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The retinas of mantis shrimps from low-light environments (Crustacea; Stomatopoda; Gonodactylidae)

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Abstract. 1. We examined microspectrophotometrically the retinas of 3 species of stomatopods in the superfamily Gonodactyloidea, all of which live in environments that are reduced both in the intensity and spectral range of natural illumination. Species examined were *Odontodactylus brevirostris*, *O. scyllarus*, and *Hemisquilla ensigera*.

2. All 3 species had the typical gonodactyloid diversity of visual pigments, with 8 different photopigments residing in the 4 tiered rows of the midband and 2 additional types in the untiered classes of photoreceptors in the midband and peripheral retina. The spectral range covered by the λ_{max} values of the visual pigments of each species was similar to that of other gonodactyloid and lysiosquilloid species.

3. Apparent retinal adaptations in species of *Odontodactylus* for vision in dimly lit, spectrally narrow photic environments were seen primarily as specializations of the intrarhabdomal filters. These were of reduced diversity, and had reduced absorption at long wavelengths compared to the filters of other gonodactyloid stomatopods. Retinas of *H. ensigera* lacked both proximal classes of intrarhabdomal filter, and had the smallest total range of visual pigment λ_{max} yet observed in mantis shrimps. These modifications decrease the spectral range and number of types of narrow-band spectral classes of photoreceptors, while increasing their sensitivity.

Key words: Photoreceptor – Retina – Spectral sensitivity – Stomatopoda – Visual ecology – Visual pigment

Introduction

The retinas of mantis shrimps (stomatopod crustaceans) possess the most complex photoreceptor system known. Species in superfamilies Gonodactyloidea and Lysiosquilloidea have up to 11 different visual pigments (Cronin and Marshall 1989a, b; Cronin et al. 1994b). The spectral absorption of the visual pigments in a single retina can range in λ_{max} from below 350 nm to beyond 550 nm. When packaged into microvilli of various arrangements and orientations, and placed in combination with an assortment of photostable filters, these photopigments produce a set of ultraviolet photoreceptors of 3 polarization classes, a set of visible-light photoreceptors of 4 or more polarization classes, and a set of 8 narrow-band spectral photoreceptors (Marshall et al. 1991a, b). Thus, the retina provides the visual system with abundant information on the distribution, polarization, and spectral composition of light.

However, to operate such a complex retina requires a reasonably well-lit environment. Most sensitivity classes have narrow sensitivity spectra, produced using short photoreceptors that are frequently heavily filtered by other receptors, or more dramatically, by the intrarhabdomal filters (Marshall et al. 1991b; Cronin et al. 1994a). Some photoreceptor classes receive light that has passed through a series of 2 dense, longpass filters; these classes necessarily have very low absolute sensitivity.

Mantis shrimps typically live in shallow, bright marine habitats, either just subtidally or in the clear waters of coral reefs (Caldwell and Dingle 1975). In such habitats, and with activity cycles that typically peak in the late morning or early evening (Basch and Engel 1989; Caldwell et al. 1989; Dominguez and Reaka 1988), there should be more than sufficient light for retinal function. Nevertheless, some very successful stomatopod species are found much deeper – from 10 m to 100 m beneath the surface of the sea. Even at midday, light in such habitats is greatly diminished both in intensity and spectral range (Jerlov 1976; Loew and McFarland 1990). Species of fish living similarly deep tend to have somewhat simpler retinas than their surface-dwelling cousins, with only one or, at best, two spectral types of cone photoreceptors (McFarland and Munz 1975; Loew and Lythgoe 1978; Lythgoe and Partridge 1991; Levine et al. 1980). Yet the stomatopod species that predominate at these depths are members of the gonodactyloid superfamily, which char-

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acteristically has the most complex retinas of all (Manning et al. 1984; Marshall et al. 1991a, b). How can such eyes continue to operate effectively under these demanding conditions?

With this question in mind, we set out to examine retinal function in 3 species of gonodactyloid stomatopods that live in deeper water or are active at night as well as by day. The species we chose were *Hemisquilla ensigera*, a large stomatopod that lives at depths from 10 to 30 m off the Channel Islands of California, and two species of *Odontodactylus*. *O. scyllarus* is a large species, active both diurnally and nocturnally, that occupies a large depth range (barely subtidal to 100 m) throughout Indopacific waters. *O. brevirostris* is a moderate-sized species that lives from at least 10 m to below 100 m in both Atlantic and Pacific waters. Our results demonstrate that the typical gonodactyloid retinal design persists even in species that inhabit photically limiting environments. Further, they suggest how the basic design can be modified for continued function in light that is both dim and spectrally narrow.

Materials and methods

Animals and experimental preparation. We hand-collected all of our study specimens of *Hemisquilla ensigera* (from near Catalina Island, California) and *Odontodactylus brevirostris* (from a site off western Oahu, Hawaii); specimens of *O. scyllarus* were obtained from suppliers. In the laboratory, animals were kept at 25°C in marine aquaria on a 12 h light:12 h dark cycle, and fed frozen shrimp. *H. ensigera*, from relatively cold waters (15°C), did not survive well in the laboratory and was examined immediately after its arrival. Both species of *Odontodactylus* were very tolerant of laboratory conditions, but were nevertheless examined as soon as possible after collection.

Animals were dark-adapted in individual containers overnight or longer; all subsequent operations were carried out in the dark or under dim red light. Such conditions maximize the concentration of rhodopsin in crustacean photoreceptors (Goldsmith 1978a; Cronin 1985; Cronin and Forward 1988). Eyes were removed and frozen immediately, using fluorocarbon spray, for cryosectioning. Eyes from *H. ensigera* were frozen without any fixation, but to stabilize retinal anatomy some eyes of both species of *Odontodactylus* were fixed briefly in 2.5% glutaraldehyde in pH 7.5 marine crustacean Ringer's solution (Cavenaugh 1956) before quick-freezing.

Frozen eyes were mounted in a cryostat at −30°C with the midband oriented vertically and sectioned at thicknesses from 10 to 14 μm. Sections were mounted between coverslips within a ring of silicone grease for microspectrophotometry. Sections to be used for measurement of filter absorption spectra were mounted in mineral oil (to reduce scattering by matching refractive index), while those used for study of visual pigments were usually mounted in pH 7.5 marine crustacean Ringer's containing 2.5% glutaraldehyde. However, in the case of Row 3 rhabdoms in *O. scyllarus*, where the rhodopsin was extremely unstable, it seemed that somewhat better data could be obtained when the section was mounted in mineral oil, so this was used for some rhabdoms.

Microspectrophotometry. We employed the single-beam instrument and procedures used previously for studies of stomatopod and crab visual pigments (see Cronin 1985; Cronin and Forward 1988; Cronin and Marshall 1989b; Cronin et al. 1993). A linearly polarized spot (diameter 1.5 or 5.0 μm) was placed in material to be scanned. When studying photoreceptors, the path was usually parallel to the rhabdom's axis, but filters were scanned either longitudinally or transversely, depending on their maximum densities. Data were obtained at 1-nm intervals from 400 to 700 nm (except for distal Row 1 photoreceptors of *O. brevirostris* and *H. ensigera*, which were scanned from 300 to 600 nm), and absorbances were computed by comparing the amount of transmitted light with a spectrum previously taken through a clear region of the specimen. Occasionally, absorption by full widths of intrarhabdomal filters was too great for accurate measurement (O.D. > 2.5). In these cases, scans were made through partial sections of the same filter classes, and the correct peak densities were determined by overlaying the undistorted scans onto measurable regions of scans taken through filters of known path length.

To learn the absorption spectra of the visual pigments, we compared absorptions of individual rhabdoms when dark-adapted and again after exposure to bright white light; the difference represented the spectrum of the photobleachable rhodopsin. First, the rhabdoms were positioned under far red light (Corning CS2-61 filter; 50% transmission at 619 nm and 1% at 602 nm). Although most crustacean rhodopsins do not undergo photoconversion under such conditions (Cronin 1985; Cronin and Forward 1988), it is possible that the long-wavelength types in midband Rows 2 and 3 were affected by such red light. However, bandwidths of our data from these regions of the eye match template spectra quite well, indicating that only the rhodopsin was present in these photoreceptors. Two scans were made of the dark-adapted photoreceptor. If these scans were identical, revealing a physically stable preparation, the data of the second scan were saved. The rhabdom was then treated with bright white light for at least 2 min, using the substage illuminator of the photometric microscope, and a third absorption spectrum was obtained. If this spectrum showed evidence of absorbance loss but also overlaid (or nearly overlaid) the spectrum of the dark-adapted rhabdom at long wavelengths (> 650 nm), where no photobleaching of visual pigments was expected, the difference between the 2nd and 3rd spectrum was taken to be that of the photobleachable rhodopsin.

Mathematical analysis. Visual pigments in all regions of the retina below the level of the 8th retinular cell were identified using our earlier techniques (Cronin and Marshall 1989b; Cronin et al. 1993). First, all difference spectra from rhabdoms of a given class that suggested the presence of a photobleachable rhodopsin were averaged. When data from rhabdoms from either glutaraldehyde-fixed or fresh-frozen retinas were compared, no differences were noted, so all data were combined for further analysis. The averaged curves were then tested for fit to rhodopsin template spectra derived by Bernard (1987). Each curve was tested against templates of λ_{max} from 400 to 600 nm (375 to 450 nm in the cases of *H. ensigera* and *O. brevirostris* Row 1 distal), at 1-nm intervals. To test the fit, the curve was first normalized to the average of the 5 data points nearest to the λ_{max} being tested. The best fit was defined as that producing the least sum of squares of deviations, from 25 nm below the wavelength of the maximum of absorption to 75 nm above it. For maxima below 425 nm (except for *H. ensigera* and *O. brevirostris* distal Row 1; see above), the sum of squares was computed from 400 nm to 75 nm beyond the maximum, and corrected for the reduced number of squared deviations. See Cronin and Marshall (1989b) for further details.

For consistency with our earlier work, we refer to the peripheral regions of the compound eye as the dorsal and ventral hemispheres. The midband which separates the hemispheres includes 6 ommatidial rows. Each is designated by the proper noun "Row", numbered dorsally to ventrally, from Row 1 (dorsal) to Row 6 (ventral). In the 4 rows of the midband where main rhabdoms are tiered, the tier nearest the cornea is "distal", that nearest the basement membrane is "proximal".

Results

Our primary objective in this study was to compare the retinas of species of gonodactyloid stomatopods that live

Table 1. Approximate lengths of rhabdoms, tiers, and filters in retinas of the study species, determined from longitudinal sections of frozen retinas. All lengths in μm. Note that *Hemisquilla ensigera* lacks proximal filters in Rows 2 and 3. Data for *Odontodactylus scyllarus* were taken from Marshall et al. (1991a)

Retinal region	*Odontodactylus brevirostris*	*Odontodactylus scyllarus*	*Hemisquilla ensigera*
Peripheral retina (Retinal Hemispheres)	300	450	800
Rows of the Midband:			
Row 1, Distal Tier	140	350	400
Row 1, Proximal Tier	80	180	360
Row 2, Distal Filter (F1)	15	29	15
Row 2, Distal Tier	170	303	470
Row 2, Proximal Filter (F2)	15	48	–
Row 2, Proximal Tier	125	190	385
Row 3, Distal Filter (F1)	25	30	30
Row 3, Distal Tier	100	240	470
Row 3, Proximal Filter (F2)	15	70	–
Row 3, Proximal Tier	190	282	415
Row 4, Distal Tier	170	390	450
Row 4, Distal Tier	130	180	365
Rows 5&6	250	520	800

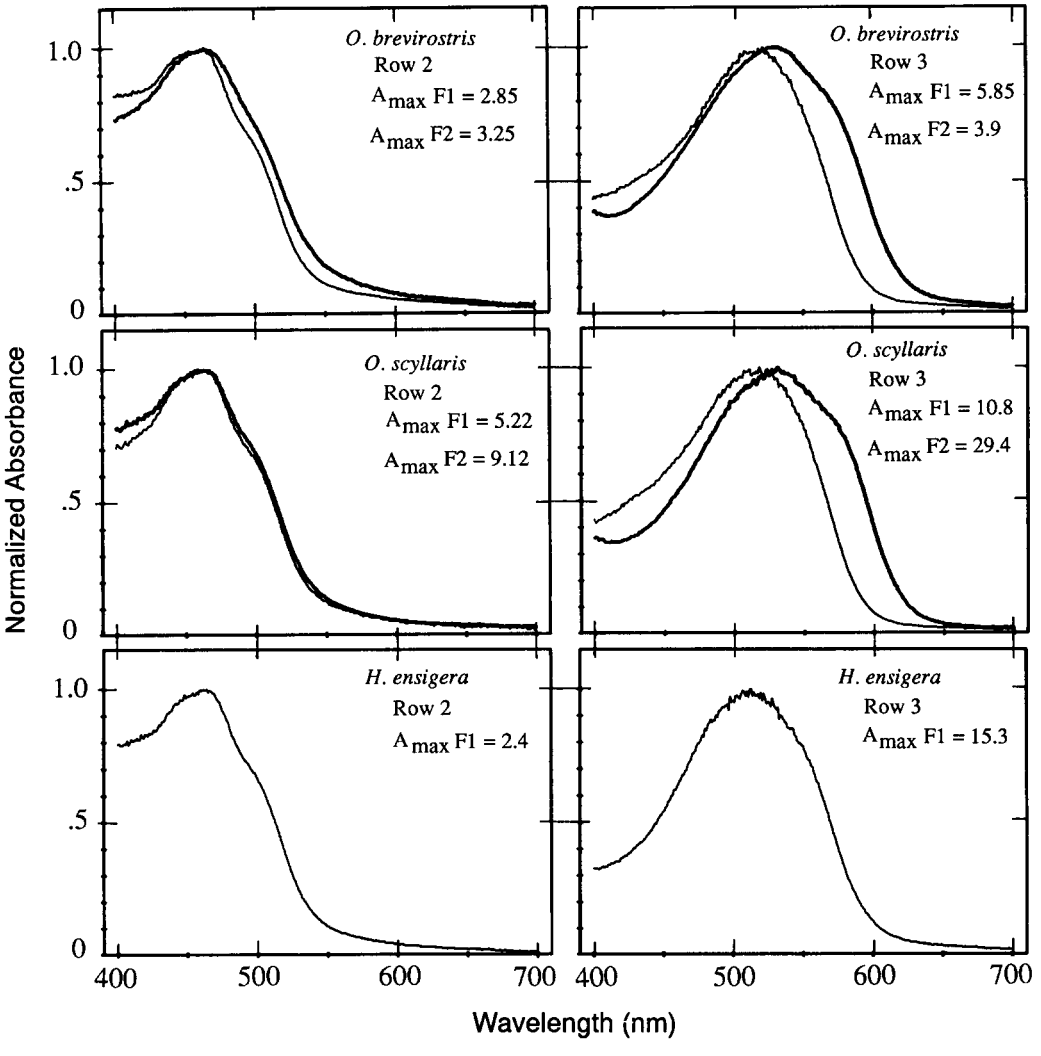

Fig. 1. Normalized, average absorption spectra of intrarhabdomal filters in Rows 2 and 3 of midbands of *Odontodactylus brevirostris* (*top panels*), *Odontodactylus scyllarus* (*middle panels*), and *Hemisquilla ensigera* (*bottom panels*). Each panel includes data from a single row of the midband; *thin traces* represent proximal filters and *thick traces* represent distal filters. The average peak absorbance for entire filters in vivo is given in each panel. Each *curve* is an average of 4 to 17 individual spectral scans

in environments having light that is restricted in both spectral range and intensity to those of their shallow-water, bright-light relatives. To make this comparison, we examined the photostable intrarhabdomal filters in Rows 2 and 3 of the midbands, and the visual pigments of all retinal regions below the level of the 8th retinular cell. Like typical stomatopods (Cronin and Marshall 1989b; Marshall et al. 1991b; Cronin et al. 1993), retinas of these species contain a diversity of colored pigments; however, since such material lay outside the path taken by light as

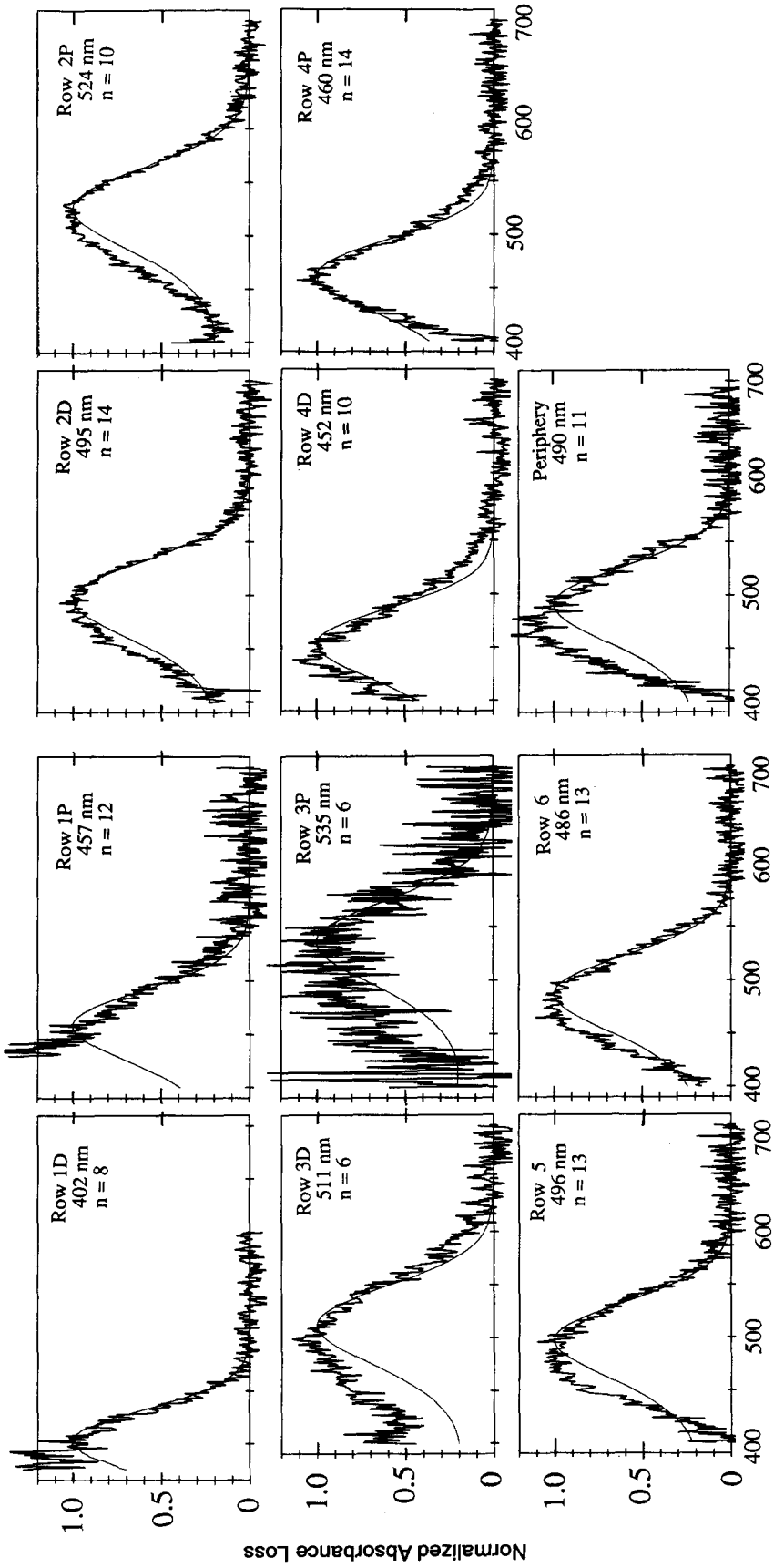

Fig. 2. *Odontodactylus brevirostris.* Normalized, average spectra for photobleaching of visual pigments in all retinal regions below the rhabdomere of the 8th retinular cell. Mean absorbance change from 651 to 700 nm is set to 0 in each *curve.* Retinal locations of measurements and number of photobleaches included in each curve are indicated in the *upper right corner of each panel.* The *smooth trace* is the best-fit template spectrum, fitted as described in the text; the wavelength of the peak absorption of the template curve is also indicated on the panel. In the distal tier of Row 1, data were originally gathered from 330 to 600 nm; only the results from 375 to 600 nm are plotted, and mean absorbance change from 551 to 600 nm was set to 0 in this case only

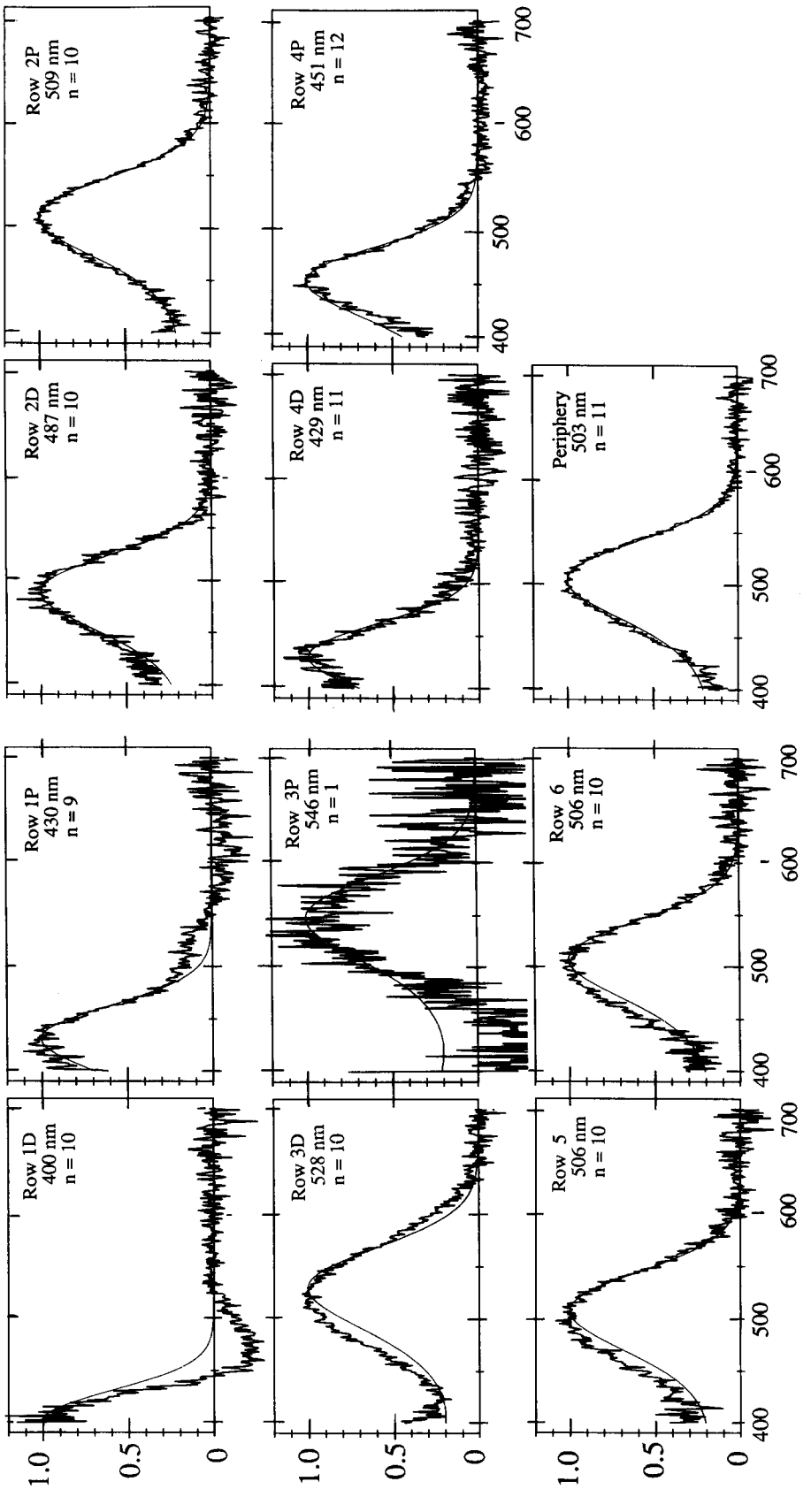

Fig. 3. *Odontodactylus scyllarus.* Normalized, average spectra for photobleaching in all retinal regions below the level of the rhabdomere of the 8th retinular cell. Otherwise as in Fig. 1, except that distal rhabdoms in Row 1 were treated identically to all other classes of rhabdoms

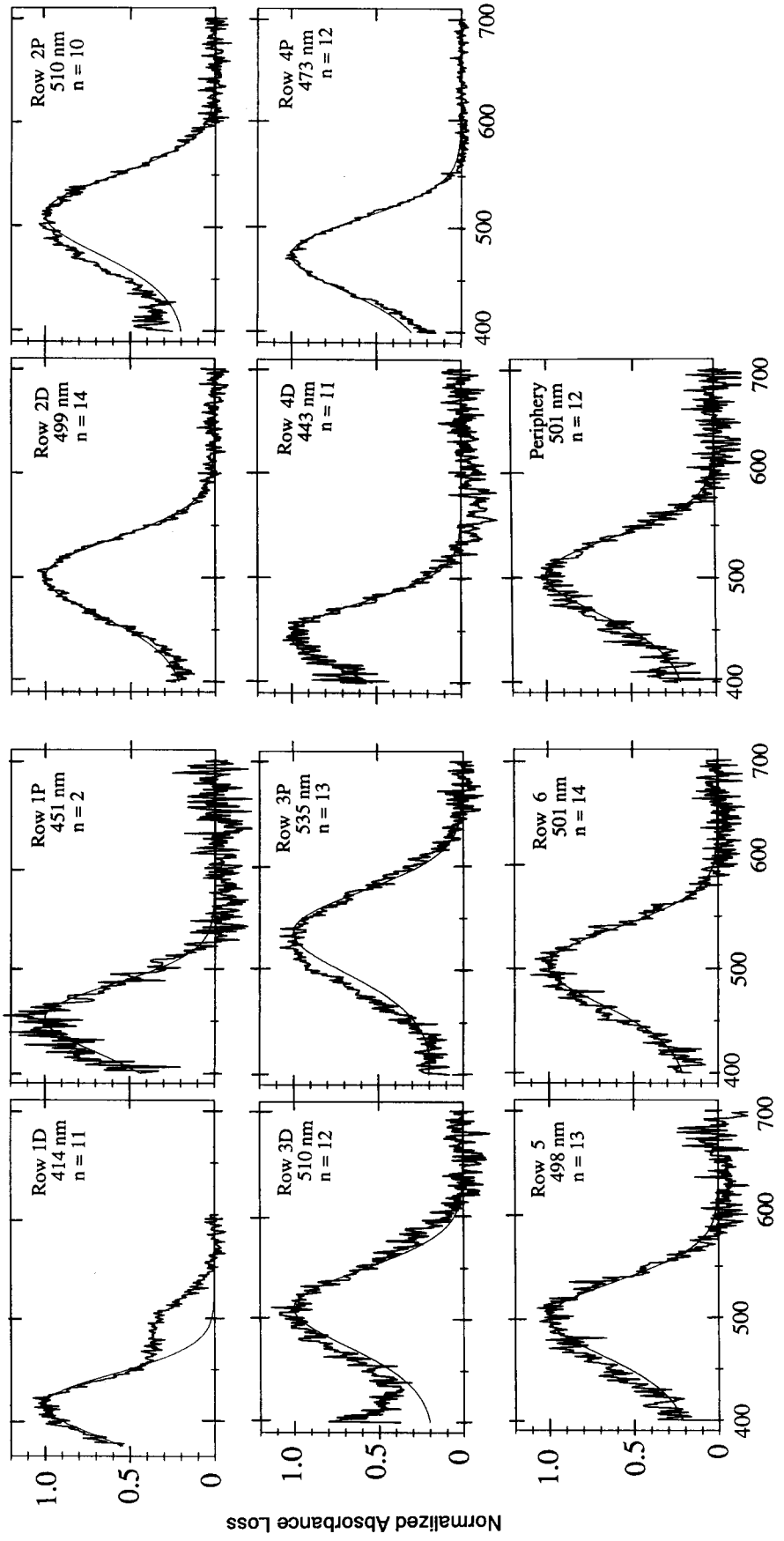

Fig. 4. *Hemisquilla ensigera.* Normalized, average spectra for photobleaching in all retinal regions below the level of the rhabdomere of the 8th retinular cell. Otherwise as in Fig. 1

it traverses the actual photoreceptors, we did not attempt to characterize it in this work. Dimensions of photoreceptors and intrarhabdomal filters were obtained from fresh-frozen, longitudinally sectioned material and are provided in Table 1. For a full description of the compound eyes of gonodactyloid stomatopod crustaceans, including details of tiering and placement of intrarhabdomal filters, see Marshall et al. (1991a, 1991b).

Intrarhabdomal filters

Stomatopod crustaceans in the superfamily Gonodactyloidea generally have 4 classes of intrarhabdomal filters in their retinas: distal Row 2, proximal Row 2, distal Row 3, and proximal Row 3 (Marshall 1988; Cronin and Marshall 1989a, b; Cronin et al. 1994a). Both species of *Odontodactylus* are typical of the superfamily, having all 4 types (Fig. 1). However, the much greater lengths of filters in *O. scyllarus* (compared to *O. brevirostris*) gives them a considerably higher axial absorbance - greater than 29 density units in one case (the 70- μm filter in the proximal Row 3 location). *Hemisquilla ensigera* is unique among species of gonodactyloid stomatopods examined thus far, in that it has only the 2 distal classes of filters; it entirely lacks the proximal classes. Nevertheless, these distal filters are long and have significant axial absorbances (Table 1, Fig. 1).

Both species of *Odontodactylus* possess identical assortments of filters. Compared to other gonodactyloid species, however, these filters span a spectrally restricted range. Row 2 contains two spectrally identical filters, although the proximal ones are longer than the distals and thus have somewhat greater total absorption. Two different filter classes occur in Row 3, but their spectra are separated by only about 25 nm on their long-wavelength limbs. Thus, the proximal filter is virtually transparent at wavelengths beyond 600 nm; proximal filters of all other known gonodactyloids are much more absorbant at long wavelengths (Cronin and Marshall 1989a, b; Cronin et al. 1994a). Both distal filters of *H. ensigera* appear to be spectrally very similar, or identical, to those of the *Odontodactylus* species.

Visual pigments

As in all gonodactyloid stomatopods, the retinas of these species include 11 classes of photoreceptors below the level of the 8th retinular cell: two tiered classes in each midband row from Row 1 to Row 4 plus the main rhabdoms of midband Rows 5 and 6 and of the peripheral retina. We examined each class of photoreceptor in retinas of all of our study species.

Odontodactylus brevirostris. This species had the smallest compound eyes and photoreceptors. The thin receptors required the use of the small scanning spot (1.5 μm), and there was an increased possibility of contamination of data by the intersection of the beam with the pigment surrounding the photoreceptor. As a result, data from *O. brevirostris* tended to be of lower quality than data from corresponding regions of retinas of the other two, larger species. In particular, Row 1 rhabdoms were unstable, and data from them was frequently contaminated by other pigments. Nevertheless, identifiable visual pigments were found in all retinal regions (Fig. 2). The distribution of visual pigments throughout the retina was qualitatively similar to what was observed in the other species (as well as in all other lysiosquilloids and gonodactyloids that we have previously examined). We shall consider these points further in the Discussion.

Odontodactylus scyllarus. Compound eyes in this species are of the same basic shape and plan as those of *O. brevirostris*, but are much larger with bigger photoreceptors. Consequently, most data were of relatively high quality (Fig. 3), although the results were occasionally distorted by the apparent presence of photoproducts produced during the bleach (e.g. distal Row 1). However, the photopigment of proximal Row 3 was extremely unstable. Despite intensive work with this class of photoreceptor, we obtained usable data from only a single photoreceptor. We cannot explain our lack of success, but it is possible that since these animals were obtained from professional suppliers, their retinas were not in optimal physiological condition. On the other hand, the histological appearance of the retina was excellent, and receptor classes throughout most of the rest of the retina had photobleach spectra closely similar to the templates.

Hemisquilla ensigera. This species is taxonomically removed from *Odontodactylus*, and has large, tall, bean-shaped eyes (those of *Odontodactylus* are globular). In general, photoreceptors of this species were very easy to work with. An exception occurred in Row 1, where a high concentration of orange-brown pigment surrounded the rhabdoms and tended to spread rapidly into the receptors after they were prepared for microspectrophotometry. Its presence and changing concentrations (due to solubilization) frequently distorted the shapes of the photobleach spectra and produced pronounced shoulder regions in spectra from the distal tier, reducing the numbers of usable scans in this region. In most cases, however, photobleach data were of exceptional quality, closely matching template spectra (Fig. 4). Once again, the distribution of classes of visual pigments was qualitatively similar to what is observed throughout stomatopods with 6-row midbands.

Spectral sensitivity

With knowledge of the visual pigments, filter absorption spectra, and dimensions of the various photoreceptor classes (Table 1), we could model the spectral sensitivities of all photoreceptors. As in our previous work (Cronin and Marshall 1989a, b; Cronin et al. 1993), we assumed that (1) absorption above the level of the main rhabdom was negligible, (2) each visual pigment's absorption spectrum was equivalent to the best-fit template spectrum, (3) all visual pigment is present as rhodopsin, with an axial

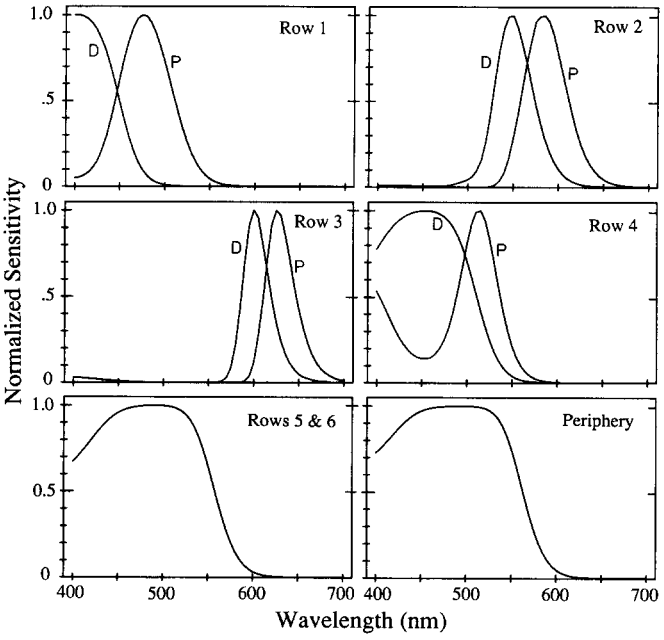

Fig. 5. *Odontodactylus brevirostris.* Computed sensitivity functions for all retinal regions below the 8th retinular cell. Method of computation explained in the text. Each row of the midband is plotted on a separate panel, except for Rows 5 and 6, which are presumed to contain the same visual pigment, with maximal absorption at 488 nm (the least-squares fit to the combined data from these 2 rows). *D* distal; *P* proximal

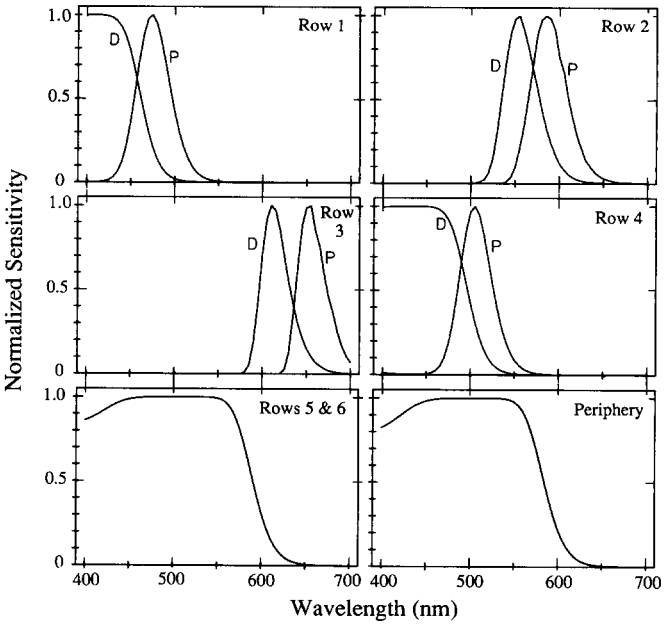

Fig. 6. *Odontodactylus scyllarus.* Computed sensitivity functions for each class of photoreceptor, as in Fig. 5. *D* distal; *P* proximal

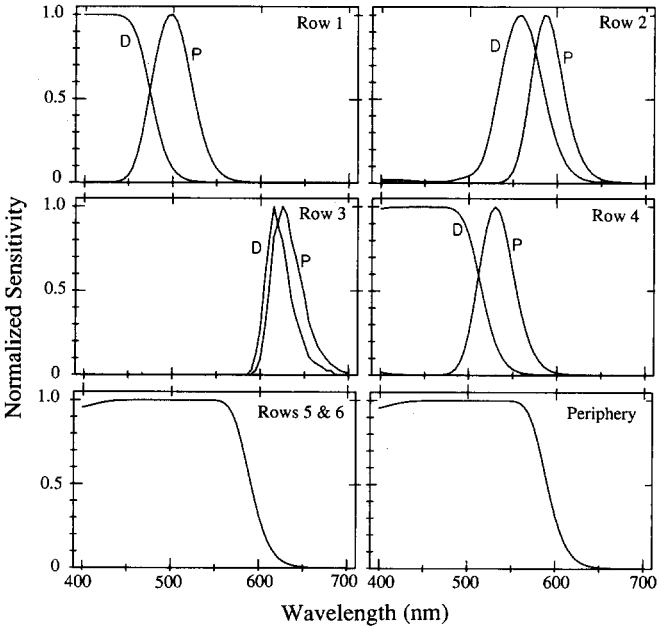

Fig. 7. *Hemisquilla ensigera.* Computed sensitivity functions for each class of photoreceptor, as in Fig. 5. *D* distal; *P* proximal. Rows 5 and 6 are presumed to contain identical visual pigments, with λ_{max} = 501 nm (the least-squares fit to the combined data from these 2 rows)

density of 0.008 μm^{-1}, (4) all light entering the distal tip of the photoreceptor remained within it and was modified only by its interactions with visual pigments and intrarhabdomal filters (rhabdoms act as light guides, having diameters of 10 μm or less and being surrounded by a palisade vacuole of low refractive index), and (5) besides the intrarhabdomal filters and visual pigments, there were no other light-absorbing materials in the rhabdom. For details of the modelling procedure, see Cronin and Marshall (1989b).

While based only on optical aspects of receptor design and light absorption, our modelling provides a reasonable estimate of actual spectral sensitivity functions, particularly in the tiered photoreceptors of the midband. This is because spectral sensitivity in stomatopods is strongly determined by filtering of light before it enters the actual photoreceptors. As demonstrated by Goldsmith (1978b), such external filtering has far greater effects than does the inclusion of a homogeneous mixture of metarhodopsin with the rhodopsin. These model spectral sensitivity functions have been confirmed physiologically in our laboratories by intracellular optical techniques (Cronin) and single-cell electrophysiological recordings (Marshall).

Our analysis reveals that in both species of *Odontodactylus*, the distribution of spectral sensitivities throughout the retina has the typical gonodactyloid pattern (see Cronin and Marshall 1989a, b). Each ommatidium of midband Rows 1 to 4 has a pair of spectrally narrow classes separated by 30 to 50 nm at their peaks; the proximal tier is sensitive at longer wavelengths than the distal tier (Figs. 5 and 6). Photoreceptors in Rows 5 and 6, and in the retinal periphery, are untiered and have broad, flat-topped spectral sensitivity functions. Because the rhabdoms of *O. scyllarus* are so much longer than those of *O. brevirostris*, the unfiltered distal tiers of Rows 1 and 4 also have distinctly flat-topped sensitivity spectra. In addition, because the proximal filter in Row 3 of *O. scyllarus* is unusually long, and is paired with a long-wavelength rhodopsin (λ_{max} = 546 nm), the proximal tier of

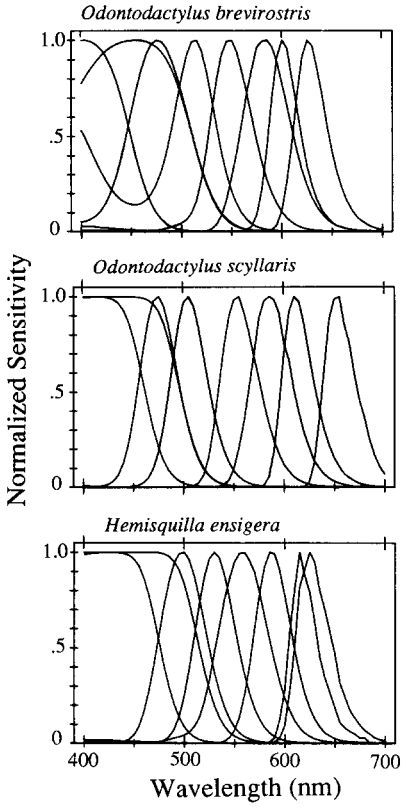

Fig. 8. Computed spectral sensitivity functions of the 4 tiered rows of the midbands for the 3 study species. All functions are plotted together to illustrate the range of spectral coverage in Rows 1 through 4

this row is more sensitive to longer wavelengths than the corresponding photoreceptor class of *O. brevirostris*. In other respects, sensitivities in the retinas of these 2 species are similar.

Hemisquilla ensigera, on the other hand, has sensitivity spectra more like those of lysiosquilloids (see Cronin et al. 1993). Receptors in the 2 tiers of the filtered rows are less separated than in *Odontodactylus*, primarily because of the lack of proximal filters in these ommatidia (Fig. 7). Again, because photoreceptors in this species are very lengthy (even tiered types; see Table 1), sensitivity spectra of the unfiltered distal tiers, and of the untiered photoreceptors, are broad and flat.

Together, the 4 tiered types of photoreceptors of all 3 species produce 8 narrow, separate spectral sensitivity functions covering a spectral range from below 400 nm to beyond 650 nm (Fig. 8). *O. scyllarus* has the broadest spectral coverage and *H. ensigera* the narrowest. Also, in *H. ensigera* most photoreceptor classes are crowded into the wavelength band from 500 to 625 nm, while receptor classes in *Odontodactylus* are more evenly spaced throughout their total spectral range. All species have the potential for high-resolution spectral discrimination through much of the visible-light spectrum.

Discussion

We selected the species described in this paper because their vision must operate under conditions that are photically demanding - these animals inhabit deep water or are active at times of reduced light intensities. All species we previously studied live in very shallow water, at depths of a few meters or less, and most are active during the day. By adding these new species to those that have already been described, we hoped to gain a broader understanding of the functional design of stomatopod retinas. In particular, we expected to learn how the narrowband spectral receptor classes may be adapted for use in low-light, spectrally restricted habitats.

Photic characteristics of the habitat of *Hemisquilla ensigera* can be devined from a description by Basch and Engel (1989) of the ecology of this species. *H. ensigera* is found at depths from 4 to 90 m, and may become most abundant at 10 to 15 m. The coastal waters it inhabits are greenish in color and generally fairly clear (though not as clear as water overlying coral reefs), but plankton blooms and turbidity can reduce visibility to a few meters or less. Based on times of burrow openings, major activity peaks occur between dawn and about 10 AM, and again from about 3 PM to dusk; few animals appear to be active at midday. Thus, light in the environment of this species can become severely reduced by a combination of depth, water clarity, and time of activity.

The waters occupied by species of *Odontodactylus* tend to be clearer than is the case with *H. ensigera*. While *O. scyllarus* is frequently encountered at shallower depths than the latter species, it is normally active by night as well as day. The optics of its eye are consistent with use primarily in dim light (Horridge 1978; Cronin 1986; Marshall and Land 1993b). *O. scyllarus* certainly is visually aware of its surroundings, exhibiting dramatic ocular activity during tracking, scanning, and optokinetic stabilization (Land et al. 1990; Cronin et al. 1991). The congeneric *O. brevirostris* lives as deep as 309 m (Manning 1969), although the population from which we collected specimens was located at the atypically shallow depth range of 10 to 20 m. At our collection site, we observed this species to be active primarily from dawn until midmorning, and again after midafternoon to dusk. Therefore, like *Hemisquilla*'s, the world of *Odontodactylus* is a light-limited one.

Modifications of intrarhabdomal filters

Retinas of the great majority of gonodactyloid species contain 4 classes of intrarhabdomal filters (Marshall et al. 1991b; Cronin et al. 1994a). In lacking proximal classes of filters in both Rows 2 and 3, *H. ensigera* is unique among the described species, although an analogous situation exists in lysiosquilloids of the genus *Lysiosquilla* (Cronin et al. 1993, 1994a). By disposing of these filter classes, *H. ensigera* compromises spectral resolution in Rows 2 and 3 of the midband; in Row 3 the proximal and distal tiers have virtually identical spectral sensitivities. Presumably, the sacrifice of spectra resolution is made to increase absolute sensitivity in these photoreceptors.

Both species of *Odontodactylus* had identical sets of filters, including all 4 possible classes. However, their filters differed spectrally from those of all other known

Table 2. Distributions of visual pigments (by wavelength of maximum absorption) in retinas of all stomatopod species examined to date. Each value is λ_{max} (nm, determined by fitting averaged data to polynomial template spectra with λ_{max} in the wavelength range 400 to 600 nm) of the rhodopsin residing in a class of photoreceptors. Data for squilloid and lysiosquilloid species are from Cronin et al. (1993). Data for *Gonodactylus oerstedii* and *Pseudosquilla ciliata* are from Cronin and Marshall (1989b); other gonodactyloids are from this work. Note that squilloid species have midbands with only 2 untiered ommatidial rows, containing the same visual pigment as the periphery

Retinal Region	Superfamily Squilloidea		Superfamily Lysiosquilloidea		Superfamily Gonodactyloidea					Average (mean ± s.d.)
	Squilla empusa	*Cloridopsis dubia*	*Coronis scolopendra*	*Lysiosquilla sulcata*	*Gonodactylus oerstedii*	*Pseudosquilla ciliata*	*Odontodactylus brevirostris*	*Odontodactylus scyllarus*	*Hemisquilla ensigera*	
Peripheral Retina	517	510	494	499	528	498	490	503	501	504.4 ± 12.0
Rows of the Midband:										
Row 1, Distal Tier[a]			407	397	400	400	402	400	414	402.9 ± 5.8
Row 1, Proximal Tier			436	434	430	433	457	430	451	438.7 ± 10.8
Row 2, Distal Tier			489	492	505	498	495	487	499	495.0 ± 6.2
Row 2, Proximal Tier			518	516	525	517	524	509	510	517.0 ± 6.2
Row 3, Distal Tier			529	517	520	535	511	528	510	521.4 ± 9.5
Row 3, Proximal Tier			533	538	551	539	535	546	535	539.6 ± 6.6
Row 4, Distal Tier			441	416	429	425	452	429	443	433.6 ± 12.3
Row 4, Distal Tier			468	461	460	452	460	451	473	460.7 ± 7.9
Row 5			519	500	493	510	496	506	498	503.1 ± 9.1
Row 6			515	501	486	510	486	506	501	500.7 ± 11.2

[a] For this tier only, *L. sulcata*, *O. brevirostris*, and *H. ensigera* were tested for best fit at wavelengths from 375 to 450 nm

gonodactyloids (Cronin et al. 1994a). Both the proximal and distal filters of Row 2 were spectrally identical. This can be viewed as an adaptation to increase the throughput of photons to the proximal tier; in fact, the 2 tiers of Row 2 maintain adequate spectral discrimination (Figs. 5 and 6). But the fact that the proximal filter acts only to reinforce the action of the distal filter, rather than to trim even more of the spectrum at long wavelengths, must increase the absolute sensitivity of the proximal tier.

The orange-red distal filter in Row 3 of *Odontodactylus* is fairly typical, but the red proximal filter in this same row transmits light at significantly shorter wavelengths than does any other known Row 3 proximal filter (see Cronin et al. 1994a). This obviously delivers more light to the proximal tier of photoreceptors than would be possible with a filter passing only far-red light. This is a clear advantage in deeper water, where downwelling red light has been strongly absorbed (Jerlov 1976; Lythgoe 1988; Loew and McFarland 1990). The two species of *Odontodactylus* provide excellent examples of the interaction between the lengths and absorption spectra of the filters and visual pigments. In the proximal tier of Row 3, both the filters and the photoreceptors of *O. scyllarus* considerably exceed those of *O. brevirostris* in length, and *O. scyllarus* has a longer-wavelength rhodopsin there (λ_{max} of 546 vs. 535 nm). This combination of factors produces a much greater difference between the sensitivity spectra of the distal and proximal tiers in *O. scyllarus* than in *O. brevirostris*. We believe that the frequent diurnal activity of *O. scyllarus*, coupled with its common appearance in shallow waters, permits this species the luxury of a red receptor class that is sensitive to longer wavelengths than the homologous receptor class of *O. brevirostris*.

Modifications of visual pigments

Distributions of visual pigments throughout the retinas of these species maintain the same pattern seen in all lysiosquilloids and gonodactyloids we've examined. Comparative data for every stomatopod species characterized to date are given in Table 2 and Fig. 9A, which indicate the computed λ_{max} for the visual pigment of each main photoreceptor class. (In addition to these data, an ultraviolet-sensitive visual pigment, with λ_{max} between 325 and 340 nm, has been localized in 8th retinular cells of *Lysiosquilla sulcata*, *Lysiosquilla maculata*, *Gonodactylus oerstedii*, *Pseudosquilla ciliata*, and *Hemisquilla ensigera*; see Cronin et al. 1994b). It is safe to assume that all these visual pigments are rhodopsins, since retinal is the only retinoid chromophore known in stomatopod eyes (Goldsmith and Cronin 1993).

A general pattern is revealed by comparing among rows of the table and among mean values for each row, as well as by an inspection of Fig. 9A. In order of wavelength of maximum absorption, the rows of the midband fall in the same order for every species: Row 1, Row 4, Row 2, and Row 3. The distal and proximal tiers contain visual pigments that tend to be separated in λ_{max} by about 25 nm, although the separation varies among rows and species. Interspecific variation in λ_{max} tends to be slightly greater in Rows 5 and 6, and in the peripheral retina, than in the tiers of the midband. In general, these untiered photoreceptors tend to absorb maximally at medium wavelengths, showing no obvious correlation with habitat or activity cycle. Nor does absorption in the 2 most ventral rows of the midband show any regular relationship to the periphery.

While differences are apparent among various retinal regions of the same species, and within the same retinal

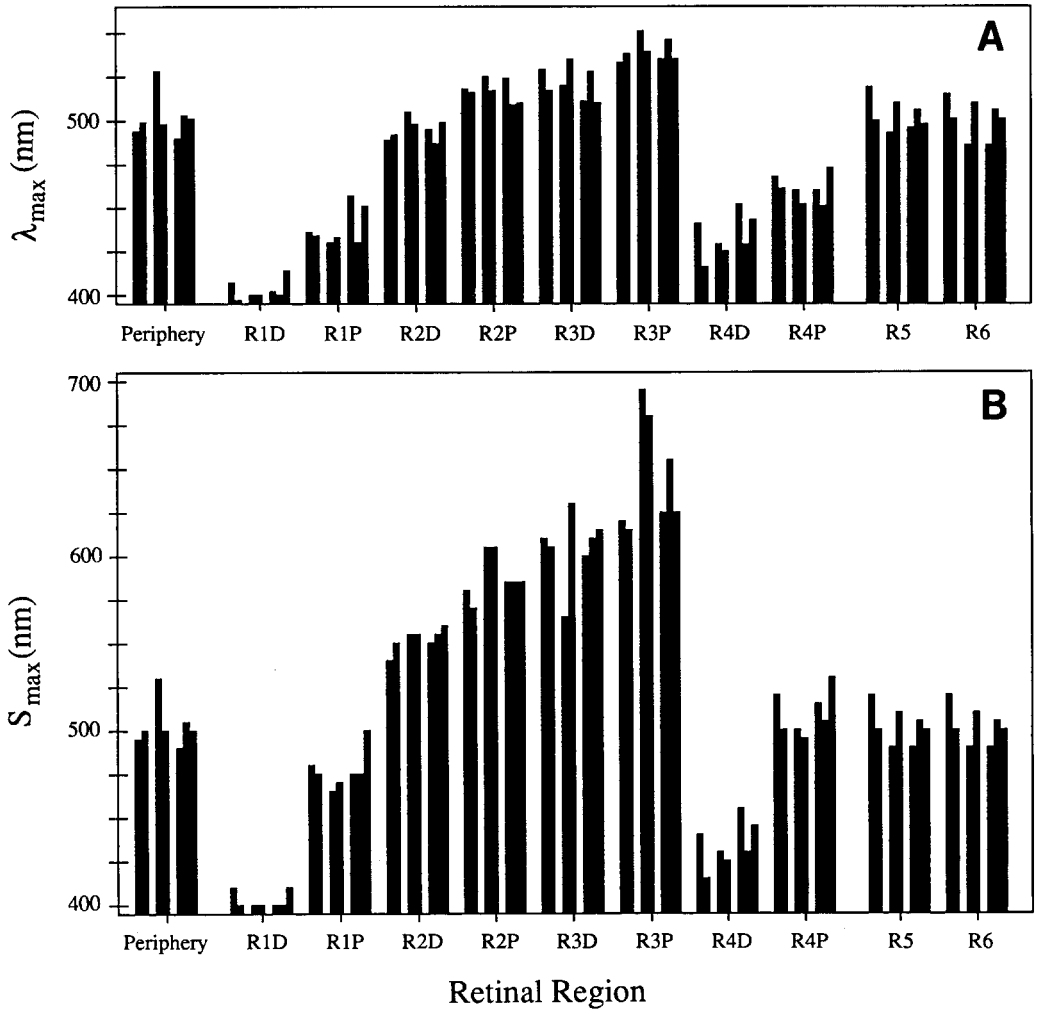

Fig. 9. A Histograms representing λ_{max} values of visual pigments in each retinal region of 7 stomatopod species. In the data of each region, lysiosquilloid species are plotted to the left, bright-light gonodactyloid species in the center, and low-light gonodactyloid species (from this paper) to the right. Species order in each region is as follows: *Coronis scolopendra*, *Lysiosquilla sulcata*; *Gonodactylus oerstedii*, *Pseudosquilla ciliata*; *Odontodactylus brevirostris*, *Odontodactylus scyllarus*, and *Hemisquilla ensigera*. *Periphery*: Peripheral retina (dorsal and ventral hemispheres). *R* Row; *D* Distal tier; *P* Proximal tier. **B** Histograms representing the wavelengths of maximum sensitivity (S_{max}) of photoreceptors in each retinal region of 7 stomatopod species (sensitivities were computed at 5-nm intervals). Otherwise as in **A**. Due to correction of an earlier computational error, data for the proximal tiers in Rows 2 and 3 of *G. oerstedii* and *P. ciliata* differ somewhat from Cronin and Marshall (1989a, b)

region of different species, it is difficult to judge which variations are significant and which reflect random outcomes of the analysis. A good indicator of the normal level of experimental variation is perhaps given by a comparison of midband Rows 5 and 6, which almost certainly contain the same visual pigment. Means of Row 5 and 6 differ by only 2.4 nm, and the average difference between values of these rows across species is 3.7 nm. The greatest difference in the results for these 2 rows from any one species is 10 nm (in *O. brevirostris*). Therefore, it seems safe to assume that differences in λ_{max} greater than 10 nm are probably real. Differences at least this large are apparent in every region of the retina across species, but no obvious trend emerges. However, species with dense or long-wavelength proximal filters in Row 3 tend to have the longest-wavelength rhodopsins in proximal tiers of this row.

A comparison of the 2 species of *Odontodactylus* shows that most homologous photoreceptor classes differ in their visual pigments by more than 10 nm, including Row 1 proximal, Row 2 proximal, both tiers of Row 3, Row 4 distal, Rows 5 and 6, and the periphery. These results from the same genus reveal a surprising amount of variation in a single receptor class in the face of a general conservation of function within retinal regions. Contrary to our expectations (see Cronin et al. 1993), it appears that evolution of the functional classes of rhodopsins can occur rapidly. Therefore, it should prove interesting to make intraspecific comparisons among populations of widespread stomatopod species such as *Pseudosquilla ciliata* or *Lysiosquilla maculata*. Similar levels of variation have been observed in homologous photoreceptor classes of congeneric hymenopterans (Peitsch et al. 1992).

Of all species, the visual pigments of *Hemisquilla ensigera* cover the most restricted total range, with λ_{max} ranging from 414 (distal Row 1) to 535 nm (proximal

Row 3). In both Row 1 and Row 4, the rhodopsins absorb at distinctly longer wavelengths than the cross-species averages. In a sense, it has "crammed" the spectral sensitivities of its photoreceptors into a fairly narrow wavelength band centering on 500 to 550 nm (see Figs. 7 and 8). This distribution of receptor classes should enhance photon capture in the green-transmitting waters inhabited by *H. ensigera* (Basch and Engel 1989). Strangely, 8th retinular cells of this species possess an ultraviolet-absorbing rhodopsin, with λ_{max} near 340 nm (Cronin et al. 1994b), which should capture very few photons at the depths it typically inhabits.

Modifications of spectral sensitivity functions

Wavelengths of computed maximum spectral sensitivity in each retinal region of seven stomatopod species are plotted in Fig. 9B. The vertical scale of Fig. 9B is identical to that of 9A, to permit a direct illustration of how the tiering and filtering greatly increase the range and diversity of receptor spectral classes. In untiered rhabdoms (peripheral retina and Rows 5 and 6), as well as the distal tiers of unfiltered rhabdoms (Rows 1 and 4), spectral sensitivity maxima are identical to visual pigment λ_{max} values. Absorption by the distal tiers of unfiltered rhabdoms (Rows 1 and 4) shift maximum sensitivities of the underlying proximal tiers by about 50 nm beyond the visual pigment λ_{max}. The inclusion of intrarhabdomal filters in Rows 2 and 3 causes even more impressive shifts in spectral sensitivity, at times well over 100 nm beyond the absorption peak of the visual pigment itself.

Because of the heterogeneity of the filter classes (see also Cronin et al. 1994a), the greatest species diversity in sensitivity occurs in Rows 2 and 3, particularly in the proximal tiers. Here, sensitivity functions of gonodactyloid species living in shallow, bright water (middle pair of histograms in each group of Fig. 9) achieve their maxima at wavelengths 25 to 75 nm beyond the homologous regions of the other species. In fact, lysiosquilloids (left pair of histograms in each group) and gonodactyloids from low-light environments (right triplet of histograms in each group) are virtually identical in their sensitivity maxima throughout the retina. Since lysiosquilloid species are frequently active during the night (see Cronin et al. 1993), it seems reasonable that their retinas functionally resemble those of the low-light gonodactyloids.

Concluding remarks

In most groups of animals, species that must see under scotopic conditions, or in environments that are light-limited in other ways, have fewer classes of photoreceptors than their bright-light relatives. As Walls (1942) noted in his classic work on vertebrate eyes, nocturnal, terrestrial vertebrates tend to possess pure-rod, or rod-dominated retinas, while diurnal species generally have one or more cone classes as well. The vertebrates that compare most directly with the mantis shrimps are fishes. Among these, shallow-water species have 3 or 4 spectral classes of cone photoreceptors, but species that live even moderately deep in coastal or open-ocean environments virtually without exception have only 2 cone classes (McFarland and Munz 1975; Loew and Lythgoe 1978; Lythgoe and Partridge 1991; Levine et al. 1980).

In maintaining a complex diversity of photoreceptors even when they live under conditions that are not fully photopic, mantis shrimps are quite different from vertebrates. Optical enhancements can play a role, delivering more light to the photically deprived receptors (Schiff et al. 1986; Marshall and Land 1993a, b). However, when photons are essentially absent, as they are at longer wavelengths in deeper water, further adaptations become necessary. Thus, our work shows that in order to maintain retinal polychromacy, several compromises must be made. The most dramatic of these is the elimination (or, at least, lack) of the proximal filter classes in *Hemisquilla ensigera*. By having a simpler retina, this species apparently trades spectral resolution for sensitivity. A further possible adaptation of *H. ensigera* is the crowding of its diversity of rhodopsins into an atypically limited spectral range (Table 2), producing a set of spectral receptors that should be well matched to the downwelling spectrum of light in its habitat.

Retinas of *Odontodactylus* possess the typical gonodactyloid quadruplex assortment of intrarhabdomal filters, but modifications are apparent in these species as well. Absolute sensitivity is enhanced in Row 2 by placing a duplicate of the distal filter in the proximal position. In Row 3, the distal and proximal filters are different, but the proximal filter absorbs only at unusually short wavelengths, transmitting more of the relatively scarce long-wavelength photons. Although spectral coverage is compressed slightly, spectral resolution remains high throughout the entire range. By including an unusually lengthy filter and long-wavelength rhodopsin in the proximal position in Row 3, *O. scyllarus* even regains some of the lost range.

In addition to these modifications to the retina, low-light species of mantis shrimps have optical modifications that enhance the delivery of photons to the photoreceptors (Horridge 1978; Marshall and Land 1993a, b). At present, however, we can only speculate about the evolutionary forces that encourage the retention of polychromatic vision in stomatopod species that inhabit environments where light is frequently low or limited. To understand how evolution constrains and defines retinal design in animals, we must learn more about the nature of their visual worlds. Since the photic environments of mantis shrimps can be precisely defined by the locations of their burrows and by their activity cycles, this unusual group of animals offers a special perspective from which to examine general problems of visual evolution.

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