UV LIGHT IN THE DEEP-SEA : *IN SITU* MEASUREMENTS OF DOWNWELLING IRRADIANCE IN RELATION TO THE VISUAL THRESHOLD SENSITIVITY OF UV-SENSITIVE CRUSTACEANS

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Several species of deep-sea crustaceans possess unusually high spectral sensitivity to UV light, measured electrophysiologically. In addition, behavioral experiments indicate that these species are equally sensitive to near-UV and blue-green light. These results raised questions about the functional significance of this short wavelength sensitivity, since it is commonly believed that UV light is virtually absent in the deep-sea. Using submersible based technology, we conducted measurements of downwelling irradiance at two wavelengths – 380 and 480 nm. These measurements indicate that the irradiance of 380 nm light at 500–600 m, the daytime depth range of the crustaceans with a UV sensitivity peak, is high enough to be detected by these species. In addition, measurements of the spectrum of the downwelling light field conducted at dusk demonstrate that spectral changes visible at the surface are not visible at 150 m. These results are discussed with respect to hypotheses on the functional significance of UV vision in deep-sea crustaceans.

KEY WORDS: Deep-sea, UV-light, crustaceans

INTRODUCTION AND REVIEW

In clear oceanic waters, the wavelength of light that penetrates best is 475 nm (Jerlov, 1976, Dartnall, 1974). The only other available source of light is bioluminescence, and the peak spectral emission of the bioluminescence from the majority of deep-sea organisms is between 460 and 490 nm (Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988). Since the available light is centered on the blue-green, one would expect the organisms inhabiting these depths to be adapted for maximum sensitivity to these same wavelengths. Studies on the visual pigments of several species of deep-sea crustaceans (Fisher and Goldie, 1958, 1960; Denys and Brown, 1982) indicated that they have single visual pigments with absorption maxima between 470 and 490 nm. However, these studies were carried out on pigment extracts, which provide information about the absorption characteristics of the primary pigment, but may miss the presence of a secondary pigment (see Menzel, 1979 for review). Extracts also do not reflect the physiological spectral sensitivity, because any effects on sensitivity due to neural processing or pre-retinal filtering are lost (see Frank and Case, 1988 for review).

In 1988, one of us (TMF) conducted electrophysiological measurements on the spectral sensitivity of 8 species of deep-sea shrimp (Table 1), utilizing the ERG, which is the mass electrical response of a large number of photoreceptor cells to a flash of light (Frank and Case, 1988). This technique, when used with selective chromatic adaptation,

is capable of revealing receptors that make up a small fraction of the total population (Goldsmith, 1986). It has proven to be particularly useful for studies of deep-sea crustaceans, because it can be conducted on shipboard, ensuring access to healthy specimens with intact visual systems. A remarkable result of this study was the discovery that four of these species, *Janicella spinacauda, Oplophorus spinosus, O. gracilirostris*, and *Systellaspis debilis*, possess two peaks of spectral sensitivity – one in the UV (380–400 nm), and the other in the blue-green (490–500 nm) (Table 1). Selective chromatic adaptation experiments, together with differences in response waveforms, indicated that the two spectral sensitivity peaks in these species were due to two independent mechanisms.

Since electrophysiologically measured spectral sensitivity cannot be used as evidence of behavioral sensitivity to these wavelengths, behavioral tests were needed to measure threshold sensitivities and to test for wavelength specific differences in behavior, such as have been found in fireflies (Frank and Case, unpublished). To this end, we developed a protocol for studying the light reflexes of deep-sea crustaceans (Frank and Widder, 1994a, b). The behaviors examined in these studies are in the category of reflexive behaviors that occur in lower level organisms in response to changes in environmental conditions. These experiments, conducted on six species whose spectral sensitivity had been determined in the previous study, demonstrated that electrophysiological differences in spectral sensitivity were reflected by differences in behavioral threshold sensitivities to near-UV and blue-green light. The three species with two peaks of electrophysiologically measured spectral sensitivity – *J. spinacauda, O. gracilirostris*, and *S. debilis* – were equally responsive to near-UV and blue-green, while the other three species with a single peak of electrophysiologically measured spectral sensitivity – *Acanthephyra curtirostris, A. smithi*, and *Notostomus elegans* – were a log unit less sensitive to near-UV compared to blue-green light (Table 1).

Table 1 Depth Distribution, Bioluminescence, and Spectral Sensitivity of Deep-sea Crustaceans

Species	*Depth Range (m)*[a]		*Bioluminescence*	*Spectral Sens.*[b] *Maxima (nm)*	*Behavioral Sens.*[c]. *(photons cm^{-2} s^{-1})*	
	Day	*Night*			*400 nm*	*500 nm*
One Putative Visual Pigment						
Acanthephyra curtirostris	500–900	175–599	spew	510	3.4×10^{7}	6.3×10^{6}
Acanthephyra smithi	650–1200	650–1200	spew	510	2.5×10^{7}	4.0×10^{6}
Notostomus gibbosus	800–1200	800–1200	spew	490	4.0×10^{8}	3.8×10^{7}
Notostomus elegans	800–1200	800–1200	spew	490	no data	
Two Putative Visual Pigments						
Janicella spinacauda	500–600	30–250	spew, photophores	390,500	2.3×10^{7}	2.7×10^{7}
Oplophorus gracilirostris	490–650	60–200	spew, photophores	400,500	8.1×10^{6}	6.5×20^{6}
Oplophorus spinosus	490–750	140–375	spew, photophores	400,500	no data	
Systellaspis debilis	600–900	100–300	spew, photophores	400,500	1.9×10^{7}	1.6×10^{7}

[a] Zieman, 1975; [b] Frank and Case, 1988; [c] Frank and Widder, 1994a, b

These results raised questions about the deep-sea visual environment, since it is commonly believed that UV light is virtually absent in the deep-sea. However, there

were no accurate measurements of the deep-sea light regime at wavelengths below 400 nm. Measurements of downwelling irradiance are generally made in the epipelagic zone, and light intensities for deeper depths (>200 m) are calculated using the surface derived attenuation coefficient for that wavelength. However, the optical properties of the ocean change with depth (Clarke and Wertheim, 1956; Clarke and Backus, 1964; Boden and Kampa, 1967; Kampa, 1970), and even slight differences in the diffuse attenuation coefficients can produce significant differences in the estimated available irradiance at great depths. One reason why so little is known about the deep-sea UV–light regime is that there is a lack of appropriate instrumentation for making the necessary measurements at great depths.

We have recently begun making *in situ* measurements of the downwelling irradiance and the spectral distribution of light, using submersible based technology, in an attempt to obtain clues as to the ecological significance of 2 visual pigments in deep-sea crustaceans. Here we present *in situ* irradiance measurements that indicate that UV light penetrates to considerable depths in the ocean. In addition, the intensity of UV light remaining at 600 m (the median daytime depth of the UV sensitive crustaceans) is within the threshold sensitivity of the crustaceans with a UV spectral sensitivity peak. We also provide measurements of the spectral distribution of light at dusk at 150 m depth, which indicate that the spectral shift in light that can be measured at the surface is not visible at this depth.

METHODS

Measurements of downwelling irradiance were conducted in Northwest Providence Channel, Bahamas, in April 1994, with the Low Light Auto-Radiometer (LoLAR). Briefly, this is a PMT-based autocalibrating radiometer, with a sensitivity range of 10^{-2} to 10^{-8} $\mu W\,cm^{-2}$ (Widder *et al.*, 1992). LoLAR was mounted on the *Johnson-Sea-Link* submersible, and iterative *in situ* irradiance measurements were conducted at 2 wavelengths – 380 and 480 nm. These wavelengths were chosen based on the spectral sensitivity peaks of the species in question (see Table 1). Measurements were made on the submersible descent only, as bubbles from the release of ballast on the ascent would have contaminated the data. Data were transmitted from the detector head to a 386SX laptop computer in the front chamber of the submersible, and stored for later analysis.

While *in situ* measurements were being made with the LoLAR, simultaneous measurements of downward irradiance of PAR (400–700 nm) at the surface were made with a Li-COR quantum sensor. These data were used to correct for the effects of cloud cover on the *in situ* irradiance measurements.

In order to determine if a spectral shift occurred in the spectrum of downwelling light at sunset, which might be utilized by vertically migrating crustaceans as a cue to trigger their migrations, measurements of the spectral distribution of light were made at one depth at various times of day. The equipment needed to conduct these measurements was not available on the earlier cruise, and these measurements were conducted off Cape Hatteras in September, 1994. An Ocean Optics Fiber Optic PS-1000 radiometer, optimized for maximum sensitivity (Widder *et al.*, in prep), was used for these measurements. Light was transmitted to the PS1000, operated from the back dive chamber of the *JSL* submersible, via a 1 mm fiber optic through-hull penetrator. Data were stored to a 486 notebook computer for later analysis.

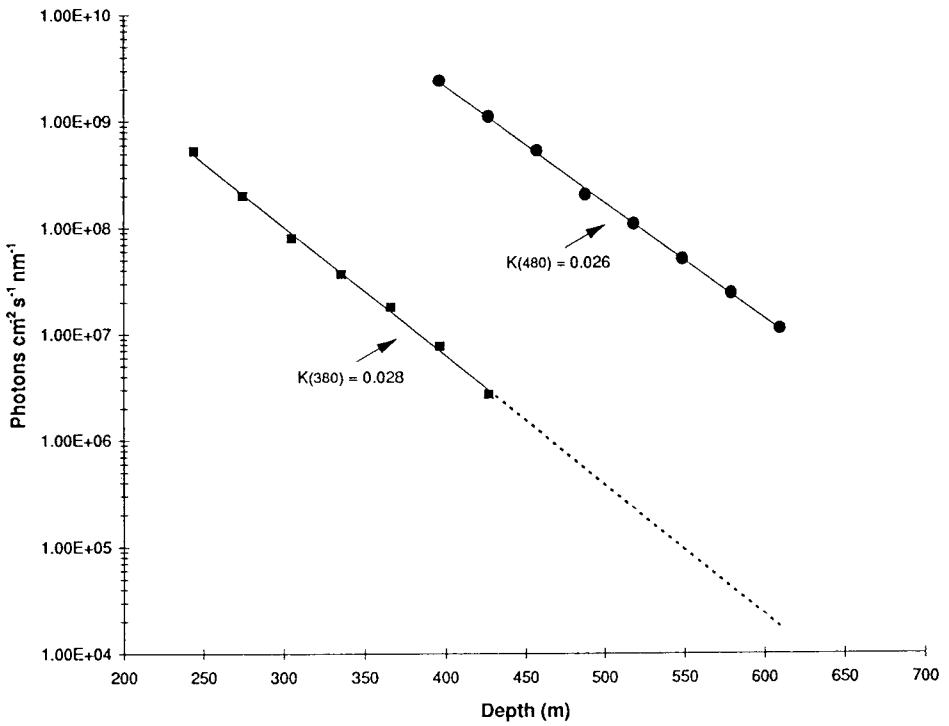

Figure 1 Measurements of downwelling irradiance below 200 m water depth. The 480 nm measurements were started at 9 : 41 – PAR was 1533 μM m^{-2} s^{-1}. The 380 nm data collection was started at 10 : 28 – PAR was 1824 μM m^{-2} s^{-1}. The 480 nm data were corrected to reflect the same surface irradiance as the 380 nm data. Downwelling irradiance attenuation coefficients – K(λ) – are the slopes of the regression lines. Measurements were made in Northwest Providence Channel Bahamas, at 26°31′ N, 78°05′ W, on April 26, 1994.

RESULTS

Measurements of downwelling irradiance in Bahamian waters at 380 and 480 nm are shown in Figure 1. These were taken at depths greater than 200 m, where the water column is optically homogeneous. At this point, it becomes valid to extrapolate the 380 nm measurements down to 500–600 m, the average daytime depth range of the deep-sea crustaceans with UV spectral sensitivity peak. At these depths, the irradiance of 380 nm light is 2.5×10^4–3.5×10^5 photons cm^{-2} s^{-1} nm^{-1}.

The penetration of 380 nm light through the water column, measured on another dive, is shown in Figure 2. As is clear from this graph, the attenuation coefficient does not assume an asymptotic state until approximately 200 m water depth, and attenuation coefficients calculated from measurements taken above vs. below 200 m depth were substantially different. The attenuation coefficient for 380 nm light, measured on three different days, in shallow (75–200 m) water was .0545 ± .0061 (n = 3), while for deep (> 200 m) water, it was .029 ± .0004 (n = 3). A similar comparison of shallow and deep water attenuation coefficients could not be carried out for 480 nm light, because above

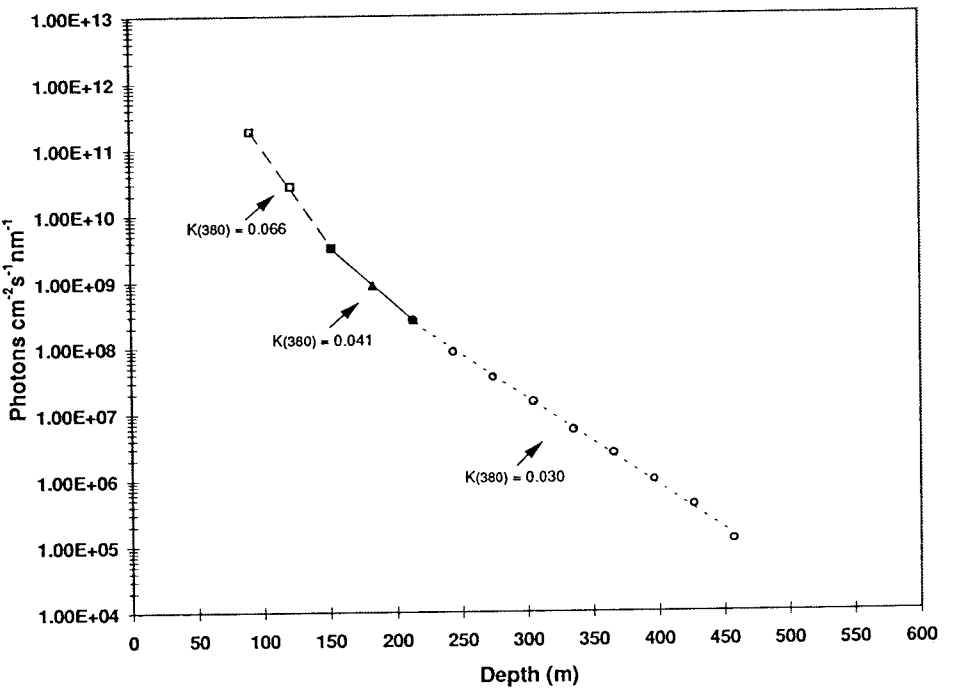

Figure 2 Measurements of downwelling irradiance at 380 nm from 75 to 450 m water depth, in Northwest Providence Channel, Bahamas. Data have been corrected for cloud cover. Data were grouped, and lines calculated, according to an iterative procedure in which data points were added or subtracted until the best fit, according to the F-test, was obtained.

200 m, the irradiance of 480 nm light saturated the LoLAR. For deep water (> 200 m), the attenuation coefficient for 480 nm light was .0263 ± .0007 (n = 3).

The spectral distribution of light was measured in waters off Cape Hatteras at several times approaching sunset. These measurements were taken at 30, 90 and 150 m depth on the same dive, at the times indicated on Figure 3. At 30 m depth (Figure 3A), there is a slight narrowing of the spectrum due to a reduction of long wavelength (yellow-orange) light. This shift is in the same direction, although of a much smaller magnitude, as that reported by McFarland and Munz (1974, 1975) at similar time intervals approaching sunset in surface waters. At 90 m depth (Figure 3B), this shift in the spectrum is barely discernible, and by 150 m depth (Figure 3C), no change in the spectral distribution is apparent.

DISCUSSION

The submersible based deployment of LoLAR and the PS1000 allowed us to avoid the effects of ship shadow on optical data, a well-known problem with "over-the-side" radiometers (Jerlov, 1976; Smith and Baker, 1986; Voss, *et al.*, 1986; Gordon, 1985,

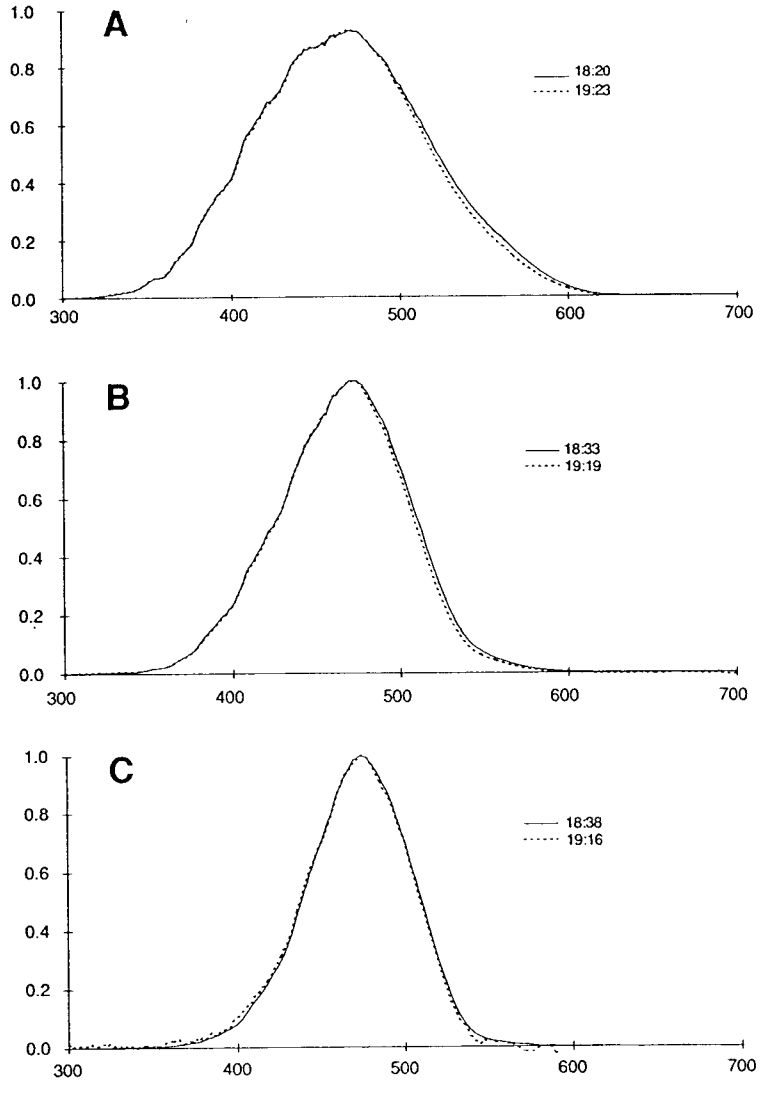

Figure 3 Spectral distribution of light at 3 depths at various times approaching sunset, off Cape Hatteras, at 34° 14′ N, 75° 44′ W, on September 12, 1994. Sunset occurred at 19 : 20. Y-axis is relative spectral irradiance (in photons cm^{-2} s^{-1} nm^{-1}); X-axis is wavelength in nm. A) 30 m depth. B) 90 m depth. C) 150 m depth.

Spinrad and Widder, 1992). In addition, measurements were made below the nonhomogeneous epipelagic zone, making the extrapolation of spectral irradiance to a depth of 600 m a valid estimate of the true photon flux at the two wavelengths measured.

Previous investigators have demonstrated that diffuse attenuation coefficients change with depth (Clark and Wertheim, 1956; Clarke and Backus, 1964; Boden and Kampa, 1967). However, prior to our investigations, the minimum wavelength measured at depths deeper than 200 m depth was 408 nm (Kampa, 1970). Our data for UV light (380 nm) indicate that utilizing attenuation coefficients measured in shallow water will greatly underestimate the true photon flux of UV light at depth. Using our own data, it can be seen that the difference between the mean shallow attenuation coefficient (.054) and deep attenuation coefficient (.029) is substantial. These results indicate that a single attenuation coefficient cannot be used to calculate the photon flux at the shorter wavelengths.

The photon flux of 380 nm light at 500–600 m (the average daytime depth range of the UV sensitive crustaceans – Table 1), extrapolated from the data in Figure 2, is approximately 2.5×10^4–3.5×10^5 photons cm^{-2} s^{-1} nm^{-1}. Our data were taken at a time when the surface water was comparatively "murky", due to high productivity and runoff, and at other times the water is more transparent. Therefore it is likely that the irradiance of 380 nm light is higher at times when there is less particulate matter in the water. Nevertheless, even under these conditions, we estimate that the UV irradiance is sufficient for vision in the crustaceans with a UV spectral sensitivity peak. The LoLAR filter for 380 nm has a full width half maximum bandwidth (FWHM) of 12 nm; therefore the actual values measured were 3×10^5–4×10^6 photons cm^{-2} s^{-1} 12 nm^{-1}. Since a visual pigment absorbs light efficiently over a waveband spanning 100 nm, a crustacean with a behavioral threshold to short wavelength light of 10^6–10^7 photons cm^{-2} s^{-1} (the mean behavioral threshold sensitivities of the crustaceans with UV spectral sensitivity peaks) should be able to detect the presence of UV light at 600 m depth.

One of the hypotheses we generated about the possible function of UV light sensitivity to the crustaceans possessing two peaks of spectral sensitivity is that it may play a role in cueing their vertical migrations. Light is considered to be the most significant factor cueing vertical migrations in most cases, but very little is known about what characteristic of the changing light field triggers the migration (see Forward, 1988 for review). Between noon and dusk, the spectral distribution of underwater light near the surface changes significantly (McFarland and Munz, 1974, 1975; Jerlov, 1976). We suggested that the deep-sea crustaceans with two peaks of spectral sensitivity may be using this spectral shift as a cue to trigger their migrations (Frank and Widder, 1994a). Our data from Cape Hatteras indicate that this is not the case. As seen in Figure 3, a slight change in the spectral distribution of downwelling light is still visible at 30 m depth, but by 150 m depth, this shift is no longer apparent. The water in Cape Hatteras is not as transparent as the water in the Bahamas (Widder *et al.* in prep), but it is unlikely that spectral changes that cannot be discerned at 150 m in Cape Hatteras water would be visible at 500–600 m (the daytime depth of the oplophorids in question) in the clearer Bahamian water. Boden (1961) describes a spectral shift between noon and sunset at 170 m depth for water off San Diego, but this spectral shift was towards longer wavelengths, which is in the opposite direction of both our data and those of McFarland and Munz (1974, 1975) for surface waters. In addition, Boden also describes a spectral shift towards shorter wavelengths at three times bracketing sunset, but a constant depth was not maintained during these measurements. Our measurements indicate that the spectral change that can be measured in shallower waters is not transmitted to the deep-sea in clear oceanic water, indicating that crustaceans living at 600 m depth in these waters must be responding to some other trigger to cue their upward migrations.

The question of the functional significance of a dual visual pigment system in deep-sea crustaceans remains. By monitoring the decrease in spectral bandwidth of downwelling light with increasing depth, such a system might function as a depth gauge, as first suggested by Wald and Rayport (1977), who discovered high near-UV and blue-green light sensitivity in a deep-sea alciopid worm. There is also the possibility that UV sensitivity serves some function related to bioluminescence since all the deep-sea crustaceans with UV spectral sensitivity peaks (that we have studied to date) possess ventrally directed bioluminescent photophores. It is thought that organisms with these photophores may attempt to camouflage their silhouette from predators below them by matching the downwelling illumination with their own bioluminescence (Clarke 1963; Herring 1990). Predators with pigmented lenses, such as the yellow lenses of numerous species of deep-sea fish (Douglas and Thorpe, 1992, for review), would be able to break this camouflage when the match between the downwelling irradiance and bioluminescent irradiance is not very good (Muntz, 1976, 1983). Therefore, since sensitivity to two wavelengths would enable these crustaceans to more closely determine and match, with their own luminescence, downwelling irradiance, dual visual pigments in these species may aid them in avoiding detection by predators with pigmented lenses.

Studies are currently underway to acquire an accurate picture of the spectral distribution and irradiance of light at depth, and how changes in these parameters, which can be replicated in the laboratory, affect the behavior of these crustaceans.

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