thin, irregular rhabdoms having low absorbances, small changes in absorbance on phototreatment, and relatively great scattering of light (see Fig. 1 B). Unexpectedly, the rhodopsins of the 2 species differed in λ_{max} by almost 20 nm (Tables 1 and 2), though some of this differences may have been due to the poor fit of the data to the nomogram spectra (Fig. 3). Both had hypsochromic metarhodopsins of elevated absorbance relative to the rhodopsins. Being of relatively low quality, coenobitid data had unusually large variances (Table 1).

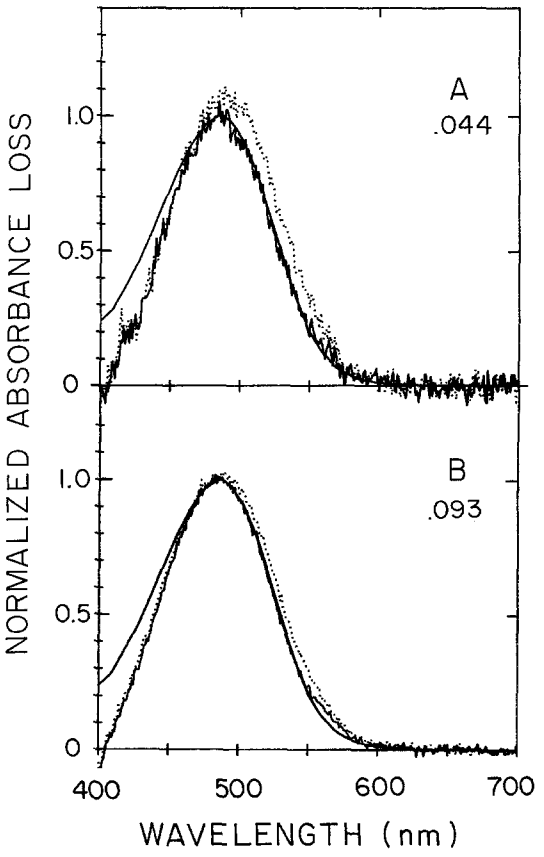

Fig. 5A, B. Visual pigments of brachyuran crabs, family Calappidae. **A** *Calappa flammea* (13 rhabdoms, 486 nm, 0.099). **B** *Hepatus epheliticus* (17 rhabdoms, 487 nm, 0.041)

Family Paguridae

Three species of *Pagurus* were collected. All had quite small eyes mounted on long stalks. Clean, spindle-shaped rhabdoms were released upon crushing the eyes, and scans were performed on rhabdoms in droplets of the glutaraldehyde fixative suspension. *P. pollicaris* provided sufficient material that some of the fixative could be drawn off and replaced with buffer, reducing glutaraldehyde concentration by about half. Rhodopsin λ_{max} values were identical in *P. pollicaris* and *P. longicarpus*, but were 20 nm shorter in *P. annulipes* (Fig. 4). The metarhodopsins of all species were similar, but had reduced absorption maxima compared to the rhodopsins (Tables 1 and 2). Unlike all other species, their visual pigment systems fit the nomogram template spectra well at all but the very shortest wavelengths.

Infraorder Brachyura

This major division of the decapod crustaceans is comprised of the 'true' crabs. Unlike the situation

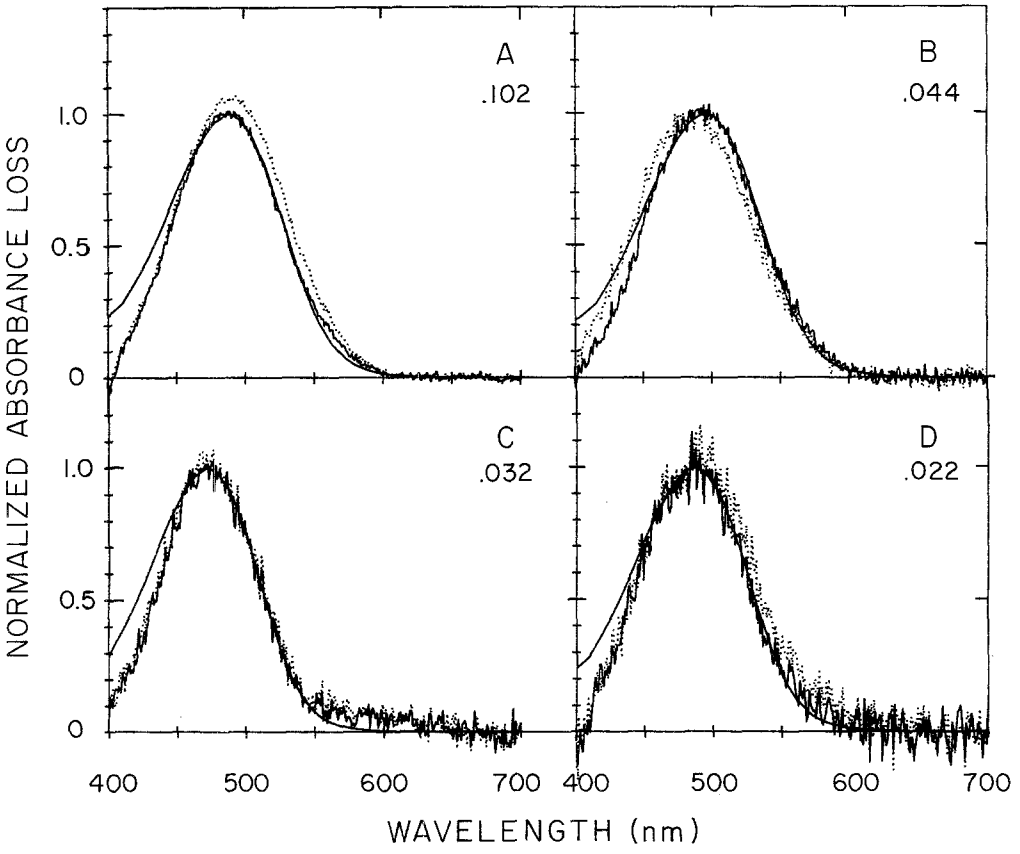

Fig. 6A–D. Visual pigments of brachyuran crabs of various families represented by single species. **A** *Libinia dubia*, family Majidae (20 rhabdoms, 489 nm, 0.052). **B** *Cancer irroratus*, family Cancridae (14 rhabdoms, 496 nm, 0.097). **C** *Geryon quinquedens*, family Geryonidae (15 rhabdoms, 473 nm, 0.111). **D** *Gecarcinus lateralis*, family Gecarcinidae (13 rhabdoms, 487 nm, 0.261)

in the anomurans, there is little doubt that all the brachyuran families we studied belong within the same taxonomic category. Brachyuran species usually had similar λ_{max} values for both *R* and *M* (Tables 1 and 2). They also had visual pigment systems with difference spectra for photobleaching characteristically lying well below the template spectra at the shortest wavelengths, suggesting the presence of bleaching products absorbing in this spectral region (see Discussion).

Family Calappidae

We obtained 2 species of box crabs from coastal waters, which yielded lovely clear cigar-shaped rhabdoms. Both had rhodopsins absorbing maximally near 486 nm, which produced bathochromic metarhodopsins (Tables 1 and 2, Fig. 5).

Family Majidae

The only spider crab species available for study was *Libinia dubia*. Its vase-shaped rhabdoms contained a rhodopsin of λ_{max} 489 nm interconvertible with a 501-nm metarhodopsin (Tables 1 and 2, Fig. 6A).

Family Cancridae

Cancer irroratus was the only species we studied in this family. It had clean cylindrical rhabdoms flecked with red or yellow oily droplets. *C. irroratus* was unique among brachyurans in that its *M* (λ_{max} = 489 nm) was distinctly hypsochromic to the 496-nm *R* (Tables 1 and 2, Fig. 6B).

Family Portunidae

The portunids, or swimming crabs, form a large and successful group having diverse lifestyles and occupying a variety of habitats. We obtained 5 species ranging from estuarine to coastal habitats. All had cylindrical or stringy rhabdoms. Of these, 4 species (*Arenaeus cribrarius, Callinectes sapidus, C. ornatus*, and *Ovalipes stephensoni*) had very similar visual pigment systems, with both *R* and *M* peaking near 500 nm (Tables 1 and 2, Fig. 7A–D). *Portunus spinimanus* (Fig. 7E) differed from these

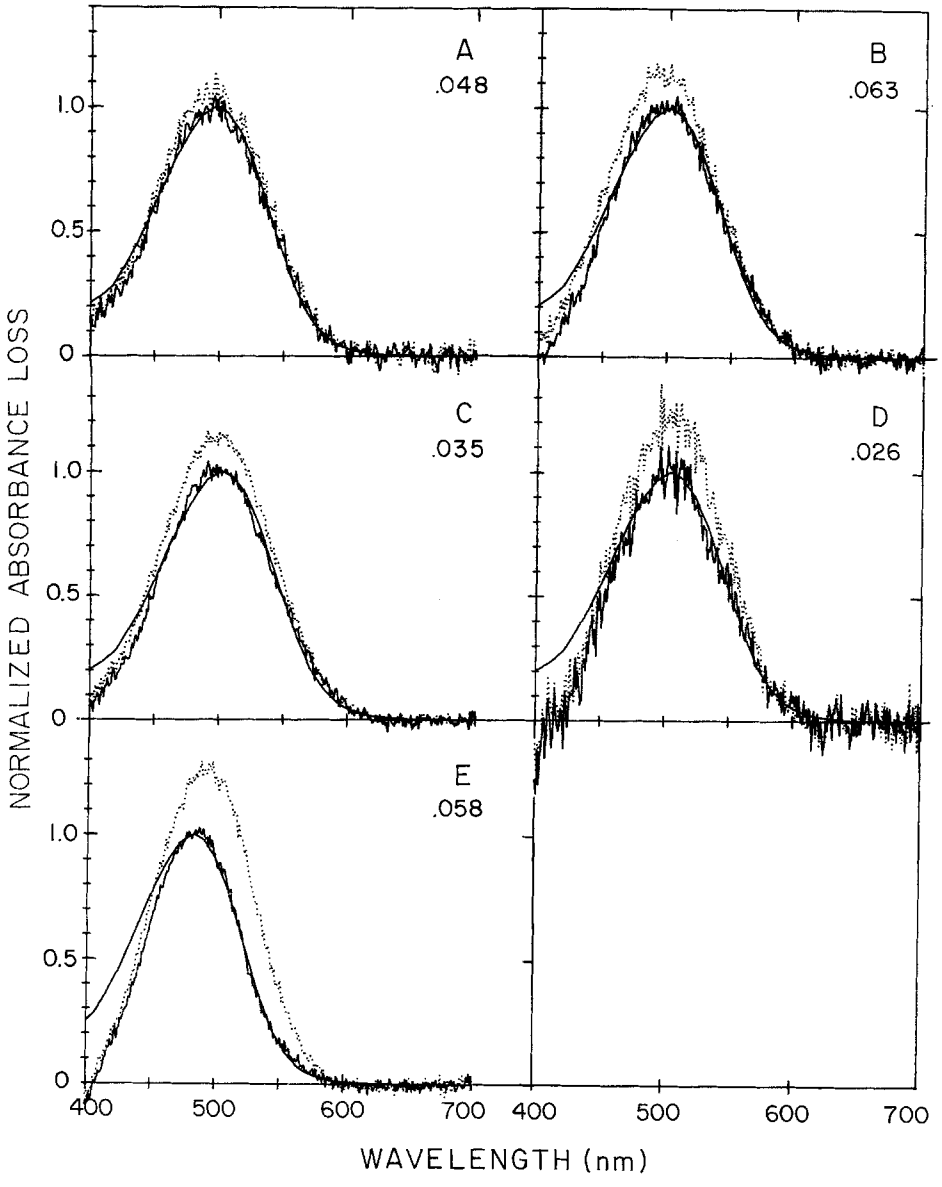

Fig. 7A–E. Visual pigments of brachyuran crabs, family Portunidae. **A** *Arenaeus cribrarius* (11 rhabdoms, 498 nm, 0.134). **B** *Callinectes sapidus* (12 rhabdoms, 503 nm, 0.105). **C** *Callinectes ornatus* (15 rhabdoms, 501 nm, 0.140). **D** *Ovalipes stephensoni* (10 rhabdoms, 505 nm, 0.312). **E** *Portunus spinimanus* (15 rhabdoms, 483 nm, 0.055)

and all other crabs, having a relatively short-wavelength rhodopsin λ_{max} and a 10-nm shift to longer wavelengths on photoconversion, together with a substantial rise in peak absorption. The $R+M$ mixture absorption spectrum cannot be analyzed for metarhodopsin since Eq. (8) predicts that $F_R = 1.323$. Possible explanations for this result are considered in the Discussion.

Family Xanthidae

Mud crabs, or xanthids, are among the most abundant small crabs in coastal or estuarine habitats and form the largest brachyuran family. Habitats of our 6 study species range from the uppermost estuarine reaches to shallow coastal waters. Xanthid rhabdoms are exceptionally clean and regular, providing data of high quality (e.g. Fig. 1A), and appear cylindrical or flask-shaped. Their visual pigments all have λ_{max} for rhodopsin near 490 nm, with metarhodopsin placed a few nm longer; and in all species except *Menippe mercenaria* the M/R extinction ratios are near 1.0 (Tables 1 and 2, Fig. 8). Xanthids illustrate the fundamental conservatism of brachyuran visual pigments more clearly than any other group.

Family Geryonidae

Geryon quinquedens, the red crab, is the only truly deep-sea species we were able to acquire for study. The individuals we obtained were caught by com-

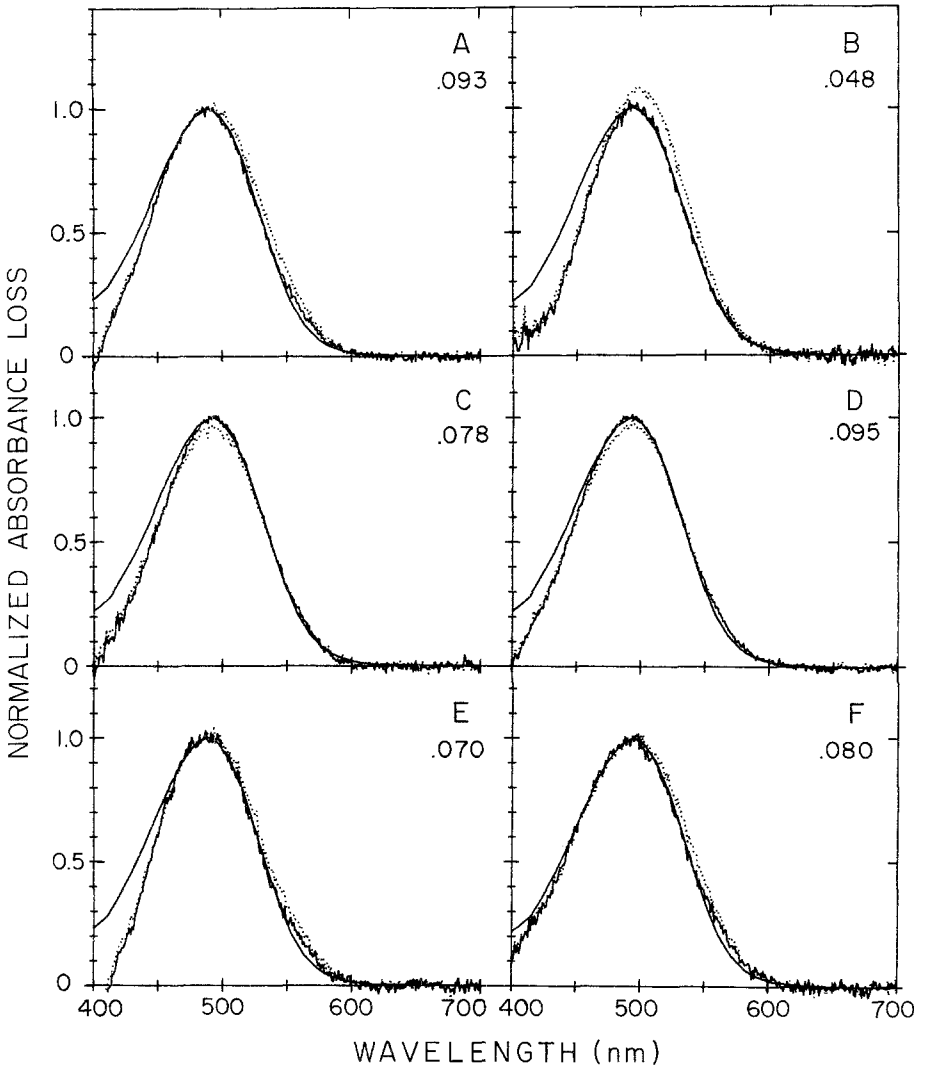

Fig. 8A–F. Visual pigments of brachyuran crabs, family Xanthidae. **A** *Eurypanopeus depressus* (20 rhabdoms, 490 nm, 0.050). **B** *Menippe mercenaria* (10 rhabdoms, 494 nm, 0.036). **C** *Panopeus herbstii* (10 rhabdoms, 493 nm, 0.018). **D** *Panopeus obesus* (10 rhabdoms, 493 nm, 0.028). **E** *Pilumnus sayi* (15 rhabdoms, 489 nm, 0.073). **F** *Rhithropanopeus harrisii* (11 rhabdoms, 495 nm, 0.089)

mercial fishermen and packed on ice the night before they arrived in the laboratory, and then allowed to dark-adapt in cold sea water for 1 to 2 nights before use. Though they survived this treatment, their condition was poor at the time their eyes were removed. Their rhabdoms were large (15×250 μm), tapered, and irregularly coated with particles of brown screening pigment. λ_{max} values for both *R* and *M* lay at 473 nm (Tables 1 and 2, Fig. 6C), the shortest values of any included species. The poor condition of the material probably led to the relatively large variation in λ_{max} among individual receptors (Table 1).

Family Grapsidae

Grapsid crabs are usually intertidal, and may spend much of their active time in air. We collected 2 species of *Sesarma: S. cinereum*, which lives in the high intertidal zone, and *S. reticulatum*, which is less commonly exposed to air. Their visual pigments were similar, with λ_{max} for *R* near 492 nm and for *M* near 500 nm (Tables 1 and 2, Fig. 9). Grapsid rhabdoms provided particularly low signal:noise ratios in spectral scans, so their individual variation in λ_{max} was large (Table 1).

Family Ocypodidae

Ocypodids include a variety of species which are usually intertidal and active at low tide on beaches and mud flats. We isolated rhabdoms from eyes of 2 species, the ghost crab *Ocypode quadrata*, and the fiddler crab *Uca pugilator*. In both species, the string-like photoreceptors were heavily coated with reddish brown granules of screening pigment. This

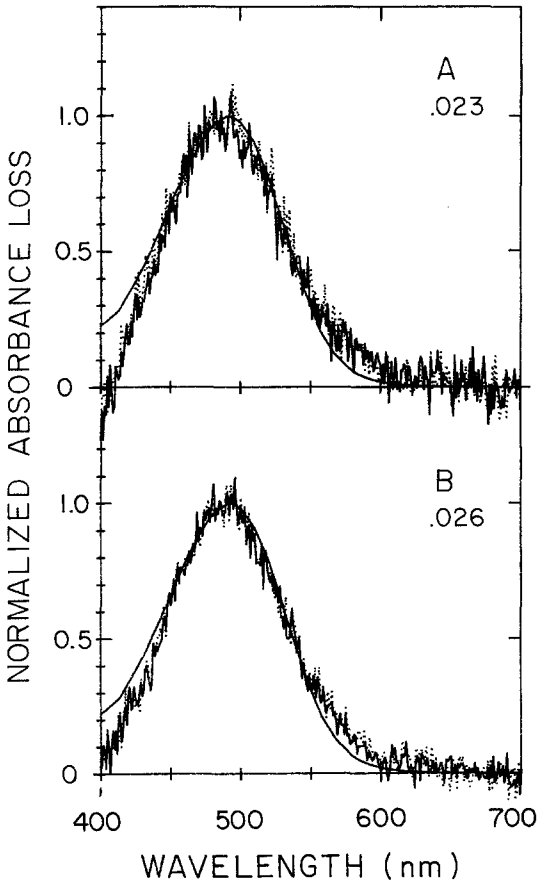

Fig. 9A, B. Visual pigments of brachyuran crabs, family Grapsidae. **A** *Sesarma cinereum* (12 rhabdoms, 492 nm, 0.486). **B** *Sesarma reticulatum* (18 rhabdoms, 493 nm, 0.368)

pigment readily photobleached, masking any changes in the underlying visual pigments, so we were unable to study the visual pigments.

Family Gecarcinidae

One species of land crab was available, *Gecarcinus lateralis*. Rhabdoms were cylindrical, somewhat disorganized, and lightly coated externally with pigment granules. The rhodopsin peaked near 485 nm and formed a typical brachyuran bathochromic metarhodopsin (Tables 1 and 2, Fig. 6D).

Discussion

The ecology of rhabdom design in crabs

We observed considerable diversity in the morphology of isolated rhabdoms. Some of this could have been caused by alterations in structure during the light:dark cycle (Nässel and Waterman 1979; Stowe 1981; Toh and Waterman 1982). Our animals were left in the dark through at least one normal dark period, and commonly for several days, before use; but since nothing is known of endogenous variations for most of our species we cannot know how typical the rhabdom structure we observed is for each species. Nevertheless, a couple of trends can be discerned. Intertidal, semiterrestrial, and terrestrial species invariably had rhabdoms of small diameter, and in the more terrestrial species the thin rhabdoms always had adhering particles of retinular cell screening pigment even after prolonged dark adaptation. In contrast, species from coastal or deeper waters had spindle-shaped, exceptionally clean and glassy rhabdoms. In the pagurid family Diogenidae, rhabdom morphology varied with habitat even though the rhodopsins were all similar.

Unlike rhabdom width, rhabdom length did not correlate with environment. However, even the shortest (40 μm) rhabdoms are long enough to trap incident photons effectively. Typical axial absorbances for crustacean rhabdoms are ~0.03 OD units/10 μm (Bruno et al. 1977; see also Forward et al. 1987). A 40-μm rhabdom could 25% or more of entering photons at the λ_{max}, and more typical rhabdoms (100–250 μm in length) could absorb 50–90% of the photons travelling along them. Summarizing, crabs requiring visual function in bright light reduce photoreceptor exposure by lining the receptors with pigments. Dim-light species increase the signal produced in each retinular cell by increasing rhabdomere cross-sectional area, thus trapping proportionately more photons.

Properties of crab rhodopsins

The name 'rhodopsin' was first given to the purplish visual pigments extractable from vertebrate retinal rods. The term is now used generically for 11-*cis* retinal-based visual pigments, no matter what their source (see Goldsmith and Bernard 1985). All but one of our rhodopsin bleaching spectra fit the Dartnall (1953) nomogram, derived for visual pigments with retinal chromophores. (The exceptional case, *Coenobita rugosa*, had a broad, noisy spectrum.) The fit is extremely close on the long-wavelength limb, and departs only on the short-wavelength limb in the region somewhat below the peak. (Explanations for this departure will be considered later.) A similar correspondence exists between our bleaching spectra and the canonical description of Mansfield (1985) for visual pigments with a retinal chromophore (Lipetz and Cronin, in press). Only retinal has been found in the eyes of the 4 crab species thus far examined

(*Hemigrapsus edwardsii* and *Leptograpsus variegatus:* Briggs 1961; *Pleuroncodes planipes:* Fernandez 1973; *Hemigrapsus sanguineus*, Arikawa et al. 1987).

The crab visual pigments qualify as rhodopsins not only for their biochemistry and spectral shape, but also for their spectral location. The range spanned by rhodopsin λ_{max} values in our test species is almost identical to that of the retinal-based rod pigments of fishes (Lythgoe 1972; Munz and McFarland 1973; Loew and Lythgoe 1978; Hobson et al. 1981; Crescitelli et al. 1985).

Goldsmith and collaborators have described the visual pigments of several crab species using MSP. Results of Hays and Goldsmith (1969) for *Libinia emarginata*, where λ_{max} for rhodopsin = 493 nm, were similar to ours for *L. dubia* (λ_{max} = 489 nm). Bruno and Goldsmith (1974) found λ_{max} = 500 nm in *Callinectes sapidus* rhodopsin; in our study it was near 503 nm. Bruno et al. (1973) examined the portunid crab *Carcinus maenas*. The rhodopsin had λ_{max} = 505 nm and it formed a photoproduct of similar absorption, which agrees well with our results for several portunid species (Tables 1 and 2, Fig. 7).

Characterizations of crab visual pigments in detergent-solubilized extracts, however, are diverse and may be misleading. Bruno and Goldsmith (1974) noted this problem when they showed that rhodopsin extracts from *C. sapidus* have λ_{max} at 477 nm. Briggs (1961) extracted photosensitive pigments from 2 species of grapsid crabs; both had apparent rhodopsin maxima at 513 nm and metarhodopsin maxima at 493 nm. These values are so unlike those we found for grapsids or any other brachyuran that they must be viewed with suspicion. On the other hand, extracts from the anomuran galatheid crab *Pleuroncodes planipes* reveal a less unusual 503-nm rhodopsin (Fernandez 1973); metarhodopsin was not observed. This value is 20 nm below the peak spectral sensitivity Fernandez measured, and should be confirmed using MSP.

MSP indicates that only one visual pigment resides in main rhabdoms (formed of the rhabdomeres of retinular cells 1–7) of crabs. We were impressed by the constancy in spectral maximum among the rhabdoms of a given species. In no species were bleach spectra observed that suggested the presence of visual pigments with clearly distinct maxima. The data of a few species did vary considerably in computed λ_{max} (Table 1), but this generally occurred in cases where the individual spectral scans were of unusually low quality (e.g. Fig. 1B). Rhabdoms of a given species, whether from one or more individuals, all appear to possess the same rhodopsin. This constancy provides further support for Cronin's (1985) conjecture that unlike insects, crustaceans in general synthesize a single rhodopsin in their 7 large retinular cells.

The smaller 8th cell, on the other hand, may be specialized for absorption in violet or ultraviolet spectral regions (Cummins and Goldsmith 1981). Martin and Mote (1982) found short-wavelength photoreceptors in *Callinectes sapidus* and *Carcinus maenas*, which they thought could be the 8th retinular cells. The presence of a second photoreceptor class could permit hue discrimination in some crab species (Hyatt 1975).

Crab metarhodopsins

Properties of the photoproducts of crab visual pigments have not previously been considered in detail, but it has been thought that brachyuran metarhodopsins have absorption spectra like those of their rhodopsins (Hays and Goldsmith 1969; Bruno et al. 1973; Bruno and Goldsmith 1974). Nothing was known of anomuran photopigment systems. Our results clearly show that for hermit crabs, at least, the metarhodopsins absorb hypsochromically to rhodopsin, usually peaking near 490 nm. In this regard, hermit crab visual pigment systems resemble those of such macruran crustaceans as lobster or crayfish (Bruno et al. 1977; Cronin and Goldsmith 1982). Anomuran metarhodopsin peak absorbances vary relative to rhodopsin, possibly for the reasons discussed below with regard to the brachyurans.

Hermit crab metarhodopsins were easily characterized in our work because saturating red irradiation converted most of the original R to M (Table 2). Such was not the case with brachyurans. With the exception of *Cancer irroratus*, formation of brachyuran metarhodopsins led to either no spectral change or a bathochromic shift. Therefore, long-wavelength irradiation caused a net conversion of only $\sim$50% of the original R to M (Table 2). This explains why the metarhodopsins have hitherto concealed themselves in photosteady-state mixtures; their spectra have been buried in the rhodopsins'.

Because the absorption spectra of $R+M$ mixtures in these cases are so similar to the R spectra, we cannot determine the precise absorption characteristics of brachyuran metarhodopsins. Under these conditions, our analytical method is very sensitive to small changes in λ_{max} or in relative absorption ratios of the mixture spectra. Additionally, crustacean metarhodopsins readily photobleach on

glutaraldehyde fixation, so as the mixture forms during red irradiation M may be preferentially removed. The range in calculated absorption ratios, $\varepsilon_{max}M/\varepsilon_{max}R$, in Table 2 is no doubt due in part to these uncertainties. But the hermit crab ratios, which are mathematically reliable, vary substantially as well, and there is one brachyuran case in which mathematical analysis is not even possible.

We propose that the variability in the ratio $\varepsilon_{max}M/\varepsilon_{max}R$ arises from changes in the preferential absorption vector of the chromophore when M is formed from R. Such changes occur in crayfish and lobster visual pigments (Goldsmith and Wehner 1977; Bruno et al. 1977), leading to decreased dichroic ratios in rhabdoms containing M as compared to those with only R. Therefore, absorption of light polarized parallel to the microvillar axes is relatively reduced. We used unpolarized red light to saturate the photopigment system, thus affecting all chromophoric orientations equally. In contrast, light polarized parallel to the axes of the rhabdomeric microvilli was used to measure absorbance, so chromophores having that preferred absorption vector dominated in the measurements. Decreased dichroism, due either to reduced alignment of chromophores with the microvillar axes or to increased randomness in overall chromophoric orientation (see Goldsmith and Wehner 1977) would then explain the low $\varepsilon_M/\varepsilon_R$ values in several brachyuran groups as well as the pagurid anomurans. The very unusual results from *Portunus spinimanus*, where the mixture absorbance was substantially increased, suggest the opposite change – much closer alignment of M than R chromophoric absorption axes to the microvilli.

Summarizing our findings, the hypsochromic spectral locations of hermit crab metarhodopsins are firmly fixed by our analyses while those of brachyuran crab metarhodopsins are more suspect. Nevertheless, in the brachyuran case M formation generally produces about a 10-nm bathochromic shift, and it appears that $\varepsilon_{max}M$ is about 1.4 times $\varepsilon_{max}R$.

Additional pigments in crab photoreceptors

Our data hint at the presence of up to 2 other photosensitive pigments in the rhabdoms of some crab species. One of these is suggested by the almost universally seen additional decrease in absorption at short wavelengths (400–450 nm) following photobleaching, below the decrease expected from the removal of rhodopsin alone as predicted by the nomogram template. The observed departure is likely caused by presence of a photoproduct, perhaps with an all-*trans* chromophore, which absorbs in this region (Lipetz and Cronin, in press). The absorbing species may occur normally in the crab visual cycle, or it may be produced only under the special circumstances of our experimental treatments. Long-lived photoproducts other than metarhodopsin have not been described in other invertebrate visual systems. The appearance of this photoproduct could be an artifact of increases in light scattering at short wavelengths produced during the phototreatments of each rhabdom. However, the form of the departure of the rhodopsin photobleach spectrum from the nomogram template is so similar throughout the brachyuran data, in spite of large differences in the magnitude of the photobleach, that it is unlikely to be due to scattering changes alone.

A second type of photosensitive pigment, absorbing in the 550–600 nm band, may have been associated with the rhabdoms in several species. Its presence is suggested by a rise in the photobleaching curve above the nomogram spectrum in this region (see Figs. 4B, 5B, 8F, and 9). The departure may have been caused by photobleaching of small particles of retinular cell pigment. We placed the scanning beam of the MSP only in clear regions of rhabdomeral microvilli, but there may occasionally have been some absorption by pigment particles lying out of the plane of focus or in regions transmitting photons scattered from the focussed beam. This interpretation is supported by our observations that screening pigment photobleaches in ocypodid rhabdoms. A comparison of the estimated extent of pigment granule content (Table 1) and Figs. 2–9 reveals that long-wavelength departures from the nomogram template are well correlated with the presence of pigment granules on isolated rhabdoms. Crustacean screening pigments have broad absorption spectra extending beyond 650 nm (Scott and Mote 1974; Goldsmith 1978; Stowe 1980). At long wavelengths their spectra lie above typical rhodopsin spectra, allowing the pigments to reveal themselves.

Crab visual pigments and spectral sensitivity

Since the retinal rod cells of fishes contain no pigment filters, and the ocular media of fish eyes are transparent at moderate to long wavelengths, fish spectral sensitivity for scotopic vision is largely a function of rhodopsin absorption. (This is not true of fish cone photoreceptors; see MacNichol et al. 1978). Crustaceans, on the other hand, often possess a variety of ocular screening pigments, having

diverse spectral absorption. These may affect the spectral distribution of light impinging on the actual photoreceptive membranes. The situation becomes quite complicated when several classes of these pigments are mobilized to varying extents during light or dark adaptation. Fortunately, there has been sufficient attention given to crustacean spectral sensitivity that we may reasonably predict how it relates to rhodopsin absorption. Spectral sensitivity, or $S(\lambda)$, functions have been measured either by recordings in single photoreceptor cells or by taking an electroretinogram (ERG) of whole-eye responses.

In dark-adapted crabs, one would expect the screening pigments to be largely removed from the photic pathway into and down the rhabdom (Ludolph et al. 1973). Data available for the dark-adapted state indicate that spectral sensitivity maxima are quite consistent with our rhodopsin absorbance data in largely aquatic crabs, but may be shifted 10 to 20 nm to longer wavelengths in semiterrestrial or terrestrial species. Scott and Mote (1974) measured $S(\lambda)$ in four crab species, including some we examined (*Callinectes sapidus, Sesarma reticulatum, Uca pugilator,* and *U. pugnax*) and found all to have identical maxima near 508 nm. Thus, *C. sapidus* maximum response is near λ_{max} for *R*, while that of *S. reticulatum* shifts from it by +15 nm. Martin and Mote (1982) found both *C. sapidus* and *Carcinus maenas* to be maximally sensitive at 508 nm when dark adapted, while Bruno et al. (1973) found the peak for *C. maenas* to be near 493 nm; they also found the rhodopsin λ_{max} near 504 nm by MSP. Another portunid, *Scylla serrata*, has its maximum dark-adapted sensitivity at 490 nm (Leggett 1979). The grapsid *Leptograpsus variegatus* is most sensitive to light of 484 nm when dark adapted (Stowe 1980). *Gecarcinus lateralis*, the most terrestrial of all our study species, has its ERG maximum near 510 nm, a shift of +23 nm from the rhodopsin peak (Lall and Cronin, in press). One exceptional aquatic example is the deep-water galatheid, *Pleuroncodes planipes*. Its λ_{max} value for rhodopsin is at 503 nm in digitonin extracts, but maximum dark-adapted sensitivity is near 523 nm (Fernandez 1973). Since a number of our anomuran rhodopsins had relatively long-wavelength maxima, it may be that the extracted rhodopsin absorbs differently than the pigment in situ (see Bruno and Goldsmith 1974).

In essentially all crab species that have had $S(\lambda)$ measured after light adaptation, maximum sensitivity is not only less than after dark adaptation, as expected, but it also shifts to longer wavelengths. This is caused by the absorption characteristics of the interposed screening pigments (Goldsmith 1978; Stowe 1980), and may produce multiple spectral classes of photoreceptors in light-adapted retinae (Wald 1968; Leggett 1979). The lesser bathochromic shifts of spectral sensitivity maxima relative to the position of the rhodopsin absorption maximum in dark-adapted semiterrestrial and terrestrial species no doubt are similarly explained, since their rhabdoms always seem to have pigment granules closely apposed. We may be confident, however, that truly aquatic crabs will have spectral sensitivity functions like their rhodopsin absorbance curves when maximally dark adapted, and perhaps when adapted to dim light as well.

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