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The Red and the Black: Bioluminescence and the Color of Animals in the Deep Sea[1]

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SYNOPSIS. The colors of deep-sea species are generally assumed to be cryptic, but it is not known how cryptic they are and under what conditions. This study measured the color of approximately 70 deep-sea species, both pelagic and benthic, and compared the results with two sets of predictions: 1) optimal crypsis under ambient light, 2) optimal crypsis when viewed by bioluminescent ''searchlights.'' The reflectances of the pelagic species at the blue-green wavelengths important for deep-sea vision were far lower than the predicted reflectances for crypsis under ambient light and closer to the zero reflectance prediction for crypsis under searchlights. This suggests that bioluminescence is more important than ambient light for the visual detection of pelagic species at mesopelagic depths. The reflectances of the benthic species were highly variable and a relatively poor match to the substrates on which they were found. However, estimates of the contrast sensitivity of deep-sea visual systems suggest that even approximate matches may be sufficient for crypsis in visually complex benthic habitats. Body coloration was generally uniform, but many crabs had striking patterns that may serve to disrupt the outlines of their bodies.

INTRODUCTION

In general, animal coloration is extremely variable and serves a multitude of functions including crypsis, thermoregulation, luring, warning, schooling, and sexual signaling (Cott, 1940). In the deep sea, however, coloration is remarkably similar among different species, families, and even phyla. Most mesopelagic species (found at $>$200 m depth) are either red or black (Herring and Roe, 1988) and most deep-sea benthic species range from pale yellow to red (Marshall, 1979). At these depths, it has generally been assumed that pigmentary coloration is almost always cryptic, with the more active uses of color (luring, warning, signaling, etc.) being performed by bioluminescence (McFall-Ngai, 1990; Widder, 1999, 2001).

In contrast to other environments, deep-sea species must be cryptic when viewed under two different forms of illumination: 1) ambient light and 2) bioluminescence. Ambient light at mesopelagic depths consists of dim, nearly monochromatic, and primarily downwelling solar radiation. In contrast, bioluminescence is often significantly brighter and more variable in spectrum and direction (Herring, 1983; Widder *et al.*, 1983). Deep-sea species can be detected under ambient light if the light reflected from them does not match the background. They can be detected by bioluminescence in at least two ways: 1) reflected light from both bioluminescent ''searchlights'' and nearby flashing organisms (Young, 1983), and 2) transmitted light from bioluminescent prey seen through the walls of their own guts (Herring, 1996).

For pelagic species, the ideal cryptic coloration under ambient light differs from the ideal coloration under bioluminescence. Under ambient light, the ideal coloration is determined by the spectrum of both the background light and the light hitting the animal (Johnsen, 2002; Johnsen and Sosik, 2003). For an animal viewed via bioluminescence, whether by a reflected beam or transmission through the gut, the ideal coloration is to reflect/transmit no light at all over the wavelength range of the bioluminescence. For benthic species, the ideal coloration is simply that which matches the background, regardless of the source and spectrum of the illumination.

The importance of bioluminescence relative to crypsis has been discussed by many researchers (*e.g.*, Herring and Roe, 1988; Widder, 1999; Widder and Johnsen, 2000). The coloration of transparent species, the lack of countershading, and the opacity of guts in deep-sea species are all hypothesized to be defenses against detection by bioluminescence. However, despite a myriad of measurements in coastal waters (*e.g.*, Marshall *et al.*, 2003), a survey of the reflectances of deep-sea species has not been performed.

In this study, the spectral reflectances (*i.e.*, color) of 29 mesopelagic and 37 deep-sea benthic species from seven phyla (Chaetognatha, Chordata, Cnidaria, Crustacea, Ctenophora, Echinodermata, Mollusca) were measured. The reflectances were then used to calculate the contrasts of the animals viewed against either the background spacelight or against the most common substrates at the collection sites. Based on these results and published data on visual physiology and bioluminescent spectra, the relative importance of ambient light and bioluminescence for crypsis was analyzed.

MATERIALS AND METHODS

Calculation of optimal reflectance for crypsis

The ideal reflectance for camouflaging a benthic animal that is viewed against the substrate is simply that which matches the reflectance of the substrate (*i.e.*, $R_{animal} = R_{substrate}$). This holds regardless of the source or the spectrum of the illumination.

Crypsis for pelagic species is more complicated be-

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cause the background light can vary independently of the light illuminating the animal (Johnsen, 2002). It also depends on the source of the illumination. If the source is a bioluminescent searchlight, the ideal reflectance depends on the irradiance of the bioluminescence striking the organism relative to the background radiance. Searchlight photophores emit approximately 10^{10}–10^{11} photons/sec, usually over a relatively narrow angle (Mensinger and Case, 1990, 1997). If the searchlight illuminates a 1 cm^2 spot on an animal, the reflected radiance is potentially equal to the background radiance at 200 m depth in extremely clear water at noon ($\sim 10^{10}$ photons/cm^2/sec/sr, Widder and Johnsen, in prep.). This background radiance is essentially an upper bound—at greater depths, lower solar elevations, and more turbid water it will be far less. At night, of course, the background radiance is very low. Therefore, the searchlights are typically far brighter than the background radiance (even if they are used over a larger distance), and the reflectance of the organism must be as low as possible to avoid detection. Ideally, it should be zero over the wavelength range of the searchlight. If the searchlight is used over long distances, or at shallow and bright depths, the ideal reflectance may be greater than zero. However, it will always be less than the predicted reflectance under ambient light alone.

The ideal cryptic reflectance for pelagic species under ambient light depends on the angular structure of the underwater light field. From Johnsen (2002), the reflected light from a diffusely reflective surface L_o matches the background light L_b when:

$$L_o = \frac{RE}{\pi} = L_b, \tag{1}$$

where R is the reflectance of the surface and E is the irradiance striking that surface (note that both depend on wavelength). Therefore, the ideal reflectance for camouflage is:

$$R = \frac{\pi L_b}{E}. \tag{2}$$

In the open ocean, downwelling light is approximately 200 times brighter than upwelling light (Johnsen, 2002). For this reason, the eyes of mesopelagic species generally look upwards or laterally (Land, 1990), making dorsal coloration relatively unimportant. Ventral surfaces of any color appear as silhouettes against the bright overhead light and so are never cryptic (Johnsen, 2002). Thus, the most interesting surfaces from a camouflage perspective are the lateral surfaces, and the most interesting viewing angle is the horizontal one. For this case:

$$R_{cryptic} = \frac{\pi L_h}{E_b}, \tag{3}$$

where $R_{cryptic}$ is the ideal reflectance of the lateral surface of the animal, L_h is the horizontal radiance behind the animal and E_h is the horizontal irradiance hitting the surface of the animal.

The horizontal radiances and irradiances were calculated from measured optical properties of the water using radiative transfer software (Hydrolight 4.2, Sequoia Scientific Inc., Bellevue, Wash.). Given the depth profiles of the absorption and scattering coefficients, the software calculates the underwater radiance distribution as a function of depth and wavelength (from 350–700 nm), taking into account solar elevation and azimuth, atmospheric parameters, sea surface conditions, and Raman scattering by the water. The accuracy of the calculations has been validated by *in situ* measurements of selected radiances and irradiances in numerous studies (*e.g.*, Mobley *et al.*, 1993; Maffione *et al.*, 1998; Stramska *et al.*, 2000; Johnsen, 2002; Johnsen and Sosik, 2003). The agreement between modeled and measured radiances is particularly good in oceanic waters, because the vast majority of the light attenuation is due to the water itself, which is easily characterized and well understood. Since they depend on the relative radiance distribution, and not absolute intensity, the predicted reflectances are particularly robust, depending primarily on absorption in the water and hardly at all on the atmospheric and surface conditions.

Depth profiles of the absorption and scattering coefficients in clear, oceanic water (Jerlov type I) were obtained from Drs. Andrew Barnard, Scott Pegau and Ronald Zaneveld (College of Oceanic and Atmospheric Sciences, Oregon State University, Corvallis, Oregon, USA), who collected them using a dual path, multiband absorption/attenuation meter (ac-9, Wetlabs Inc., Philomath, OR) in the Equatorial Pacific Ocean (10:05 A.M. local time, 30 April, 1996; 0°0′N 177°21′W). Absorption and beam attenuation coefficients at eight wavelengths (412, 440, 488, 510, 532, 555, 650, and 676 nm) were measured at 1 m intervals to a depth of 138 m. Measurements were corrected for temperature and salinity, and absorption measurements were corrected for scattering errors (Zaneveld *et al.*, 1994; Pegau *et al.*, 1997).

Using this profile, underwater radiance distributions were calculated from 0 to 450 m depth at 50 m intervals (the measured coefficients at 138 m were used for all deeper depths). The sun was assumed to be at the zenith, the sky was assumed to be cloudless, and the sea was assumed to be calm. The sky irradiance was calculated using the Radtran model (Gregg and Carder, 1990) and the sky radiance distribution was calculated using the model given in Harrison and Coombes (1988). Pure water absorption was taken from Pope and Fry (1997). Petzold's average particle was used for the scattering phase function (Mobley *et al.*, 2002). At each depth, radiance was calculated from 400–570 nm at 5 nm intervals with an angular resolution of 15° (azimuth) by 10° (elevation). The horizontal irradiance was calculated from the radiance distribution. This irradiance and the radiance in the opposite direction were then inserted into equation (3) to calculate the ideal reflectance. Because the spectra at mesopelagic depths are quite narrow, light at wavelengths longer or shorter than the wavelength of peak transmission contributes very little to visibility. This was taken into consideration by calculating only the ideal reflectances over the wavelengths at which the intensity was at least 5% of the peak intensity at that depth. The total light outside these ranges was less that 4% of the total. Its contribution to vision is even less since the deep-sea visual pigments have little sensitivity at these short and long wavelengths.

Unfortunately measurements of absorption and scattering coefficients at mesopelagic depths in oceanic waters ($>$200 m) do not exist. Because oceanic water tends to get clearer with increasing depth, the predictions based on the profile used, which only goes down to 138 m, may not be entirely accurate. Therefore, a second set of calculations was performed using absorption and scattering coefficients from the clearest known oceanic waters—the Sargasso Sea (taken from Smith and Baker, 1981). Since the clarity of mesopelagic waters is between that found at 138 m and that found in the clearest oceanic waters, the true predicted reflectance is bounded by the predictions of these two sets of calculations.

Animal collection

With the exception of the shrimp *Systellaspis debilis*, all mesopelagic species were obtained from Oceanographer Canyon (48°19′N 68°08′W, on the southern edge of Georges Bank) and Wilkinson Basin (42°30′N 69°32′W, in the Gulf of Maine) during two cruises of the R. V. *Seward Johnson I* (June 2000; June 2001). Approximately one-third of the species were collected at mesopelagic depths with the *Johnson Sea-Link* research submersible using 11-liter plexiglass cylinders with hydraulically activated, sliding lids. The remaining species were collected using an opening/closing Tucker trawl (4.3 m^2 opening, ¼ inch knotless nylon mesh) fitted with a thermally insulated collecting container that could be closed at depth. Benthic species were obtained from several deep-sea *Lophelia* reefs, brine pools, and chemosynthetic sites in the northern Gulf of Mexico and the Gulf Stream region of the South Atlantic Bight during two cruises of the R. V. *Seward Johnson II* (August 2002, August 2004). These benthic species were collected at depths ranging from 250 to 650 m using the *Johnson Sea-Link*'s robot arm and suction sampler. The oplophorid shrimp *Systellaspis debilis* was also collected during the 2002 cruise using the trawl net described above. Figures 1 and 2 show the pelagic and benthic species collected. Both benthic and pelagic specimens were maintained in cold seawater (collected at depth) and measured within an hour of collection.

Reflectance measurements

The spectral reflectances of the collected specimens and of mud, coral rubble, and sand from the collection sites (from 300 to 700 nm) were measured using a fiber optic reflectance probe (R400-7 reflection probe, Ocean Optics. Inc., Dunedin, FL) coupled with a pulsed xenon source (PX-2, Ocean Optics) and a multichannel spectrometer (USB2000, Ocean Optics) (Fig. 3). The reflectance probe contained seven 400 μm diameter optical fibers in a six-around-one arrangement (Fig. 3, inset A). The six outer fibers were coupled to the light source and illuminated the specimen. The central fiber collected the light reflected from the specimen and was coupled to the spectrometer. The end of the reflectance probe was always placed at a distance of 3 mm from the measured surface and held at an angle of 45° to the surface using a rigid optical mount (not shown). Therefore, the probe measured back-reflection from an object illuminated at an angle of 45°. Because the angle of collection did not equal the angle of incident light, this arrangement did not collect the light that is specularly reflected from the shiny, wet surface of the specimen. Instead it measured the diffuse reflectance, which is relatively independent of the angles of illumination and measurement (Palmer, 1995). All nonopaque specimens were placed on a filter that absorbed all visible light (Melles-Griot Inc.) to eliminate reflected light from the surface underneath the specimen. The reflectance measurements were calibrated using a Spectralon™, plastic standard that reflects nearly 100% of the light at all wavelengths from 200 to 800 nm (WS-1 Diffuse Reflection Standard, Ocean Optics).

For logistical reasons, all measurements were performed in air. Reflectance measurements of wet surfaces in air were then converted into submerged reflectances using Duntley's formula that relates the two:

$$R_{submerged} = \frac{R_{wet}}{0.42R_{wet} + 0.564}, \tag{4}$$

where R_{wet} is the measured reflectance of the wet surface and $R_{submerged}$ is the actual reflectance of the object when viewed underwater (Duntley, 1952). Equation (4) assumes that the angle of incidence is 45° and the angle of measurement is 90°, which is quite close to the experimental conditions (angle of incidence and measurement both equal 45°), particularly for diffuse reflectance, which is relatively angle-independent.

The lateral surfaces of the pelagic species and the dorsal surfaces of the benthic species were measured. In the cnidarians, chaetognaths, and ctenophores, the opaque gut wall was the surface measured. With the exception of three shrimp (*Meganyctiphanes norvegica*, *Nematoscelis megalops*, and *Pasiphaea multidentata*), all the measured surfaces were opqaue. A total of 125 pelagic and 85 benthic specimens were measured from 29 and 37 species respectively. Pelagic species were from the Chaetognatha, Cnidaria, Crustacea, Ctenophora, and Mollusca, and benthic species were from the Chordata, Crustacea, Mollusca, and Echinodermata.

FIG. 1. The pelagic species collected and measured. The image of *Meningodora sp.* is a colored figure; the rest are photographs. Images are courtesy of E. Widder (HBOI), T. Frank (HBOI), S. Haddock (MBARI), and K. Raskoff (MBARI).

Calculation of the contrast of the measured species

The contrast of an object is its incremental radiance relative to the background, or:

$$C = \frac{L_o - L_b}{L_b} \tag{5}$$

In the case of the lateral surface of a pelagic animal, $L_o = RE_h/\pi$ and $L_b = L_h$. So:

$$C = \frac{\frac{RE_h}{\pi} - L_h}{L_h} = \frac{RE_h}{\pi L_h} - 1 = \frac{R}{R_{cryptic}} - 1, \tag{6}$$

since $R_{cryptic} = \pi L_h/E_h$. In the case of the dorsal surface of a benthic animal, $L_o = RE_d/\pi$ and $L_b = R_sE_d/\pi$, where R_s is the reflectance of the substrate. Substituting in (5) gives:

$$C = \frac{\frac{RE_h}{\pi} - \frac{R_sE_h}{\pi}}{\frac{R_sE_h}{\pi}} = \frac{R}{R_s} - 1. \tag{7}$$

So, given the measured reflectance and the predicted reflectance, one can calculate the contrasts of the collected species in the water. Similarly, given the measured reflectance and the reflectance of the substrate, one can determine the contrasts of the collected benthic species.

RESULTS

Predicted reflectance for pelagic crypsis

The predicted reflectance for pelagic camouflage ranged from 20% to 40% in the Equatorial Pacific water and from 40% to 60% in the clearest known oceanic waters (Fig. 4a). In both water types, the predicted reflectance decreased with depth, with this effect more pronounced in the Equatorial Pacific water. The predicted reflectance in the Equatorial Pacific water decreased with increasing wavelength, with a shoulder at approximately 480 nm. In the clearest water, the predicted reflectance decreased with increasing wavelength, with a slight increase at the longest wavelengths.

FIG. 2. The benthic species collected and measured. The images are of the actual specimen measured (with the exceptions of *Stenorhynchus seticornis* and *Ophiacantha* sp.). Certain images are courtesy of E. Heine (HBOI).

The predicted reflectance for benthic camouflage (which equals the reflectance of the substrates) ranged from 20% to 40% for a sand substrate and from 15% to 30% for a coral rubble substrate, and from 6% to 12% for a mud substrate (Fig. 4b). For all three substrates, the reflectance increased with increasing wavelength.

Measured reflectance of pelagic species

The reflectance of the pelagic species was generally less than 20% at all wavelengths and less than 5–10% at the blue and green wavelengths relevant for deep-sea vision (Fig. 5). While the variation in reflectance among species at blue-green wavelengths was quite low (standard deviation equaled ~1% from 440–500

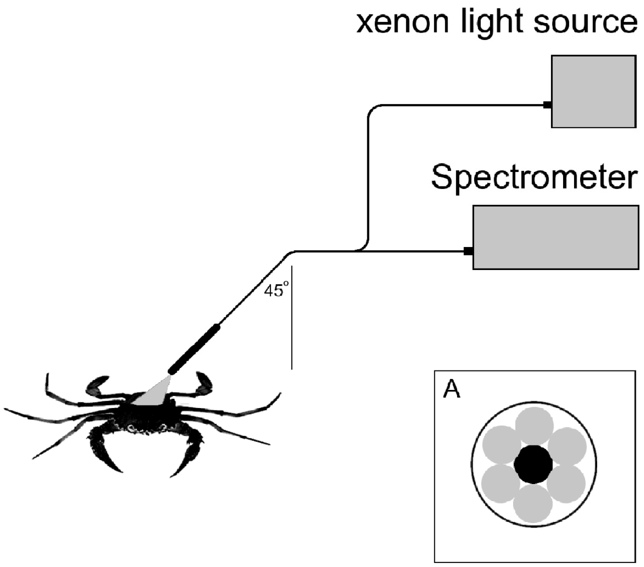

FIG. 3. Apparatus for measuring reflectance. Thick black lines are fiber optic cables. a) Cross-section of reflectance probe consisting of six illumination fibers (gray) surrounding one measurement fiber (black).

nm), there was considerable variation at both longer and shorter wavelengths, with many ‘‘red’’ surfaces having a secondary peak at ultraviolet wavelengths. Among the crustaceans, all three oplophorid shrimp (*Acanthephyra pelagica*, *Meningodora sp.*, *Systellaspis debilis*), the benthesicymid shrimp *Gennadas sp.*, and the mysid shrimp *Gnathophausia zoea* had extremely low reflectances at blue-green wavelengths (<0.5%) with much higher reflectances at longer wavelengths (Fig. 5a, c). The sergestid shrimp *Sergestes arcticus*, the isopod *Anuropus sp.*, and the unidentified copepod followed a similar pattern, but had slightly higher reflectances at blue-green wavelengths (~3%) (Fig. 5c). The two euphausiid shrimps (*Meganyctiphanes norvegica*, *Nematoscelis megalops*), the pasiphaeid shrimp, *Pasiphaea multidentata*, and the hyperiid amphipod *Themisto compressa* were translucent and had low and spectrally flat reflectances averaging ~3% (Fig. 5b, d).

The reflectances of the guts of the cnidarians, the ctenophore *Ctenoceros*, and the chaetognath *Eukrohnia fowleri* also generally were minimal at blue-green wavelengths (~2%), sometimes with substantially higher reflectances at longer and shorter wavelengths (Fig. 5e–g, i). Peaks at ultraviolet wavelengths were more pronounced and common among these species than among the crustaceans. There was little systematic variation between the major clades, though the highest long-wavelength reflectances were found among the hydromedusae and the blue-green reflectances of the guts of the two scyphozoans (*Atolla vanhoeffeni*, *Periphylla periphylla*) were lower than the average (~1%) (Fig. 5g). The body walls of the two gymnosomatous pteropods (*Clione limacina*, *Cliopsis krohni*) had reflectances of about 3% at blue-green wavelengths, with slightly higher reflectance at longer wavelengths (Fig. 5h).

Measured reflectance of benthic species

The reflectances of the benthic species were both substantially higher and more variable than those of the pelagic species (Fig. 6). Approximately half the species had a dip in reflectance at blue-green wavelengths. The galatheid crabs had particularly high reflectances, relatively spectrally neutral on the small carapaces, and quite red on the large legs (Fig. 6a, b). The carapaces of the non-galatheid crabs had lower reflectances (Fig. 6c). The legs of these crabs often had two colors, one with a spectrally neutral reflectance, and one with very low reflectance at blue-green wavelengths (Fig. 6d, e). The asteroids had high reflectances, with generally higher reflectance at longer

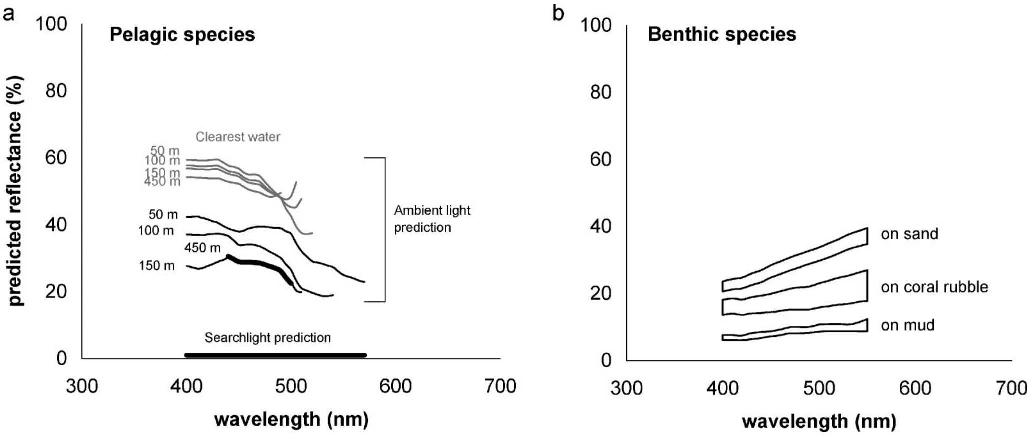

FIG. 4. Predicted reflectances for ideal crypsis. a) Predicted reflectances for the lateral surfaces of pelagic animals as a function of depth and wavelength. The black lines denote the predictions based on data from the Equatorial Pacific Ocean. The grey lines denote the predictions based on data from the clearest known natural waters (from Smith and Baker, 1981). The thick line at 0 denotes the prediction for crypsis when viewed by bioluminescent searchlights. b) Predicted reflectances for the dorsal surfaces of benthic animals viewed on three different substrates. The lines bound the 95% confidence intervals.

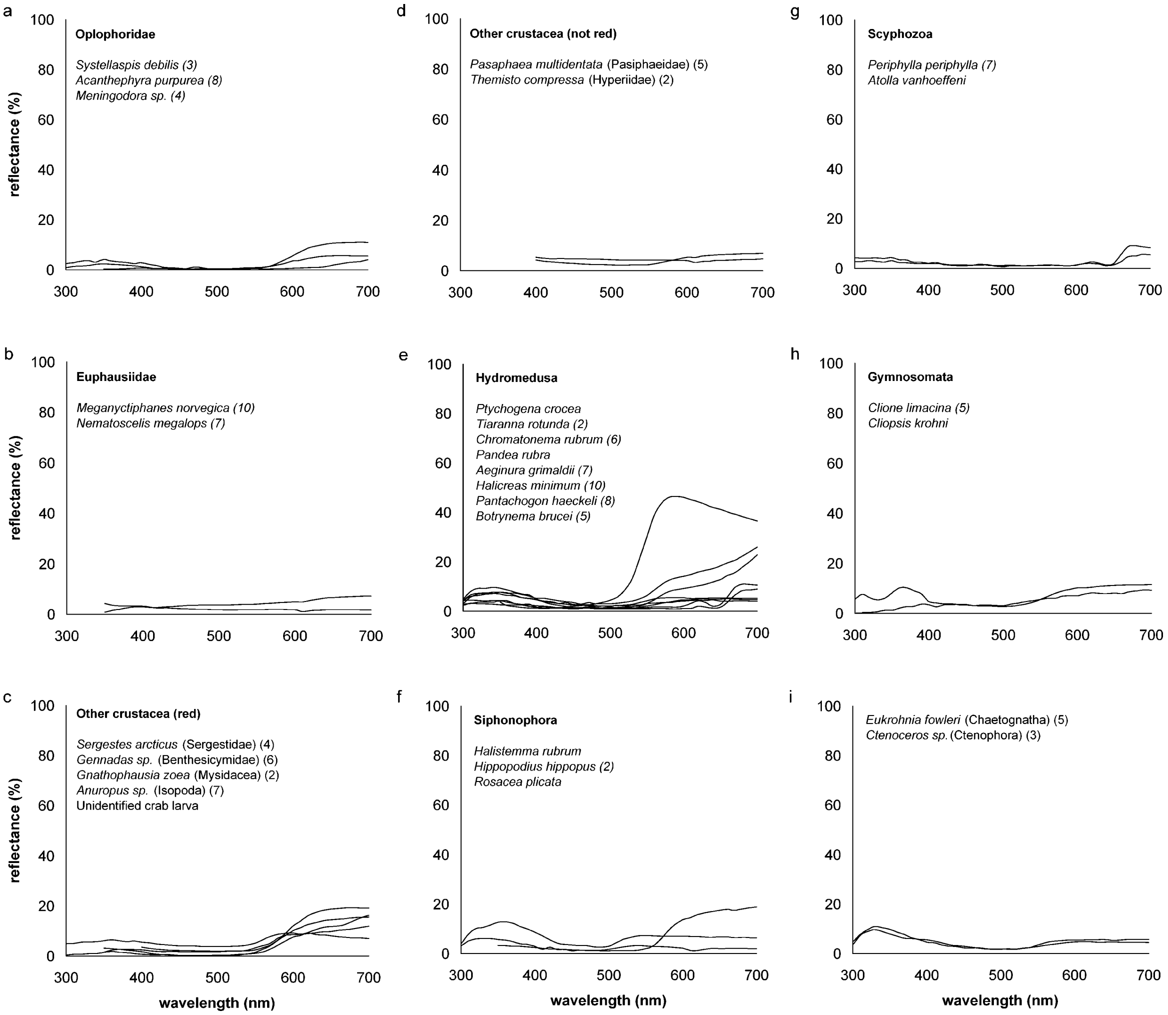

FIG. 5. Measured reflectances of the lateral surfaces of mesopelagic species. a)–d) Crustaceans. e)–g) The gut walls of cnidarians. h) Gymnosomatous pteropods. i) One ctenophore and one chaetognath. The species listed in each graph are in order of the reflectances at 700 nm. *i.e.,* the first species listed has the highest reflectance at 700 nm. Numbers in parentheses show the number of specimens measured (if greater than 1).

wavelengths (Fig 6f). The two asteroids (*Lophaster verilli*, *Coronaster briareus*) had significant dips in reflectance at blue-green wavelengths. The echinoids, ophiuroids and gastropods had slightly lower reflectances with no dips at blue-green wavelengths (Fig. 6g, h). With the exception of *Nezumia sp.,* all of the fish had lower reflectance in the blue-green (Fig. 6i). *Nezumia sp.* was the only measured deep-sea species with an inverse relationship between reflectance and wavelength.

Contrasts of pelagic and benthic species

The contrasts of the pelagic species when viewed under ambient light were all negative and close to the theoretical minimum of −1 (*i.e.,* black silhouette) (Fig 7a). The variation in contrast was quite small, with a standard deviation always less than 0.05. The contrasts of the benthic species were quite variable, with standard deviations usually ranging from 0.5 to 1. On average, the species were generally lighter than the mud and the coral rubble and approximately matched the brightness of the sand. (Fig. 7b, c, d). In certain cases, the contrast was quite high, especially at ultraviolet wavelengths. The contrast at blue-green wavelengths was generally closer to zero than at other wavelengths, but the effect was not strong.

DISCUSSION

The relative importance of downwelling light vs. bioluminescence for pelagic species

The reflectances of the pelagic species were all far lower than even the lowest predicted reflectances for crypsis under ambient light (*i.e.,* those at 450 m in the Equatorial Pacific). Indeed, they were so much lower that all the measured species would appear as silhouettes against the background horizontal light. The reflectances of the oplophorid shrimp and several other crustaceans were a particularly bad fit to the predicted reflectances, being too low by approximately two orders of magnitude. Even the highest measured reflec-

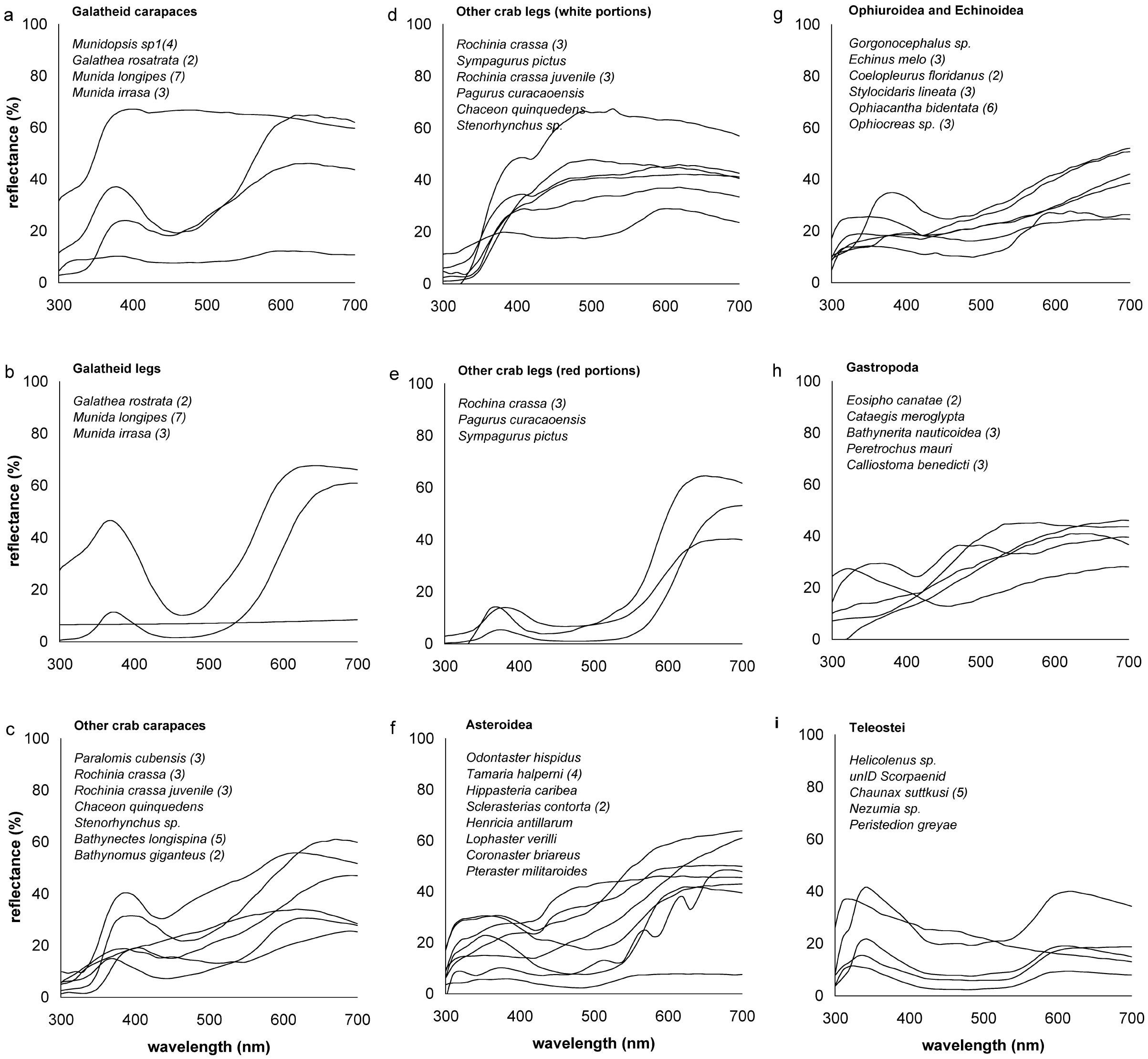

FIG. 6. Measured reflectances for the dorsal surfaces of deep-sea benthic species. a)–e) Crab carapaces and legs. f)–g) Echinoderms. h) Gastropods. i) Fish. See Figure 4 caption for further details.

tances at blue-green wavelength were five times too low.

Although the reflectances are a poor fit for crypsis in ambient light, their uniformity (in contrast to the reflectances of the benthic species), suggests they are advantageous for another purpose. Indeed, the reflectances more closely matched the prediction for crypsis against bioluminescence (*i.e.*, 0% reflectance).

Because the intensity of the bioluminescence striking an animal is highly variable (depending on distance, photophore intensity, and light collimation), generally applicable contrasts cannot be calculated. In addition, the contrast of an illuminated object on a black background does not decrease with distance. This property, due to the lack of veiling light, makes contrast a less useful concept in this case (see Warrant and Locket, 1994 for the influence of this on deep-sea visual systems). Broadly speaking however, the visibility of an animal viewed by bioluminescence depends on at least four factors: 1) the spectrum and intensity of the light striking the animal, 2) the reflectance of the animal, 3) the spectral sensitivity of the viewer, and 4) the transmission properties of the water. Figure 8a shows a summary of all four as a function of wavelength, with the transmission properties of the water depicted as detection distances (which are proportional to $1/c$, where c is the beam attenuation coefficient). These detection distances are maximal at 490 nm, the same wavelength at which most bioluminescent emission peaks and visual pigment absorption maxima are found. The presence of the minimum reflectance at this same wavelength strongly suggests that there is an adaptive significance of mesopelagic coloration, despite its mismatch with the predictions for crypsis under ambient light.

The importance of bioluminescence relative to am-

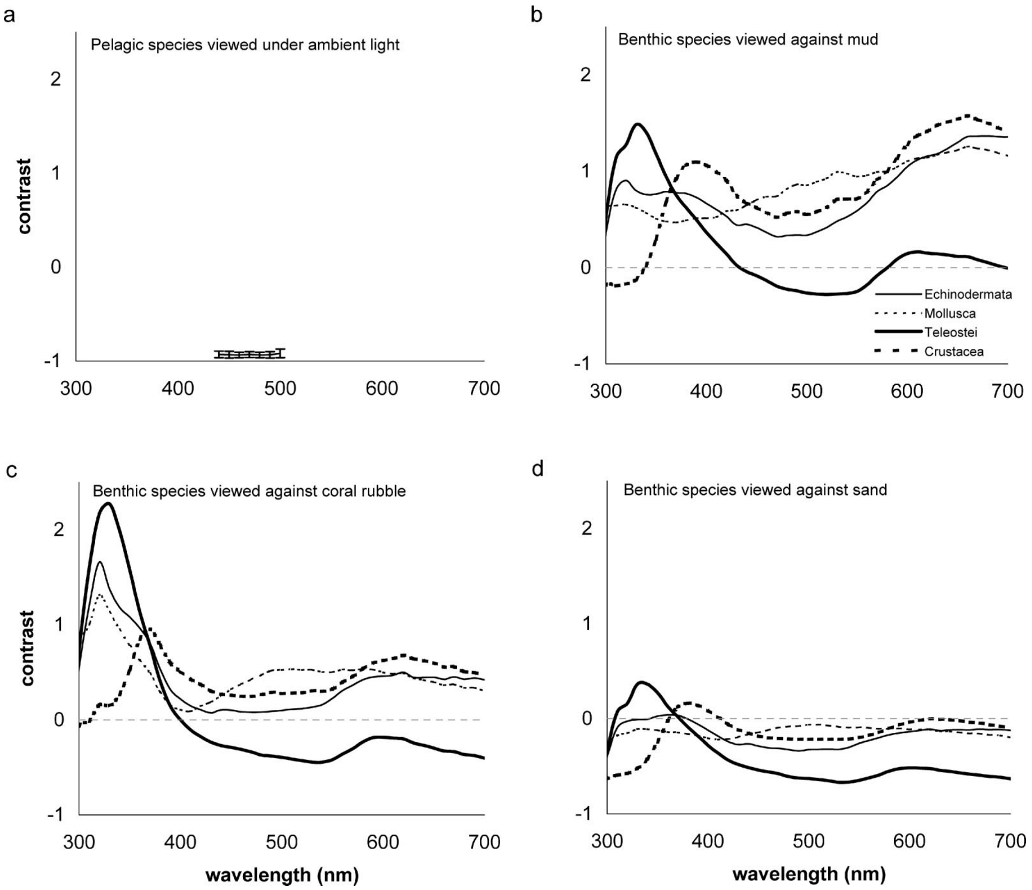

FIG. 7. The contrasts of pelagic and benthic species. a) The average contrast (± standard deviation) of the lateral surfaces of the measured pelagic species when viewed horizontally at 450 m. b) The contrasts of the dorsal surfaces of the measured benthic species when viewed against the mud substrate. c) The contrasts of the benthic species when viewed against the coral rubble substrate. d) The contrasts of the benthic species when viewed against the sand substrate.

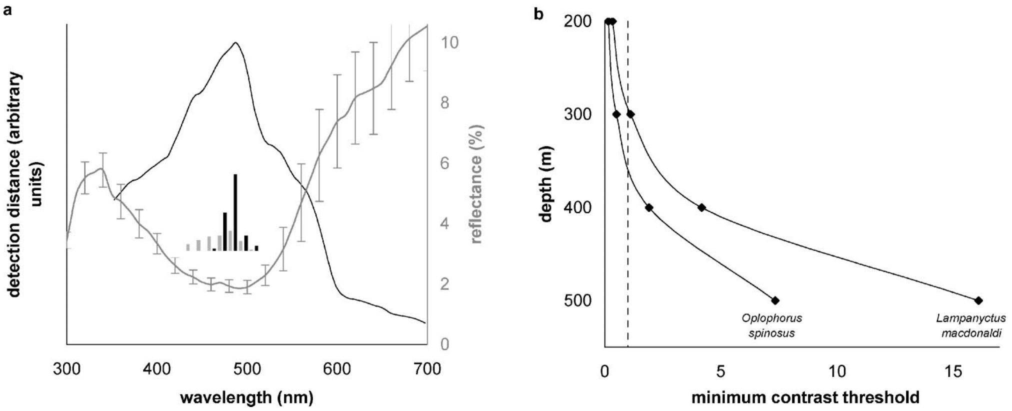

FIG. 8. a) Factors affecting crypsis when viewed by bioluminescent searchlights. Black line indicates transmissibility of light through the water. Gray line denotes average reflectance (± standard error) of the 29 pelagic species. The grey bar chart is a histogram of the wavelengths of peak emission from non-counterilluminating photophores (n = 124) (based on data from Nicol, 1960; Swift *et al.*, 1973, 1977; Biggley *et al.*, 1981; Herring, 1983; Denton *et al.*, 1985; Widder *et al.*, 1983; Herring *et al.*, 1992, 1993; and Haddock and Case, 1999). The black bar chart is a histogram of the wavelengths of maximal absorption of known visual pigments in deep-sea fish and crustaceans (n = 203) (based on data from Douglas *et al.*, 1998; Marshall *et al.*, 1999). b) Estimate of minimum contrast threshold as a function of depth for two mesopelagic species. The vertical, dashed line denotes a contrast threshold of one.

TABLE 1. *The visual parameters used to estimate minimum contrast threshold. The upper and lower citations for each parameter refer to Lampanyctus macdonaldi and Oplophorus spinosus respectively.*

	Lampanyctus macdonaldi	Oplophorus spinosus	Source
spatial resolution	0.5°	8°	Collin and Partridge (1996) Land (1976)
pupil diameter	0.25 cm	0.1 cm	Wagner *et al.* (1998) Land (1976)
integration time	1.5 s	0.16 s	Aho *et al.* (1988, 1993)[a] Moeller and Case (1994, 1995)[c]
quantum efficiency	0.34	0.5	Aho *et al.* (1988, 1993)[a] Warrant (1999)[b]
transmission of optics	0.95	0.8	Aho *et al.* (1988, 1993)[a]
absorbance of visual pigment	0.064 μm^{-1}	0.01 μm^{-1}	Warrant and Nilsson (1998)[c] Warrant and Nilsson (1998)[c]
wavelength of peak absorbance	490 nm	490 nm	Douglas *et al.* (1998) Cronin and Frank (1996)
length of photoreceptor	50 μm	200 μm	Wagner *et al.* (1998)[d] Land (1976)

[a] for nocturnal toads.
[b] for insects.
[c] average for deep-sea fish and crustaceans.
[d] based on values for similarly sized fishes from the same depth habitat.

bient light for mesopelagic crypsis has been discussed by other workers (Nicol, 1958; Herring and Roe, 1988; Widder, 1999). Indeed, the presence of bioluminescence explains several otherwise paradoxical characteristics of mesopelagic body coloration. First, it explains why the members of most transparent pelagic taxa become red or black at greater depths, since Johnsen (2003) showed that the reflections of a beam of light from the outer surface of a transparent tissue can be greater than the reflections from an opaque, colored tissue. It also explains why the coloration of deep-sea species is essentially uniform rather than countershaded, since bioluminescent searchlights may strike the animal from any angle. As previously discussed by many researchers (*e.g.,* Herring, 1996), it also explains why the guts of transparent species that eat bioluminescent prey have such low reflectances.

The contrast and visibility of benthic species

With a few exceptions (mostly against the relatively rare mud substrate) contrasts of the benthic species ranged from −1 to 1. The contrast cannot drop below −1 (black object), but it can be greater than 1 if the animal is brighter than the background. The fact that the absolute values of the contrasts were frequently lower than 1 suggests the possibility that this is adaptive and that the potential viewers of these species cannot detect these levels.

Unfortunately, minimum contrast thresholds ($C_{\min}$) have not been determined for any deep-sea species. However, they can be estimated using methods developed by Warrant (1999). From Warrant and Locket (2004), eyes can detect a photon difference equal to $1.96\sqrt{2N_0}$, where N_0 is the number of photons absorbed by a photoreceptive field over a given time interval. From this:

$$C_{\min} = \frac{N_0 + 1.96\sqrt{2N_0} - N_0}{N_0} = \frac{2.77}{\sqrt{N_0}}, \qquad (8)$$

where:

$$N_0 = 2.7 \times 10^{-4} \cdot \Delta\rho^2 D^2 \Delta t \times \int \kappa\tau[1 - e^{-kR(\lambda)l}]L_b(\lambda)\, d\lambda, \qquad (9)$$

where $\Delta\rho$ is the spatial resolution of the eye (in degrees), D is the pupil diameter, Δt is the integration time, κ is the quantum efficiency of transduction, τ is the fractional transmission of the optics, k is the absorption coefficient of the photoreceptor, $R(\lambda)$ is the visual pigment absorbance, and l is the length of the photoreceptor (Warrant and Nilsson, 1998; Warrant, 1999). $L_b(\lambda)$ is the background quantal radiance, which in this case equals $E_d R/\pi$, where E_d is the downwelling irradiance and R is the reflectance of the substrate.

Figure 8b shows the minimum contrast thresholds calculated from measured values and reasonable estimates for the visual parameters of the mesopelagic fish *Lampanyctus macdonaldi* and the shrimp *Oplophorus spinosus* (Table 1) and measurements of downwelling irradiance in the South Atlantic Bight (Widder and Johnsen, in prep.). Using these parameters, the minimum contrast threshold is greater than 1 at depths greater than 300 m (Fig. 8b). The minimum contrast threshold is inversely proportional to the spatial resolution $\Delta\rho$ and inversely proportional to the square root of the integration time Δt. Therefore, either species can improve its contrast sensitivity by increasing spatial summation or greatly increasing temporal summation (see Warrant, 1999 for details). For example, the myctophid fish *Lampanyctus macdonaldi* can lower its minimum contrast threshold from 4 at 400 m depth to 1 by either increasing its spatial resolution from 0.5°

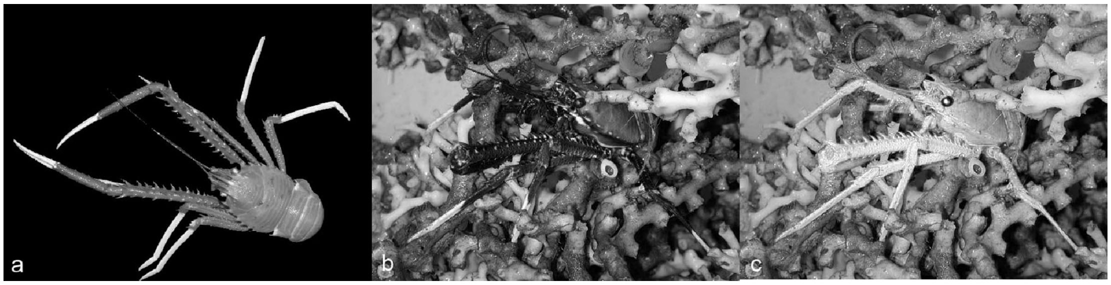

FIG. 9. a) The red and white galatheid crab *Munida longipes*. b) The same crab in *Lophelia* rubble viewed at blue and green wavelengths. c) Same image as b), but viewed at red wavelengths (where the red and white portions of the legs have the same reflectance). Note that the variable reflectance of the legs at blue-green wavelengths makes the animal harder to detect against the *Lophelia*.

to 2° or increasing its integration time from 1.5 sec to 24 sec. These increases, however, greatly diminish the animal's ability to find small and/or moving animals. Thus, while these estimates are necessarily approximate, they do suggest that contrast vision under ambient light at depth is difficult and that a contrast less than 1 may be a sufficient condition for crypsis in many cases.

The case may be different for directed bioluminescence however. As mentioned above, the light reflected from searchlight photophores can be as bright as or brighter than the background radiance at 200 m depth (Mensinger and Case, 1990, 1997). At these light levels, the minimum contrast threshold is considerably less than 1 (0.15 and 0.34) for the two species. The requirements that the beam be both narrow and close to the animal, however, imply that the spot size must be quite small. Also, unlike the pelagic case, where any reflection implies the presence of some object, a searchlight aimed at the seafloor will always be reflected, making object detection and identification more difficult. For this reason, bioluminescent searchlights may be less useful for benthic species.

Distribution of coloration

The distribution of coloration over the bodies of the measured species was generally unremarkable. The cnidarians were generally transparent with opaque, colored guts, and most of the remaining species were uniformly colored. However, there were a few exceptions. The fishes *Chaunax suttkusi* and *Helicolenus* sp., the urchin *Coelopleurus floridanus*, and the starfish *Tamaria halperni* all had mottled patterns with two primary colors. The reflectances of the two colors of these species differed significantly at blue-green wavelengths and so are potentially distinguishable by the monochromatic visual systems found in deep-sea organisms. However, given the coarse resolution of these visual systems, the viewer would have to be quite close to the animal to detect these patterns.

The most interesting color patterns were found in the benthic crustaceans (Fig. 1). Many of these were bi-colored, one color having a high blue-green reflectance and the other having a low reflectance. It is possible that this is some form of disruptive coloration (in which the body outline is broken by variable coloration) (Fig 9). While some of the collection sites were featureless plains of sand, most of the crustaceans were found in more complex habitats, where disruptive coloration would be highly advantageous. Other functions, perhaps involving species recognition or mate choice, are possible but currently speculative.

Conclusions

The measured reflectances of the pelagic species at the blue-green wavelengths important for deep-sea vision are far lower than the estimated reflectances required for crypsis under ambient light. This suggests that directed bioluminescence and the transmission of bioluminescence through gut walls are more important than ambient light for detecting animals at mesopelagic depths. These results are consistent with those of other researchers showing the importance of bioluminescence at depth. The reflectances of the benthic species were highly variable and a relatively poor match to the substrates on which they were found. However, estimates of the contrast sensitivity at these low light levels suggest that a poor match may be sufficient for crypsis in the visually complex benthic habitat. In general, body coloration was uniform. However, many crustaceans had striking color patterns with unknown significance.

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