The Journal of Experimental Biology 215, 3344-3353

doi:10.1242/jeb.072033

RESEARCH ARTICLE

Light and vision in the deep-sea benthos: II. Vision in deep-sea crustaceans

Tamara M. Frank[1,*], Sönke Johnsen[2] and Thomas W. Cronin[3]

[1]Oceanographic Center, Nova Southeastern University, Dania Beach, FL 33004, USA, [2]Biology Department, Duke University, Durham, NC 27708, USA and [3]Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21250, USA

*Author for correspondence (tfrank1@nova.edu)

SUMMARY

Using new collecting techniques with the *Johnson-Sea-Link* submersible, eight species of deep-sea benthic crustaceans were collected with intact visual systems. Their spectral sensitivities and temporal resolutions were determined shipboard using electroretinography. Useable spectral sensitivity data were obtained from seven species, and in the dark-adapted eyes, the spectral sensitivity peaks were in the blue region of the visible spectrum, ranging from 470 to 497 nm. Under blue chromatic adaptation, a secondary sensitivity peak in the UV portion of the spectrum appeared for two species of anomuran crabs: *Eumunida picta* (λ_{max}=363 nm) and *Gastroptychus spinifer* (λ_{max}=383 nm). Wavelength-specific differences in response waveforms under blue chromatic adaptation in these two species suggest that two populations of photoreceptor cells are present. Temporal resolution was determined in all eight species using the maximum critical flicker frequency (CFF_{max}). The CFF_{max} for the isopod *Booralana tricarinata* of 4 Hz proved to be the lowest ever measured using this technique, and suggests that this species is not able to track even slow-moving prey. Both the putative dual visual pigment system in the crabs and the extremely slow eye of the isopod may be adaptations for seeing bioluminescence in the benthic environment.

Key words: vision, deep-sea, benthic, bioluminescence.

Received 1 March 2012; Accepted 18 June 2012

INTRODUCTION

The large eyes of deep-sea animals have been remarked upon since the first specimens were pulled up in trawl nets. In addition to large eyes, early structural studies on mesopelagic species (i.e. those living in the water column) demonstrated further adaptations for increasing sensitivity to the dim environmental light field, including larger apertures, longer photoreceptor cells, reductions in screening pigments and the presence of reflecting tapeta (reviewed in Warrant and Locket, 2004). Studies on visual pigment absorption maxima from mesopelagic species have shown a shift in peak sensitivity to shorter, 'bluer' wavelengths compared with those of shallow-water species [e.g. fish (reviewed in Douglas et al., 2003; Turner et al., 2009) and crustaceans (reviewed in Marshall et al., 2003)]. This shift to blue sensitivity is an adaptation to both downwelling light, which becomes more monochromatic and blue with increasing depth (Jerlov, 1976), and bioluminescence, the vast majority of which lies between 450 and 510 nm (Herring, 1983; Widder et al., 1983; Haddock and Case, 1999).

The only vision studies that have been conducted on benthic (bottom-dwelling) species are structural, and these show that there are similar structural adaptations for increasing light sensitivity, including reflecting tapeta [e.g. crustaceans (Hiller-Adams and Case, 1985; Eguchi et al., 1997) and fish (Denton and Shaw, 1963; Somiya, 1980)], enlarged photoreceptive membranes (Hiller-Adams and Case, 1985; Eguchi et al., 1997) and aphakic spaces [allowing light to hit the retina without going through the lens (Denton, 1990)]. However, in mesopelagic crustaceans, eye growth rates relative to body growth rates decline with depth, as does the eye size relative to body length (Hiller-Adams and Case, 1984; Adams and Case, 1988), whereas in benthic species, there is a depth-related increase in both parameters, resulting in the eyes of many benthic crustaceans being larger, relative to body length, than those of pelagic crustaceans (Hiller-Adams and Case, 1985). Similar trends are found among deep-sea fish: eyes decrease in size with increasing habitat depth among a variety of pelagic species, but many deep-sea benthic or near-benthic species retain large, well-developed eyes (Murray and Hjort, 1912; Locket, 1977). Many of these benthic species have depth ranges well below the depths at which there is sufficient daylight remaining to have any significance for vision (Denton, 1990), suggesting that these species may be adapted for seeing bioluminescence.

However, there is virtually no information on the visual pigments of benthic species because of the difficulty in collecting them without exposing their eyes to damaging light levels. Even minimal exposure to light (1 min of daylight) can cause irreparable damage to photoreceptors adapted for dim-light environments (Loew, 1976; Lindström et al., 1988). Photoreceptors of hydrothermal vent shrimp caught under the lights of a submersible also show significant light-induced damage, with disordered microvilli and disruption to photoreceptor membranes (O'Neill et al., 1995; Lakin et al., 1997), whereas those of their pelagic larvae, caught with a net that prevented exposure to light, show orthogonal layers of microvilli with intact membranes typical of crustacean rhabdoms (Gaten et al., 1998). The structural organization of the compound eye of the giant deep-sea isopod *Bathynomus giganteus*, collected with a dredge and brought up in bright sunlight, showed similar severe disruption of the microvilli, even after 2 months maintenance in constant darkness (Chamberlain et al., 1986). Therefore, it is imperative that

these benthic species be collected without exposure to light. Deep-living pelagic species have successfully been collected alive and with intact eyes with the aid of insulated and light-tight containers attached to the end of trawl nets (Childress et al., 1978; Frank and Widder, 1994), but trawling in the benthic environment (other than sparsely populated muddy bottom) with such a device would seriously damage the environment and destroy the net.

Using new technologies developed for the *Johnson-Sea-Link* (*JSL*) submersible, the photoreceptor function of eight species of deep-sea benthic crustaceans were examined on a series of research expeditions funded by the NOAA Ocean Exploration program. These studies demonstrated unusual spectral sensitivity in two species of deep-sea crabs, in addition to extraordinarily low temporal resolution in a species of deep-sea isopod, indicative of an integration time so long that this species may not be able to track even slow-moving prey. Preliminary data from this study have been published in abstract form (Frank, 2006; Frank, 2008; Frank et al., 2010).

MATERIALS AND METHODS

Animal collections

One species of isopod, two species of caridean shrimp, four species of anomuran crabs and one species of brachyuran crab were collected on a series of research cruises in the Gulf of Mexico and in the Bahamas (Fig. 1, Table 1). Crustaceans were collected with the *JSL* submersible using several techniques. Baited traps were deployed in several locations, collected 6 to 24 h later under red light and placed into light-tight Bio-Boxes. These containers were constructed out of 1.5 cm thick black Plexiglas with lids fitted with O-ring seals, making the boxes light-tight, water-tight and thermally insulated. Pressure relief valves on the Bio-Boxes released excess pressure as the submersible ascended from the collection depths to the surface. In addition, animals attracted to a bait bag placed near the traps were collected under red light with the suction sampler on the *JSL*, and deposited directly into the Bio-Box. The red lights used in collections were two submersible lights, one filtered with a Schott OG 590 nm cutoff filter (transmission below 560 nm <0.001%; Dalton, GA, USA) and the other with a HOYA O-58 cut-off filter (transmission below 560 nm <0.1%; THK Photo Products Inc., Huntington Beach, CA, USA). Once back at the surface, the Bio-Boxes were removed from the submersible and brought into a light-tight cold room, where specimens were removed under dim red light, placed in individual light-tight chambers, and maintained at 7°C until used for experiments.

Spectral sensitivity

Electrophysiological recordings were made shipboard in a light-tight compartment constructed out of polyvinylchloride and black plastic sheeting, with all preparations carried out under dim red light. Animals were clamped into a holder of the appropriate size and suspended in a recording chamber containing chilled seawater (7°C). With this configuration, electroretinograms (ERGs) were recorded with subcorneal metal microelectrodes (FHC, Bowdoin, ME, USA) from live animals for 2–3 days. Signals were amplified with an X Cell-3 Microelectrode Amplifier (FHC) used in conjunction with a high-impedance probe to eliminate electrode polarization artifacts (Kugel, 1977). Low-frequency filters were set to minimal filtering (0.01–0.1 Hz) to minimize distortion of the AC-amplified signal. Recordings were digitized and stored for later analysis using a data acquisition program written in LabView (National Instruments, Austin, TX, USA).

Monochromatic [full-width half-max (FWHM)=5 nm] test flashes (CM110 monochromator, Spectral Products, Putnam, CT, USA) were conducted to the eye through one end of a bifurcated light guide composed of randomized fused silica fibers (EXFO, Quebec, QC, Canada). The light guide entered through a small slit in the light-tight compartment, and was taped in place with black tape to prevent any stray light from entering through the slit. The other branch of the light guide transmitted light filtered with the appropriate filter (see below) from an Ocean Optics LS-1 lamp (Ocean Optics, Dunedin, FL, USA) to the eye through another small baffled slit during chromatic adaptation experiments, ensuring that both the stimulus and adapting lights were impinging on the same receptors. Flash durations of 100 ms were controlled by a Uniblitz Shutter (Model VS14 S, Vincent Associates, Rochester, NY, USA) under computer control. The stimulus irradiance was controlled with a neutral-density wheel driven by a stepper motor under computer control and calibrated at 10 nm intervals with a UDT Optometer (UDT Instruments, San Diego, CA, USA) and calibrated radiometric probe.

Spectral sensitivity measurements were initiated when the amplitude of the response to a standard dim test flash of constant wavelength and irradiance had not changed for an hour. The irradiance of the stimulus light at each test wavelength (350–600 nm,

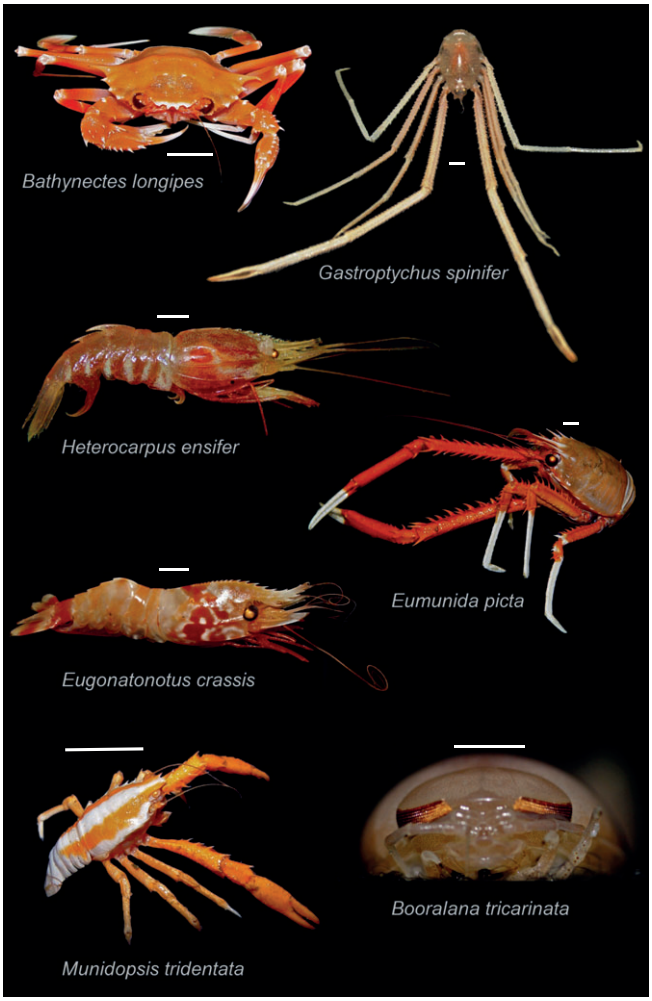

Fig. 1. Seven of the eight species of deep-sea crustaceans examined in this study. These are the seven species for which spectral sensitivity data are shown in Fig. 2. Scale bars are 1 cm, to show the relative sizes of the species.

Table 1. Taxonomic classification and collection information

Taxonomic classification	Location	Latitude, Longitude	Depth (m)
Order Isopoda			
Suborder Flabellifera			
Family Cirolanidae			
Booralana tricarinata (Camp and Heard)	Goulding's Cay, Bahamas	25.02°N, 77.60°W	635, 520
Order Decapoda			
Suborder Pleocyemata			
Infraorder Caridea			
Family Eugonatonotidae			
Eugonatonotus crassus (Milne-Edwards)	Goulding's Cay, Bahamas	25.02°N, 77.60°W	635
Family Pandalidae			
Heterocarpus ensifer (Milne-Edwards)	Burrows Cay, Bahamas	26.42°N, 77.86°W	690
Infraorder Anomura			
Family Chirostylidae			
Gastroptychus spinifer (Milne-Edwards)	SW Florida Shelf lithoherms	29.20°N, 84.46°W	560
	Burrows Cay, Bahamas	26.58°N, 78.19°W	700
	Memory Rock, Bahamas	27.06°N, 77.32°W	700
Family Eumunididae			
Eumunida picta (Smith)	Vioska Knoll, Gulf of Mexico	29.15°N, 88.01°W	620
	Memory Rock, Bahamas	27.08°N, 79.32°W	580
Family Munidopsidae			
Munidopsis erinacea (Milne-Edwards)	Vioska Knoll, Gulf of Mexico	29.16°N, 88.01°W	530
Munidopsis tridentate (Esmark)	Vioska Knoll, Gulf of Mexico	29.15°N, 88.01°W	525
Infraorder Brachyura			
Family Polychiidae			
Bathynectes longipes (Risso)	Vioska Knoll, Gulf of Mexico	29.15°N, 88.01°W	530

every 10–20 nm) was adjusted such that a defined criterion response of 20 μV above background noise was obtained (Frank and Case, 1988). This criterion was chosen as it could reliably be measured against background noise (ranging from 10 to 20 μV), but was near the threshold sensitivity of the eye, so that the light flashes would not light-adapt the eye. Stimulus flashes were given at 1 min intervals, with a standard dim test flash presented throughout the experiment to ensure that the physiological state of the eye had not changed (Frank and Widder, 1999).

Chromatic adaptation experiments were conducted by filtering the white adapting light with a 400 nm bandpass filter (ESCO S914000, FWHM=10 nm) or a 486 nm bandpass filter (Melles Griot 03FIR022, FWHM=10 nm). The irradiance was adjusted with neutral density filters such that the sensitivity of the eye at the adapting wavelength was reduced by 1.5 to 2 log units. After the adapting light was turned on, another spectral sensitivity curve was recorded once the response amplitude to a standard test flash had not changed for 1 h.

Spectral sensitivity curves were generated by plotting the inverse of irradiance (photons $cm^{-2} s^{-1}$) required to produce the criterion response at each wavelength. When data from several animals of the same species were available, data from individuals were normalized to the peak wavelength responses before being combined to produce the final curve. These averaged data were best-fit to an A1-based visual pigment absorbance template (Govardovskii et al., 2000), using the Solver function of Excel (Microsoft, Redmond, WA, USA) to determine the lowest residual sum of squares fit to the data. Absorbance was calculated instead of absorptance because of the lack of information on photoreceptor rhabdom path length and visual pigment optical density.

Microspectrophotometry

Eyes from the crabs *Eumunida picta* Smith 1883, *Gastroptychus spinifer* (A. Milne Edwards 1880) and *Bathynectes longipes* (Risso 1816) were excised shipboard, quick-frozen with a cryogenic spray (SHUR/Freeze, Triangle Biomedical Sciences, Durham, NC, USA) and stored in a freezer until they could be shipped to a shore-based laboratory for microspectrophotometry (MSP) analysis. Frozen eyes were sectioned at 14 μm on a cryostat, and sections were transferred to microscope slide cover slips in a drop of marine crustacean Ringer's solution (Cavenaugh, 1956), pH 7.0, containing 1.25% glutaraldehyde to enhance photobleaching of the visual pigment. Photoreceptors were selected under dim, far-red light and scanned from 400 to 700 nm at 1 nm intervals in a single-beam microspectrophotometer using a beam placed in each rhabdom. Rhabdoms were scanned when fully dark-adapted and after full photobleaching under 2 min of bright white light. Absorbance spectra of the rhodopsin in the dark-adapted receptor were taken as difference spectra between the appropriate scans and the scan of the bleached receptor. These difference spectra were fitted to standard rhodopsin templates using a least-squares procedure (Cronin et al., 2002; Cronin and Frank, 1996).

Temporal resolution and sensitivity

Temporal resolution of the eye was determined in two ways. Firstly, the critical flicker frequency (CFF; also known as the critical flicker fusion frequency) in response to square pulses of light with a constant 0.5 duty cycle (50:50 light:dark ratio) was measured. CFF is the highest stimulus rate at which the eye can produce electrical responses that remain in phase with a flickering light of a certain irradiance. CFF increases as the irradiance of the stimulating light increases (Bröcker, 1935; Crozier and Wolf, 1939; Crozier et al., 1939), until a plateau is reached, at which point further increases in irradiance do not result in further increases in CFF, and may actually result in a decrease (Glantz, 1968; Frank, 1999). The maximum CFF (CFF_{max}), which is the highest flicker rate that the eye is capable of following at any irradiance, was used to compare temporal resolution between different species. The wavelength of light used for these experiments was 490 nm, at an initial irradiance that generated a 100 μV response to a single flash. Flash duration was controlled by the Uniblitz shutter and the duration of the pulse trains was 2 s. The response to a dim test flash was monitored between every pulse train, and subsequent flickering stimuli were not given until the response to the test flash had recovered to the

dark-adapted level. At the brightest flickering stimuli, recovery took up to 1 h. After every pulse train, the output was immediately analyzed visually to determine whether the eye was able to produce a modulated electrical signal that remained in phase with the flickering light for 0.5 s. Flicker stimulus rate was increased by 1–2 Hz for every pulse train until fusion occurred. At this point, irradiance was increased by 0.5 log units, and the flicker rate was increased until fusion was again achieved. The CFF_{max} was defined as the point at which three successive irradiance increases did not result in a faster flicker frequency. The critical duration (r), which is the time period over which light is summed in the eye (i.e. the integration time of the eye), was estimated as the inverse of CFF_{max} (Matin, 1968).

Temporal resolution was also determined by measuring the response latency, defined as the time elapsed between the onset of the stimulus flash and the onset of the photoreceptor response. Because response latency decreases as stimulus intensity increases (Moeller and Case, 1995), latencies were calculated for flashes yielding response amplitudes of 50% of the maximum amplitude of the V/log$I$ curve (Frank, 1999; Frank, 2003).

Irradiance sensitivity was determined by presenting the eye with 100 ms flashes of monochromatic light (chosen to match the peak sensitivity of each species) of varying irradiances and measuring the amplitude of the ERG response. To ensure that the eye was fully dark-adapted for each stimulus, subsequent stimulus flashes were only presented when the response to a standard dim test flash had recovered to dark-adapted levels. The response *versus* irradiance data were plotted on semilogarithimic coordinates to generate V/log$I$ curves. These curves were fit with the Zettler modification of the Naka–Rushton equation, which describes the intensity response function of photoreceptors (Naka and Rushton, 1966; Zettler, 1969):

$$\frac{V}{V_{max}} = \frac{I^m}{I^m + K^m}\ , \qquad (1)$$

where I is the stimulus irradiance (photons cm^{-2} s^{-1}), V is the response amplitude at irradiance I, V_{max} is the maximum response amplitude, m is the slope of the linear part of the V/log$I$ curve and K is the stimulus irradiance evoking a response that was 50% of the V_{max}. In some cases, V_{max} was not reached, but if the recorded V_{max} was 90% of the calculated V_{max}, these data were included in the analysis. Log$K$ was used to compare physiological photoreceptor sensitivity between species (Frank, 2003).

RESULTS

Spectral sensitivity and MSP

Eight species of deep-sea crustaceans were examined in this study (Table 1) and usable spectral sensitivity curves were obtained from seven of them (Figs 1, 2). Spectral sensitivity curves were judged usable if replicates were available (five species), or the preparation was viable enough to conduct chromatic adaptation experiments in addition to dark-adapted spectral sensitivity, and chromatic adaptation did not shift the spectral peak (two species). Based on these criteria, the spectral sensitivity data from *Munidopsis erinacea* were not usable. The spectral sensitivity peaks of the seven remaining species were in the blue region of the visible spectrum, ranging from 470 to 497 nm. The spectral sensitivity peaks of the two shrimp species were the longest of all the species tested.

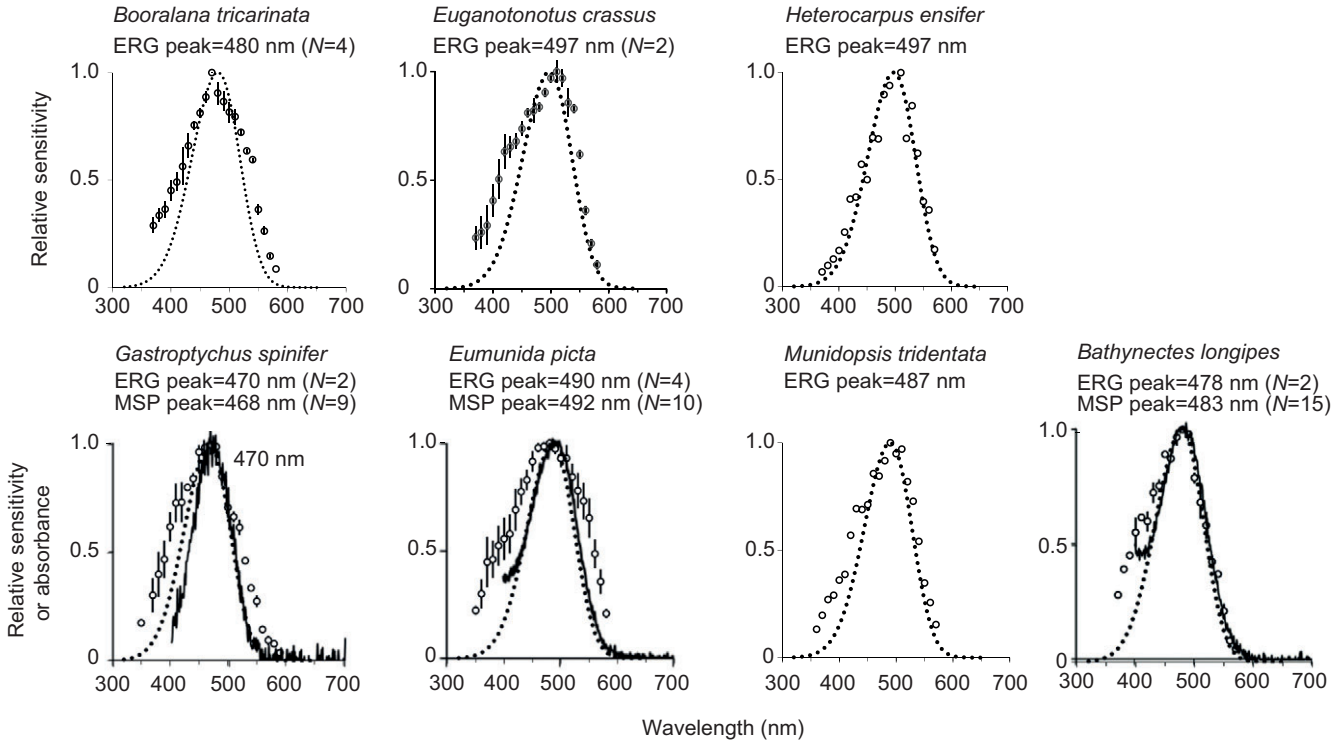

Fig. 2. Spectral sensitivities of seven species of deep-sea benthic crustaceans. Species are arranged according to taxonomic group (see Table 1). The open circles represent the normalized spectral sensitivity data; for those species with sample sizes >1 (indicated in parentheses), the data represent averaged normalized spectral sensitivity data, with the bars representing the standard error. The dotted lines represent calculated best-fit absorbance curves for 400 through 600 nm (see Materials and methods). The solid lines are the relative absorbances of the visual pigments measured *via* microspectrophotometry (MSP) for the three species for which these data are available, with the number of bleaches performed shown in parentheses. ERG, electroretinogram.

The visual pigments' absorption peaks were determined *via* MSP for three species, and are very close to the sensitivity peaks measured electrophysiologically. For *G. spinifer*, the MSP peak was at 468 nm *versus* 470 nm measured electrophysiologically; for *E.*, 492 nm *versus* 490 nm; and for *B. longipes*, 483 nm *versus* 478 nm (Fig. 2).

Chromatic adaptation experiments were conducted on all seven species with the 486 nm bandpass filter. This blue chromatic adaptation had no effect on spectral sensitivity in the isopod *Booralana tricarinata*, the carideans *Eugonatonotus crassus* and *Heterocarpus ensifer*, or two of the crab species, *Munidopsis tridentata* and *B. longipes*. Chromatic adaptation reduced overall sensitivity by 1.5 to 2 log units, but did not affect the shape or peak of the spectral sensitivity curve. The curves for *B. longipes* are given in Fig. 3A as a representative example for these five species, plotted on a log scale so that both curves could be plotted on the same graph. However, the effects of blue chromatic adaptation on the photoreceptors of *E. picta* and *G. spinifer* revealed the presence of secondary peaks in the ultraviolet portion of the spectrum – at 363 and 383 nm, respectively (Fig. 3B,C). To demonstrate that the effects of light adaptation were specific to blue adaptation, spectral sensitivity curves under chromatic adaptation with a 400 nm bandpass filter (FWHM=20 nm) were also recorded. In both species, adaptation with this violet light had no wavelength-specific effects on spectral sensitivity: overall sensitivity decreased but the wavelength of the peak response remained the same.

Wavelength-specific effects of chromatic adaptation

Response waveforms to short wavelength (370 nm) and long wavelength (570 nm) stimuli were identical in the dark-adapted eyes of all the species (Fig. 3D,F,H). Blue chromatic adaptation had no wavelength specific effects on the five species whose spectral sensitivity was not affected by this adaptation light. However, in *G. spinifer* and *E. picta*, the two species in which blue chromatic adaptation revealed a UV sensitivity peak, a wavelength-specific effect on the response waveforms is also evident (Fig. 3G,I). In *G. spinifer*, under blue chromatic adaptation, the mean (±s.e.m.) time to peak for responses to short-wavelength light (360–390 nm) was 193±3 ms (N=4) compared with 180±4 ms (N=5) for long-wavelength light (560–590 nm). For *E. picta*, the times to peak were 215±11 ms (N=6) and 176±7 ms (N=5) for short- and long-wavelength light, respectively. These differences were statistically significant for both species (P=0.042 and 0.009, respectively). In contrast, adaptation with violet light did not affect the response waveforms in either species.

Photoreceptor sensitivity and temporal resolution

Because temporal resolution depends upon temperature, the following data were obtained from animals that had been maintained at 7°C throughout the experiment. In addition, temporal resolution also depends on the irradiance of the test flash. While the irradiance of the light source was calibrated, there was no way to ensure, when working on live animals suspended in a chilled water bath, that the tip of the light guide was in the identical position for each preparation. Therefore, only CFF_{max}, which is the highest flicker rate that the eye is capable of following at any intensity, was used for species comparisons. Flicker fusion data could not be obtained from *M. tridentata*. As shown in Table 2, the CFF_{max} was highest for the two caridean shrimp species, ranging from 16 to 24 Hz (Fig. 4A). The crabs, both anomuran and brachyuran, had somewhat lower temporal resolutions, with CFF_{max} ranging from 10 to 14 Hz. The isopod *B. tricarinata* had a CFF_{max} of 4 Hz, the lowest ever measured in a marine crustacean (Fig. 4B).

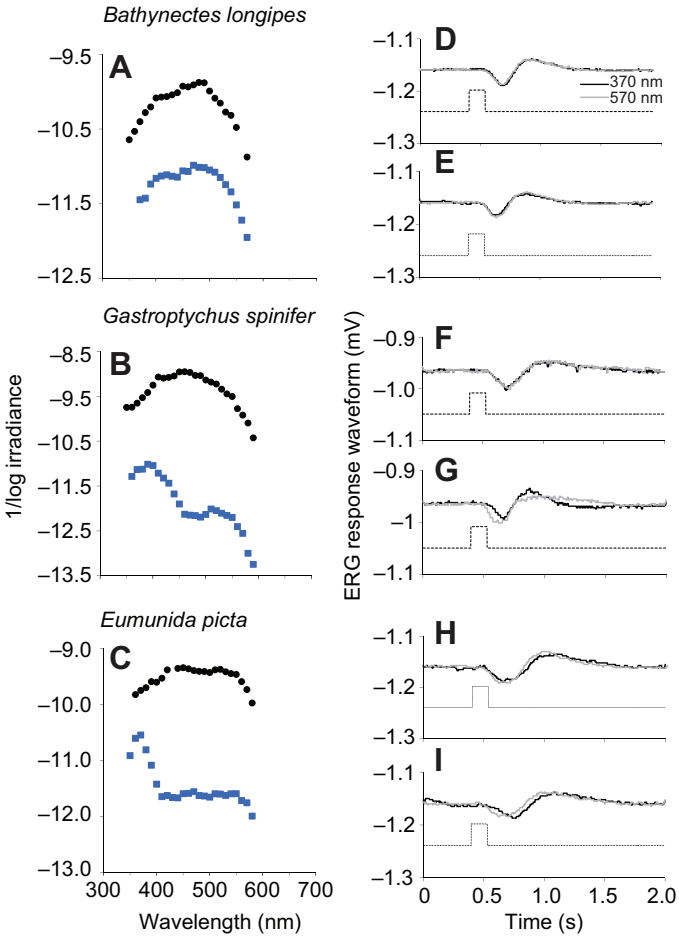

Fig. 3. (A–C) Dark-adapted (black circles) and blue-adapted (blue squares) spectral sensitivity curves measured in the same individual within a species. Data are plotted on a log axis so that both data sets could be plotted together. (A) Blue-chromatic adaptation had no effect on the spectral sensitivity of the brachyuran crab *Bathynectes longipes*. Similar results were obtained from the anomuran crab *Munidopsis tridentata*, the caridean shrimp *Heterocarpus ensifer* and *Eugonatonotus crassus*, and the isopod *Booralana tricarinata*. (B,C) Blue-chromatic adaptation unveiled an ultraviolet sensitivity peak in both *Gastroptychus spinifer* and *Eumunida picta*. (D–I) ERG response waveforms matched for equal amplitude in the dark-adapted (D,F,H) and blue-adapted (E,G,I) eye in response to short wavelength (370 nm) and long wavelength (570 nm) stimuli. There were no wavelength-specific effects of chromatic adaptation in *B. longipes* (data shown in D,E), *M. tridentata*, *H. ensifer*, *E. crassus* or *B. tricarinata*. There were no differences in response waveforms to any wavelength of light in the dark-adapted eyes of *G. spinifer* (F) or *E. picta* (H), but blue-adaptation resulted in a significantly faster times to peak in short wavelength (370 nm) *versus* long wavelength (570 nm) responses (G,I).

Response latency, an indication of the integration time of the eye, is also a function of irradiance, so response latencies were determined for responses whose amplitudes were 50% of the V_{max} (as determined by plotting the V/log$I$ curves; Fig. 4C). V_{max} is the largest response that the eye is capable of generating at any irradiance; once V_{max} is reached, a plateau is seen in the V/log$I$ curves. V_{max} was not reached in the shrimp *H. ensifer* or the crab *M. tridentata*, and therefore the 50% V_{max} could not be determined for these species. As seen in Table 2, response latency mirrored the CFF_{max} results: the species with the highest CFF_{max} (the two shrimp species) also had the shortest response latencies.

Table 2. Maximum critical flicker frequency (CFF_{max}), critical duration and half-maximum latency in the dark-adapted photoreceptors

Species	CFF_{max} (Hz)	Critical duration (ms)	Latency (ms) at 50% V_{max}
Booralana tricarinata	4±1 (*N*=4)	250±34	161±13 (*N*=3)
Gastroptychus spinifer	10±2 (*N*=3)	105±21	74±3 (*N*=2)
Eumunida picta (*N*=3)	11±2	90±10	57±5
Munidopsis erinacea (*N*=1)	12	83	69.5
Bathynectes longipes (*N*=1)	14	71	65
Heterocarpus ensifer (*N*=1)	16	63	n.a.
Eugonatonotus crassus (*N*=2)	24	42	47±6

CFF_{max} is the highest flicker rate at which the eye can still stay in phase with the flashing light. Critical duration ($1/CFF_{max}$) is an indication of the integration time of the photoreceptor. Latency is the delay between the onset of the stimulus and the initiation of the photoreceptor response at an irradiance that produces a response that is 50% of the maximum response the eye is capable of producing.

K, the irradiance required to generate a response that is 50% of the V_{max}, has been used an indicator of absolute sensitivity in intracellular studies on insects (Laughlin, 1976; Autrum, 1981), and an indicator of relative sensitivity when utilizing the ERG (Eguchi and Horikoshi, 1984; Frank and Case, 1988). As expected, the isopod *B. tricarinata*, which had the slowest eye, had half a log unit greater sensitivity than any of the other species (Table 2). The crabs had considerably less sensitive eyes with concurrent faster response latencies. The apparent anomaly was the caridean shrimp *E. crassus*, which had the fastest eye with the fastest response latency, but was second in sensitivity to the isopod.

DISCUSSION

Spectral sensitivity

The dark-adapted spectral sensitivity data from all the benthic species in this study showed a sensitivity maximum in the blue region of the spectrum, between 470 and 497 nm. For the three species in which the visual pigments were analyzed *via* microspectrophotometry, the absorbance peak of the visual pigment was remarkably close to the spectral sensitivity peak, suggesting that there was not much pre-retinal filtering occurring. The hypsochromatic shift to bluer wavelengths, compared with the 490–550 nm visual pigments found in shallow-water crustaceans (reviewed in Goldsmith, 1972; Cronin, 1986), is similar to what has been found in most species of mesopelagic crustaceans (Frank and Case, 1988; Frank and Widder, 1999; Cohen and Frank, 2007) and fish (reviewed in Douglas et al., 2003). This sensitivity shift to bluer wavelengths would confer greater sensitivity to both the remaining downwelling irradiance as well as bioluminescence, which is considered by some to be the major visual stimulus in the deep sea (Beebe, 1935; Clarke and Hubbard, 1959; Jerlov, 1976; Widder, 1999; Johnsen, 2005).

Blue chromatic adaptation had no effect on spectral sensitivity in five of the species in this study: the isopod *B. tricarinata*, the caridean shrimp *E. crassus* and *H. ensifer*, and the crabs *M. tridentata* and *B. longipes*. These results indicate that a single, blue-sensitive visual pigment is present in these species, similar to what has been found for most species of deep-sea pelagic crustaceans (Frank and Case, 1988; Frank and Widder, 1999; Cohen and Frank, 2007; Frank et al., 2009) as well as the only two deep-sea benthic crab species that have been studied to date *via* MSP [*Geryon quinquedens* (Cronin and Forward, 1988) and *Bythograea thermydron* (Jinks et al., 2002)]. Although the results of experiments involving a sample size of one (*H. ensifer* and *M. tridentata*) should be discussed with caution, in both cases, the animals were alive at the end of the experiment, and the adapting light depressed sensitivity by over 1.5 to 2 log units, which is sufficient to reveal the presence of a secondary sensitivity maximum if one is present (Frank and Case, 1988; Frank and Widder, 1999).

Unexpected responses to blue chromatic adaptation were discovered in the other two crab species, *E. picta* and *G. spinifer*. Blue chromatic adaptation revealed a secondary sensitivity peak at 363 and 383 nm, respectively. In addition, violet adaptation had no wavelength-specific effects on spectral sensitivity. These results can be explained if, as in deep-sea pelagic crustaceans with two visual pigments, the blue-sensitive cells vastly outnumber the ultraviolet-sensitive cells (Frank and Case, 1988; Cronin and Frank, 1996; Gaten et al., 2003). In this situation, blue chromatic adaptation would diminish the contribution of the blue-sensitive cells to the ERG response, unmasking the presence of the minority ultraviolet-sensitive cells. Conversely, violet chromatic adaptation would only diminish the already small contribution of the ultraviolet-sensitive cells to the ERG response, and no change in spectral sensitivity would be visible.

The conclusion that there are two classes of receptor cells is supported by the wavelength-specific effects of blue adaptation on the response waveforms. In the dark-adapted eyes of all species, the time to peak response at all wavelengths was identical within a species. The five species that showed no spectral sensitivity shift in response to blue adaptation also had identical times to peak at all wavelengths under blue adaptation. However, for the two species in which blue adaptation uncovered a secondary sensitivity peak at the ultraviolet portion of the spectrum, the time to peak was significantly longer at the shorter wavelengths than at the longer wavelengths. Again, if two classes of receptor cells are present in different quantities, blue chromatic adaptation would diminish the contribution of the blue-sensitive receptor cells to the ERG response, and the contribution of the ultraviolet-sensitive cells would become visible in response to short-wavelength light. Single cells do not respond differentially to different wavelengths of light (Graham and Hartline, 1935; Naka and Rushton, 1966; Stark and Wasserman, 1974), and if several different receptors with different time courses contribute to the ERG, equal amplitude responses at all wavelengths can never be matched (Chapman and Lall, 1967). Therefore, differences in response waveforms to short- *versus* long-wavelength light in crustaceans can only be attributed to two different populations of receptor cells with different membrane properties (Wald, 1968).

Although a second visual pigment was not found during the MSP analysis of *G. spinifer* and *E. picta* photoreceptors, the same situation occurred for the initial discovery of a short-wavelength-sensitive visual pigment in several species of mesopelagic shrimp in the family Oplophoridae. Electrophysiological studies indicated the presence of two receptor classes (Frank and Case, 1988), whereas the initial MSP analysis found only a single visual pigment (Hiller-Adams et al., 1988). It took a re-examination of fresh-frozen material (which was not available in the present study) to detect the presence of the second visual pigment in *Systellaspis debilis* (Cronin and Frank, 1996).

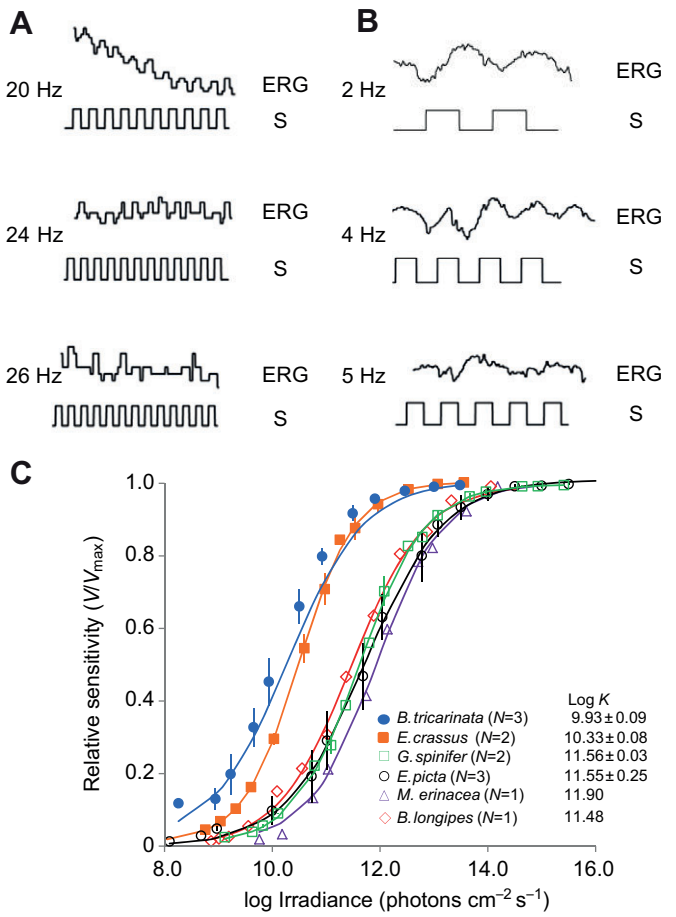

Fig. 4. (A) Flicker fusion frequency of *Eugonatonotus crassus*. ERG designates the response recorded from the eye, whereas S is the flickering light stimulus. The time period shown is 0.5 s. This photoreceptor is able to follow 24 Hz, but by 26 Hz, it can no longer respond to every stimulus. (B) Flicker fusion frequency of *Booralana tricarinata*. The time period shown is one second. This species is barely able to follow 4 Hz, with a long delay between the start of the stimulus and the response, and by 5 Hz, is unable to respond to every stimulus. (C) *V*/log*I* curves for the six species for which data were available. The curves for *B. tricarinata* and *E. crassus* are shifted to the left along the *x*-axis, indicating greater photosensitivity in these species compared to the other four species. The log*K* is the log of the irradiance generating a half-maximal response, with a smaller log*K* indicating greater sensitivity.

Sacrificing retinal space for a UV photoreceptor suggests that UV sensitivity plays an important role in the visual ecology of these species, and at these depths (400–600 m), UV sensitivity is most likely related to bioluminescence. As mentioned above, several species of mesopelagic oplophorid shrimp have both UV-sensitive and blue-sensitive visual pigments. Those species with the dual visual pigment system possess both light-emitting organs called photophores, which are used for counterillumination and hypothesized to play a role in sexual signaling (reviewed in Herring, 2007) and a bioluminescent spew (i.e. vomit), which probably serves a defensive function (Herring and Barnes, 1976). Further analysis by Cronin and Frank (Cronin and Frank, 1996) indicated that the absorption maxima of the two pigments may be optimized for distinguishing between the broader emission spectrum of the spew and the narrower emission spectrum of the photophores. The other species in this family possess the bioluminescent spew but lack photophores, and those that have been studied to date have a single blue-sensitive visual pigment (Frank and Case, 1988), supporting the hypothesis that the dual visual pigment system is used to distinguish between different bioluminescent 'colors'.

This cannot be the case for the deep-sea crabs, as neither species is bioluminescent. However, at the Little Bahamas Bank Lithoherms, both species of crabs were frequently seen on pennatulaceans (sea whips) or zoanthids (colonial anemones known as gold corals) (Fig. 5A,B), both of which emit a greenish bioluminescence (Johnsen et al., 2012). In a remarkable long exposure image taken by S.J. from the front sphere of the *JSL* submersible, the green bioluminescence of the zoanthid (formerly *Gerardia* sp.) is clearly distinguishable from the blue luminescence of the bioluminescent plankton striking it (Fig. 5C). A series of observations (under both red and light white) and videos (under white light) made from the *JSL* indicate that the crabs are stationary on these benthic structures for long periods of time, and then periodically use their claws to pick organisms off the structure and bring them to their mouths. *Eumunida picta* has also been observed in association with other benthic structures that would impose a similar impediment to plankton flowing by in the water column. In the Mississippi Canyon in the northern Gulf of Mexico, 81% of the >100 *E. picta* observed during the study occurred on the deep coral *Lophelia pertusa* (Kilgour and Shirley, 2008). Although there are clearly other possibilities to explain the relationship between the crabs and stationary benthic structures that project up into the water column, one intriguing possibility is that these crabs may be using their putative dual visual pigment systems to distinguish the green benthic bioluminescence of their preferred substrate, which they apparently do not eat, from the blue bioluminescence of the planktonic organisms impacting these structures, which may be their preferred food source. This hypothesis is supported by measurements of emission spectra of gelatinous zooplankton, the most likely organisms to be impacting benthic structures. These emission spectra are considerably bluer than the bioluminescence from benthic animals, with a mean (±s.e.m.) λ_{max} of 473.8±2.9 nm for medusae (N=34), 486.1±1.6 nm for ctenophores (N=41), and two modes for siphonophores, centered at 450.5±1.3 nm (N=16) and 486±2.3 nm (N=9) (Haddock and Case, 1999). *Lophelia pertusa* is not bioluminescent (Johnsen et al., 2012), but would still provide a raised substrate for the plankton to impact against, and a dual visual pigment system may help enhance contrast between the bioluminescence and the remaining bluish downwelling light as well.

Temporal resolution and photosensitivity

The temporal resolution of most of the species was within the range of what one would anticipate for deep-sea species, based on previous studies utilizing the CFF_{max} techniques (reviewed in Marshall et al., 2003). All of the species had relatively low temporal resolution compared with shallow species, with that of the more mobile shrimp approximately equal to that of mesopelagic decapod shrimp (Frank, 1999), at ~20 Hz. The 10–14 Hz found in the crabs is considerably lower than that of the shrimp, which could be a function of their less mobile lifestyle, and fits well with Autrum's hypothesis that the response dynamics of the retina match the habitat and lifestyle of the organism (Autrum, 1958). This is similar to the situation demonstrated in the only other physiological study of deep-sea benthic species, which used intermediate light levels (Johnson et al., 2000). Because temporal resolution depends so strongly on the irradiance of the stimulus light (Bröcker, 1935; Crozier and Wolf, 1939; Crozier et al., 1939; Frank, 2000), results from that study cannot be directly compared with results from the studies utilizing the invariant CFF_{max}

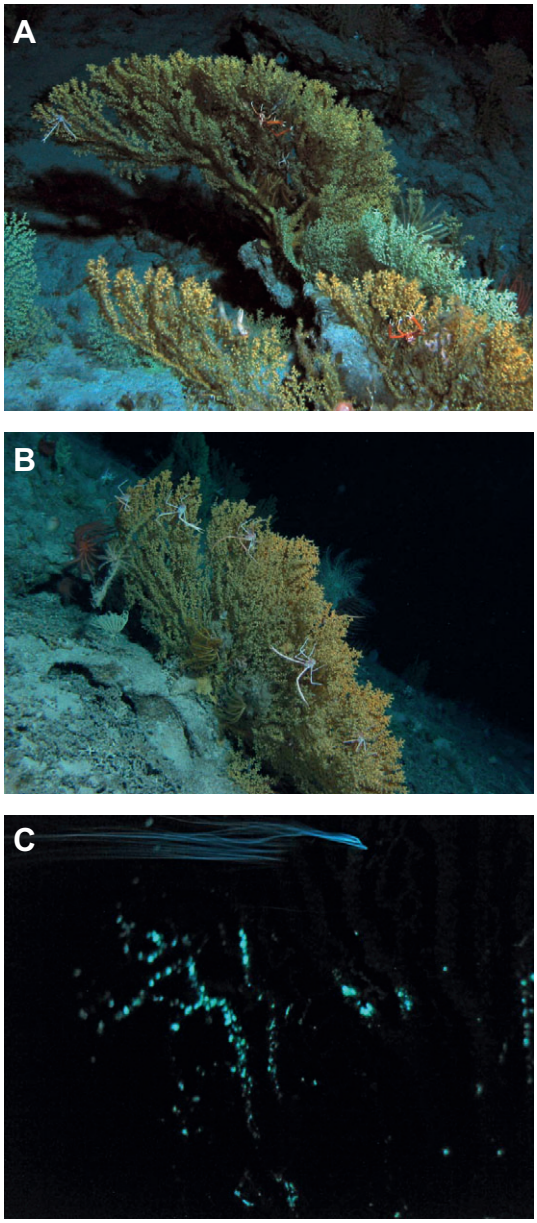

Fig. 5. Representative images of *Eumunida picta* (A) and *Gastroptychus spinifer* (B) in their characteristic stances among the colonial zoantharian. Images were taken at the Memory Rock, Bahamas, dive sites. (C) *In situ* photograph of the blue-green bioluminescence of the zoantharian fan (formerly known as *Gerardia* sp.) along with blue bioluminescence generated by an unknown planktonic animal (likely a gelatinous species) striking the fan. The water current is passing from right to left. The image was taken from inside the passenger sphere of the *JSL* submersible using a Nikon D700 fitted with a 50 mm f1.8 lens (exposure details: 10 s at f1.8 at ISO 6400).

technique, but within the present study, the same correlations were seen. The eyes of the slower-moving scavenger crab species had slower response dynamics than the eyes of the deeper-living crab species whose stomach contents indicated it was an active fast-moving predator.

The real surprise was the isopod *B. tricarinata*. With a CFF_{max} of only 4 Hz, it has the slowest eye measured thus far in a crustacean, with the next slowest being found in the deep-sea crabs in the present study. The integration time, the time period over which incoming photons are summed, could not be directly measured using the ERG technique, but the critical duration, which is the inverse of the CFF_{max} (Matin, 1968), can be directly related to integration time, such that a long critical duration indicates a long integration time (de Souza and Ventura, 1989). The critical duration was 250 ms for these isopods, more than twice that of the crabs and shrimps, with ranges from 42 to 105 ms (Table 2). A further indication of the very long integration time is the response latency, as a long response latency is an indication of a long integration time. The response latency for the isopods was more than twice as long as those of the other species in this study, as well as those of deep-sea pelagic crustaceans that have been examined using the same methodology (Frank, 2003; Cohen and Frank, 2006; Cohen and Frank, 2007). This long integration time would give this eye very high sensitivity, but likely limited ability to track moving prey or separate fast visual stimuli, such as rapid flashes of bioluminescence (Warrant, 1999). One intriguing possibility for the adaptive significance of this extremely slow eye has to do with bioluminescence and food preferences of isopods. Lampitt et al. (Lampitt et al., 2001) suggested that bacterial luminescence may be present on aggregations of organic matter deposited on the deep-sea floor, based on previous observations that luminous bacteria are present on crustacean and fish carcasses (Wada et al., 1995) and that fecal pellets in sediment traps have been observed to luminesce (Andrews et al., 1984). This colonization of organic matter by bioluminescent bacteria may produce a background glow that could be used by deep-sea scavengers (Nishida et al., 2002), if their eyes were sensitive enough to see it. Deep-sea isopods are thought to be scavengers (reviewed in Barradas-Ortiz et al., 2003), and an eye with an extremely long integration time might be able to visualize dimly glowing detritus, aiding the animal in finding a target initially tracked *via* chemoreception.

An interesting anomaly is seen in the caridean shrimp *E. crassus*, which has the shortest response latency amongst the benthic species, generally indicative of a less sensitive eye, but this species was second in sensitivity to the isopod. However, sensitivity is a function not only of temporal resolution, but also of other factors such as size of the eye, interommatidial angle, facet size, length of the photoreceptor, amount of visual pigment present, etc. (reviewed in Land and Nilsson, 2002). This species had the largest stalked eye of all the species in the present study, and further studies are currently underway to determine whether there are other optical adaptations present to increase light capture.

In summary, this study of only a few of the denizens of the deep-sea benthic environment demonstrated some unexpected physiological adaptations to the deep-sea light environment. Although the number of benthic bioluminescent species is relatively rare in the location occupied by many of the crustaceans in this study (Johnsen et al., 2012), evidence suggesting that at least two species of deep-sea anomuran crabs possess both UV- and blue-sensitive visual pigments indicates that bioluminescence still may have been a driving force behind their visual adaptations. Further studies are needed to determine whether UV sensitivity is common in the deep-sea Chirostyloidea, the superfamily to which *E. picta* and *G. spinifer* belong, as well as other deep-sea ‘stalk sitters’. Because so little work has been done on deep-sea benthic bioluminescence, there are, in all probability, many unknown sources of benthic bioluminescence yet to be discovered, including the intriguing possibility of dimly glowing mats of marine snow and detritus on the seafloor bottom, as suggested by the remarkably slow eye of the isopod *B. tricarinata*.

ACKNOWLEDGEMENTS

We thank the captain and crew of the RV *Seward Johnson,* and the pilots and crew of the deep-sea submersible *Johnson-Sea-Link II.* We also thank two anonymous reviewers for comments on earlier drafts of the manuscript.

FUNDING

Funding for this research was provided by the National Oceanographic and Atmospheric Administration, Office of Ocean Exploration [NA050AR4601059 to T.M.F. et al., NA07OAR46000289 to S.J. et al. and NA09OAR4600095 to T.M.F. et al.]. S.J. was also supported in part by grants from the National Science Foundation [OCE-0852138 to S.J. et al.] and the Office of Naval Research [N00014-09-1-1053 to S.J. et al.]. T.W.C. was supported in part by grants from the National Science Foundation [IOS-0721608 to T.W.C. et al.] and the Air Force Office of Scientific Research [FA9550-09-1-0149 to T.W.C.].

REFERENCES

Andrews, C. C., Karl, D. M., Small, L. F. and Fowler, S. W. (1984). Metabolic activity and bioluminescence of oceanic faecal pellets and sediment trap particles. *Nature* **307**, 539-541.

Autrum, H. (1958). Electrophysiological analysis of the visual systems in insects. *Exp. Cell Res.* **14 Suppl. 5**, 426-439.

Autrum, H. (1981). Light and dark adaptation in invertebrates. In *Comparative Physiology and Evolution of Vision in Invertebrates* (ed. H. Autrum), pp. 2-91. Berlin: Springer-Verlag.

Barradas-Ortiz, C., Briones-Fourzán, P. and Lozano-Álvarez, E. (2003). Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatán peninsula. *Deep Sea Res. Part I* **50**, 495-513.

Beebe, W. (1935). *Half Mile Down.* London: John Lane, The Bodley Head.

Bröcker, H. (1935). Untersuchungen iiber das Sehvermogen der Ein-siedlerkrebse. *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere* **55**, 399-430.

Cavenaugh, G. M. (1956). Formulae and methods of the marine biological laboratory chemical room. Woods Hole, MA: Woods Hole Oceanographic Institution.

Chamberlain, S. C., Meyer-Rochow, V. B. and Dossert, W. P. (1986). Morphology of the compound eye of the giant deep-sea isopod *Bathynomus giganteus. J. Morphol.* **189**, 145-156.

Chapman, R. M. and Lall, A. B. (1967). Electroretinogram characteristics and the spectral mechanisms of the median ocellus and the lateral eye in *Limulus polyphemus. Gen. Physiol.* **50**, 2265-2267.

Childress, J. J., Barnes, A. T., Quetin, L. B. and Robison, B. H. (1978). Thermally protecting cod ends for the recovery of living deep-sea animals. *Deep-Sea Res.* **25**, 419-422.

Clarke, G. L. and Hubbard, C. J. (1959). Quantitative records of the luminescent flashing of oceanic animals at great depths. *Limnol. Oceanogr.* **4**, 163-180.

Cohen, J. H. and Frank, T. M. (2006). Visual physiology of the Antarctic amphipod *Abyssorchomene plebs. Biol. Bull.* **211**, 140-148.

Cohen, J. H. and Frank, T. M. (2007). Vision in the hyperiid amphipod *Scina crassicornis. J. Mar. Biol. Assoc. U. K.* **87**, 1202-1206.

Cronin, T. W. (1986). Photoreception in marine invertebrates. *Am. Zool.* **26**, 403-415.

Cronin, T. W. and Forward, R. B., Jr (1988). The visual pigments of crabs. I. Spectral characteristics. *J. Comp. Physiol. A* **162**, 463-478.

Cronin, T. W. and Frank, T. M. (1996). A short-wavelength photoreceptor class in a deep-sea shrimp. *Proc. R. Soc. Lond.* **263**, 861-865.

Cronin, T. W., Caldwell, R. L. and Erdmann, M. V. (2002). Tuning of photoreceptor function in three mantis shrimp species that inhabit a range of depths. I. Visual pigments. *J. Comp. Physiol. A* **188**, 179-186.

Cronin, T. W., Marshall, N. J. and Frank, T. M. (2003). Visual adaptations in crustaceans: chromatic, developmental, and temporal aspects. In *Sensory Processing in Aquatic Environments* (ed. S. P. Collin and N. J. Marshall), pp. 343-372. New York: Springer.

Crozier, W. J. and Wolf, E. (1939). The flicker response contour for the crayfish. *J. Gen. Physiol.* **23**, 1-10.

Crozier, W. J., Wolf, E. and Zerrahn-Wolf, G. (1939). The flicker response contour for the isopod *Asellus. J. Gen. Physiol.* **22**, 451-462.

de Souza, J. M. and Ventura, D. F. (1989). Comparative study of temporal summation and response form in hymenopteran photoreceptors. *J. Comp. Physiol. A* **165**, 237-245.

Denton, E. J. (1990). Light and vision at depths greater than 200m. In *Light and Life in the Sea* (ed. P. J. Herring, A. K. Campbell, M. Whitfield and L. Maddock), pp. 127-148. Cambridge: Cambridge University Press.

Denton, E. M. and Shaw, T. I. (1963). The visual pigments of some deep-sea elasmobranchs. *J. Mar. Biol. Assoc. U. K.* **43**, 65-70.

Douglas, R. H., Bowmaker, J. K. and Hunt, D. M. (2003). Spectral sensitivity tuning in the deep-sea. In *Sensory Processing in Aquatic Environments* (ed. S. P. Collin and N. J. Marshall), pp. 323-342. Berlin: Springer-Verlag.

Eguchi, E. and Horikoshi, R. (1984). Comparison of stimulus-response (V-logI) functions in five types of lepidopteran compound eyes (46 species). *J. Comp. Physiol.* **154**, 3-12.

Eguchi, E., Dezawa, M. and Meyer-Rochow, V. B. (1997). Compound eye fine structure in *Paralomis multispina* Benedict, an anomuran half-crab from 1200 m depth. *Biol. Bull.* **192**, 300-308.

Frank, T. M. (1999). Comparative study of temporal resolution in the visual systems of mesopelagic crustaceans. *Biol. Bull.* **196**, 137-144.

Frank, T. M. (2000). Temporal resolution in mesopelagic crustaceans. *Philos. Trans. R. Soc. Lond. B* **355**, 1195-1198.

Frank, T. M. (2003). Effects of light adaptation on the temporal resolution of deep-sea crustaceans. *Integr. Comp. Biol.* **43**, 559-570.

Frank, T. M. (2006). UV photosensitivity in a deep-sea benthic crab. Ocean Sciences Meeting, Honolulu, HI, USA.

Frank, T. M. (2008). Vision in the deep. Ocean Sciences Meeting, Orlando, FL, USA.

Frank, T. M. and Case, J. F. (1988). Visual spectral sensitivities of bioluminescent deep-sea crustaceans. *Biol. Bull.* **175**, 261-273.

Frank, T. M. and Widder, E. A. (1994). Comparative study of behavioral-sensitivity thresholds to near-UV and blue-green light in deep-sea crustaceans. *Mar. Biol.* **121**, 229-235.

Frank, T. M. and Widder, E. A. (1999). Comparative study of the spectral sensitivities of mesopelagic crustaceans. *J. Comp. Physiol. A* **185**, 255-265.

Frank, T. M., Porter, M. L. and Cronin, T. W. (2009). Spectral sensitivity, visual pigments and screening pigments in two life history stages of the ontogenetic migrator *Gnathophausia ingens. J. Mar. Biol. Assoc. U. K.* **89**, 119-129.

Frank, T. M., Johnsen, S., Widder, E. A., Messing, C. G. and S. H. D. Haddock. (2010). Bioluminescence 2009: living light on the deep-sea floor. Ocean Sciences Meeting, Portland, OR, USA.

Gaten, E., Herring, P. J., Shelton, P. M. J. and Johnson, M. L. (1998). The development and evolution of the eyes of vent shrimps (Decapoda: Bresiliidae). *Cah. Biol. Mar.* **39**, 287-290.

Gaten, E., Shelton, P. M. J. and Nowel, M. S. (2003). Interspecific variations in the morphology and ultrastructure of the rhabdoms of oplophorid shrimps. *J. Morphol.* **257**, 87-95.

Glantz, R. M. (1968). Light adaptation in the photoreceptor of the crayfish, *Procambarus clarkii. Vision Res.* **8**, 1407-1421.

Goldsmith, T. H. (1972). The natural history of invertebrate visual pigments. In *Handbook of Sensory Physiology*, Vol. VII/1 (ed. H. J. A. Dartnall), pp. 727-742. New York: Springer-Verlag.

Govardovskii, V. I., Fyhrquist, N., Reuter, T., Kuzmin, D. G. and Donner, K. (2000). In search of the visual pigment template. *Vis. Neurosci.* **17**, 509-528.

Graham, C. H. and Hartline, H. K. (1935). The response of single visual sense cells to lights of different wavelengths. *J. Gen. Physiol.* **18**, 917-931.

Haddock, S. H. D. and Case, J. F. (1999). Bioluminescence spectra of shallow and deep-sea gelatinous zooplankton: ctenophores, medusae, and siphonophores. *Mar. Biol.* **133**, 571-582.

Herring, P. J. (1983). The spectral characteristics of luminous marine organisms. *Proc. R. Soc. Lond. B* **220**, 183-217.

Herring, P. J. (1985). Bioluminescence in the Crustacea. *J. Crustac. Biol.* **5**, 557-573.

Herring, P. J. (2007). Sex with the lights on? A review of bioluminescent sexual dimorphism in the sea. *J. Mar. Biol. Assoc. U. K.* **87**, 829-842.

Herring, P. J. and Barnes, A. T. (1976). Light-stimulated bioluminescence of *Thalassocaris crinita* (Dana). *Crustaceana* **31**, 107-110.

Hiller-Adams, P. and Case, J. F. (1984). Optical parameters of euphausiid eyes as a function of habitat depth. *J. Comp. Physiol. A* **154**, 307-318.

Hiller-Adams, P. and Case, J. F. (1985). Optical parameters of the eyes of some benthic decapods as a function of habitat depth (Crustacea, Decapoda). *Zoomorphology* **105**, 108-113.

Hiller-Adams, P. and Case, J. F. (1988). Eye size of pelagic crustaceans as a function of habitat depth and possession of photophores. *Vision Res.* **28**, 667-680.

Hiller-Adams, P., Widder, E. A. and Case, J. F. (1988). The visual pigments of four deep-sea crustacean species. *J. Comp. Physiol. A* **163**, 63-72.

Jerlov, N. G. (1976). *Marine Optics*. Amsterdam: Elsevier Scientific.

Jinks, R. N., Markley, T. L., Taylor, E. E., Perovich, G., Dittel, A. I., Epifanio, C. E. and Cronin, T. W. (2002). Adaptive visual metamorphosis in a deep-sea hydrothermal vent crab. *Nature* **420**, 68-70.

Johnsen, S. (2005). The red and the black: bioluminescence and the color of animals in the deep sea. *Integr. Comp. Biol.* **45**, 234-246.

Johnsen, S., Frank, T. M., Haddock, S. H. D., Widder, E. A. and Messing, C. G. (2012). Light and vision in the deep-sea benthos: I. Bioluminescence at 500–1000 m depth in the Bahamian Islands. *J. Exp. Biol.* **215**, 3335-3343.

Johnson, M. L., Shelton, P. M. J. and Gaten, E. (2000). Temporal resolution in the eyes of marine decapods from coastal and deep-sea habitats. *Mar. Biol.* **136**, 243-248.

Kilgour, M. J. and Shirley, T. C. (2008). *Eumunida picta* S. I. Smith, 1883, *and Lophelia pertusa* (Linnaeus, 1758): a relationship or just good friends? *Crustaceana* **81**, 587-593.

Kugel, M. (1977). The time course of the electroretinogram of compound eyes in insects and its dependence on spectral recording conditions. *J. Exp. Biol.* **71**, 1-6.

Lakin, R. C., Jinks, R. N., Battelle, B.-A., Herzog, E. D., Kass, L., Renninger, G. H. and Chamberlain, S. C. (1997). Retinal anatomy of *Chorocaris chacei*, a deep-sea hydrothermal vent shrimp from the Mid-Atlantic Ridge. *J. Comp. Neurol.* **385**, 503-514.

Lampitt, R. S., Bett, B. J., Kiriakoulakis, K., Popova, E. E., Ragueneauc, O., Vangriesheim, A. and Wolf, G. A. (2001). Material supply to the abyssal seafloor on the northeast Atlantic. *Prog. Oceanogr.* **50**, 27-63.

Land, M. F. and Nilsson, D. E. (2002). *Animal Eyes.* Oxford: Oxford University Press.

Laughlin, S. B. (1976). The sensitivities of dragonfly photoreceptors and the voltage gain of transduction. *J. Comp. Physiol.* **111**, 221-247.

Lindström, M., Nilsson, H. L. and Meyer-Rochow, V. B. (1988). Recovery from light-induced sensitivity loss in the eye of the crustacean *Mysis relicta* in relation to temperature: a study of ERG-determined *V*/log*I* relationships and morphology at 4°C and 14°C. *Zool. Sci.* **5**, 743-757.

Locket, N. A. (1977). Adaptations to the deep-sea environment. In *Handbook of Sensory Physiology*, Vol. VII/5 (ed. F. Crescitelli), pp. 67-192. Berlin: Springer-Verlag.

Loew, E. R. (1976). Light, and photoreceptor degeneration in the Norway lobster, *Nephrops norvegicus* (L.). *Proc. R. Soc. Lond.* **193**, 31-44.

Marshall, N. J., Cronin, T. W. and Frank, T. M. (2003). Visual adaptations in crustaceans: chromatic, developmental, and temporal aspects. In *Sensory Processing in Aquatic Environments* (ed. S. P. Collin and N. J. Marshall), pp. 343-372. Berlin: Springer-Verlag.

Matin, L. (1968). Critical duration, the differential luminance threshold, critical flicker frequency, and visual adaptation: a theoretical treatment. *J. Opt. Soc. Am.* **58**, 404-415.
Moeller, J. F. and Case, J. F. (1995). Temporal adaptation in visual systems of deep-sea crustaceans. *Mar. Biol.* **123**, 47-54.
Murray, J. and Hjort, J. (1912). *The Depths of the Ocean*. London: Macmillan.
Naka, K. I. and Rushton, W. A. H. (1966). S-potentials from colour units in the retina of fish (Cyprinidae). *J. Physiol.* **185**, 536-555.
Nishida, S., Ohtsuka, S. and Parker, A. R. (2002). Functional morphology and food habits of deep-sea copepods of the genus *Cephalophanes* (Calanoida: Phaennidae): perception of bioluminescence as a strategy for food detection. *Mar. Ecol. Prog. Ser.* **227**, 157-171.
O'Neill, P. J., Jinks, R. N., Herzog, E. D., Battelle, B.-A., Kass, L., Renninger, G. H. and Chamberlain, S. C. (1995). The morphology of the dorsal eye of the hydrothermal vent shrimp, *Rimicaris exoculata*. *Vis. Neurosci.* **12**, 861-875.
Somiya, H. (1980). Fishes with eye shine: functional morphology of guanine type tapetum lucidum. *Mar. Ecol. Prog. Ser.* **2**, 9-26.
Stark, W. S. and Wasserman, G. S. (1974). Wavelength-specific ERG characteristics of pigmented- and white-eyed strains of *Drosophila*. *J. Comp. Physiol.* **91**, 427-441.
Turner, J. R., White, E. M., Collins, M. A., Partridge, J. C. and Douglas, R. H. (2009). Vision in lanternfish (Myctophidae): adaptations for viewing bioluminescence in the deep-sea. *Deep Sea Res. Part I* **56**, 1003-1017.
Wada, M., Yamamoto, I., Nakagawa, M., Kogure, K. and Ohwada, K. (1995). Photon emission from dead marine organisms monitored using a video recording system. *J. Mar. Biotechnol.* **2**, 203-209.
Wald, G. (1968). Single and multiple visual systems in arthropods. *J. Gen. Physiol.* **51**, 125-156.
Warrant, E. J. (1999). Seeing better at night: life style, eye design and the optimum strategy of spatial and temporal summation. *Vision Res.* **39**, 1611-1630.
Warrant, E. J. and Locket, N. A. (2004). Vision in the deep sea. *Biol. Rev. Camb. Philos. Soc.* **79**, 671-712.
Widder, E. A. (1999). Bioluminescence. In *Adaptive Mechanisms in the Ecology of Vision* (ed. S. N. Archer, M. B. Djamgoz, E. Loew, J. C. Partridge and S. Vallerga), pp. 555-58. London: Kluwer Academic Publishers.
Widder, E. A., Latz, M. I. and Case, J. F. (1983). Marine bioluminescence spectra measured with an optical multichannel detection system. *Biol. Bull.* **165**, 791-810.
Zettler, F. (1969). Die Abhängigkeit des Übertragungsverhaltens von Frequenz und Adaptationszustand; Gemessen am einzelnen Lichtrezeptor von *Calliphora erythrocephala*. *Z. Vgl. Physiol.* **64**, 432-449.