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Spectral Sensitivity of Vision and Bioluminescence in the Midwater Shrimp *Sergestes similis*

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Abstract. In the oceanic midwater environment, many fish, squid, and shrimp use luminescent countershading to remain cryptic to silhouette-scanning predators. The midwater penaeid shrimp, *Sergestes similis* Hansen, responds to downward-directed light with a dim bioluminescence that dynamically matches the spectral radiance of oceanic downwelling light at depth. Although the sensory basis of luminescent countershading behavior is visual, the relationship between visual and behavioral sensitivity is poorly understood. In this study, visual spectral sensitivity, based on microspectrophotometry and electrophysiological measurements of photoreceptor response, is directly compared to the behavioral spectral efficiency of luminescent countershading. Microspectrophotometric measurements on single photoreceptors revealed only a single visual pigment with peak absorbance at 495 nm in the blue-green region of the spectrum. The peak electrophysiological spectral sensitivity of dark-adapted eyes was centered at about 500 nm. The spectral efficiency of luminescent countershading showed a broad peak from 480 to 520 nm. Both electrophysiological and behavioral data closely matched the normalized spectral absorptance curve of a rhodopsin with λ_{max} = 495 nm, when rhabdom length and photopigment specific absorbance were considered. The close coupling between visual spectral sensitivity and the spectral efficiency of luminescent countershading attests to the importance of bioluminescence as a camouflage strategy in this species.

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Introduction

In terrestrial, aquatic, and aerial environments, vision is central to many predator-prey interactions. Thus, camouflage (crypsis) is a common method of predator avoidance used by a wide variety of invertebrates and vertebrates. The oceanic midwater environment offers few structural refuges from predation. Visual predators commonly search for prey silhouetted against dim downwelling irradiance. Camouflage strategies adopted by midwater animals include transparency, reflective camouflage, and photophore-mediated counterillumination (reviewed by McFall-Ngai, 1990). For example, some animals such as gelatinous zooplankton have a refractive index similar to that of seawater, making them optically transparent (Chapman, 1976). Many fish use reflective camouflage to blend with the optical environment by simulating the angular distribution of oceanic light (Denton, 1970). Finally, animals may produce downward-directed bioluminescence, disrupting or minimizing their silhouette (Clarke, 1963; Herring, 1982; Young, 1983); this behavior is termed luminescent countershading or counterillumination.

For luminescent countershading to be effective, the optical properties of the bioluminescence must match those of the optical environment. Laboratory studies have demonstrated that the angular distribution, intensity, and spectral emission of luminescent countershading from many midwater animals match those of oceanic downwelling irradiance (reviewed by Young, 1983). Under certain conditions, some squid modify the spectral emission of their bioluminescence, presumably to match diel changes in the spectral composition of downwelling light (Young *et al.*, 1980).

The optical properties of the oceanic midwater environment derive from downwelling light and bioluminescence, and the relative importance of these components varies with

time of day, depth, and distance from the source of light. Far-field illumination consists of diffuse, dim downwelling light that decreases exponentially with depth and has an irradiance spectrum centered on 475 nm (Jerlov, 1968). Near-field illumination consists mainly of bioluminescence, manifested as point sources of light with emission spectra peaking primarily between 460 and 490 nm, depending on species (Herring, 1983; Widder *et al.*, 1983; Latz *et al.*, 1988). Deep-sea and midwater animals have well-developed eyes with unique adaptations for dealing with the *in situ* light conditions they encounter. Specialized features include large or tubular eyes (squids and fishes: Bowmaker, 1976; Locket, 1977), yellow-pigmented lenses that increase the contrast between bioluminescence and downwelling light (fishes: Muntz, 1976), rod-dominated retinae in fishes for greater light sensitivity (Bowmaker, 1976), and visual pigments with blue-shifted (470–490 nm) absorption maxima (fishes: Partridge *et al.*, 1989; Douglas *et al.*, 1995; cephalopods: Kito *et al.*, 1993; crustaceans: references in Frank and Case, 1988). These adaptations attest to the importance of bioluminescence and vision in the deep sea.

Thus there may be a close link between visual spectral sensitivity and the spectral efficiency of luminescent countershading (*i.e.*, behavioral spectral sensitivity) if luminescent countershading is to be a successful means of camouflage. In the simplest case, the visual system, bioluminescence emission, and oceanic downwelling light will all operate in the same spectral range. This hypothesis has never been tested, largely due to the difficulty in obtaining quantifiable behavioral data from midwater and deep-sea animals. Typically, these organisms survive for very short periods when brought to the surface, and controlled experimental studies of meaningful behaviors have been difficult to achieve. Studies by Land (1992) and Frank and Widder (1994a,b) are the only investigations of visually mediated swimming responses by midwater and deep-sea crustaceans under environmentally relevant illumination levels. The only examination of the spectral efficiency of bioluminescence in a marine organism was performed by Kay (1965) with the euphausiid crustacean, *Meganyctiphanes norvegica,* although the intensity of the photoflash stimulus employed was considerably brighter than *in situ* levels experienced by the animal.

The present study investigates visual and behavioral sensitivity in the bioluminescent penaeid shrimp, *Sergestes similis,* a common member of the midwater community in the northeast Pacific Ocean. Bioluminescence in *S. similis* originates from modified portions of the hepatopancreas, called organs of Pesta, which produce ventrally directed light. Previous laboratory studies indicate that the emitted light is consistent with a camouflage function. Bioluminescence is tuned to the optical properties of the midwater environment, matching the spectral distribution (Widder *et al.*, 1983), irradiance (Warner *et al.*, 1979), and angular distribution (Latz and Case, 1982) of downwelling oceanic light. In addition, ventrally directed dim glowing is produced only when a downward-directed light stimulus is present and is extinguished within seconds of the cessation of the stimulus (Warner *et al.*, 1979; Latz and Case, 1992).

Luminescent countershading in *Sergestes similis* is clearly dependent on vision; covering the eyes reversibly abolishes the response (Warner *et al.*, 1979). Shrimp that are completely dark adapted are initially unresponsive to light, but continued light exposure induces bioluminescence after a latency of several minutes, reaching maximum intensity about 20 min later (Latz and Case, 1992). Once luminescence is induced, responses exhibit the typical fast kinetics of luminescent countershading observed in squid and fishes; luminescence increases within several seconds of light stimulation and reaches maximum intensity in approximately 30 s (Latz and Case, 1992). Thereafter, the lack of eye shine suggests that the eyes are light adapted when luminescent countershading occurs.

In the present study, behavioral and physiological approaches were used to characterize the link between vision and luminescent countershading behavior in *Sergestes similis.* Results demonstrate a close coupling between visual spectral sensitivity and the spectral efficiency of luminescent countershading, further supporting the hypothesized role of bioluminescence in camouflaging this species.

Materials and Methods

Collection of animals and tissue

Adult specimens of *Sergestes similis* Hansen were collected at night from depths of 55 to 300 m in the San Diego Trough (8 Dec 1996, 12 Jun 1997, and 25 Jun 1997), the San Clemente Basin (10 Aug 1997), both near San Diego, California, USA, and in the Santa Barbara Basin (28 Sep 1997 and 24 Oct 1997), near Santa Barbara, California, during cruises of the *RV R.G. Sproul.* Animals were collected using a modified Tucker trawl with a closing light-proof cod end (after Childress *et al.*, 1977). The cod end was closed at depth and animals were brought to the surface and sorted in 5°C seawater under dim red light. Animals were placed in light-proof containers filled with chilled seawater and transported to the shore laboratory, where they were maintained in constant darkness in aquaria with flow-through, 5 μm filtered seawater at a temperature of 10°C. All experiments were performed within one week of collection; only actively swimming specimens were used for testing. Animals were not fed, and except for brief exposure to dim red light during handling, they remained in constant darkness.

For electroretinogram studies, animals collected on 8 Dec 1996 from the San Diego Trough were shipped in light-tight containers to the Harbor Branch Oceanographic Institution

(HBOI), Fort Pierce, Florida. At HBOI, shrimp were maintained in chilled (10°C) filtered seawater in constant darkness.

For microspectrophotometry experiments, adult *S. similis* were collected off the coast of southern California during a cruise of the *RV New Horizon* between 12 May and 26 May 1996 using similar equipment and methods (see Kent, 1997). Eyes were removed and cryopreserved for subsequent microspectrophotometry, which was carried out at the University of Bristol, UK. Eyes were orientated in plastic wells filled with cryomount (Tissue Tek, OCT Compound, Miles Inc., USA) and rapidly frozen with fluorocarbon spray (Cryospray 22, Bright Instrument Co., UK). Frozen blocks were individually sealed in plastic bags to avoid desiccation, placed in light-tight aluminum tubes, and maintained at −70°C until sectioned for microspectrophotometry.

Microspectrophotometry (MSP) experiments

Frozen eyes from adult *Sergestes similis* were sectioned using a cryostat and cut sections (average thickness 14 μm) were transferred to a 22 × 50 mm No. 1 coverslip, mounted in Tropic Marin™ artificial seawater, covered with a 19 mm diameter No. 1 coverslip, and sealed with a ring of silicone grease. Sectioning proceeded from the region of the cornea most distal to the eyestalk towards the center of the eye.

Visual pigment absorption was measured with a single beam, wavelength scanning, computer-controlled microspectrophotometer described by Hart *et al.,* (1998). For invertebrate MSP, the instrument was modified by the incorporation of a high intensity substage lamp that was used for photoconversion of the visual pigment in the rhodopsin (R) state to the metarhodopsin (M) state using red light, and for the photobleaching of R and M mixtures to photoproducts of non-physiological importance using actinic white light exposure. This protocol is fully described by Kent (1997) and follows the methods of Cronin and Goldsmith (1982).

Initial "baseline" scans were first made from 350 to 750 nm at 1-nm intervals in a tissue-free area of the cell preparation and then followed by several "sample" scans from rhabdomeric tissue. Sample scans were first made from an unexposed rhabdom which was then exposed to red light (wavelength of cut-on *ca.* 610 nm) for approximately 20 s to photoconvert the rhodopsin pigment to a stable R/M mixture, after which the rhabdom was re-scanned. The tissue was then photobleached by an exposure of approximately 30 min to white light and re-measured. Difference spectra between these data sets were calculated, from which the absorption spectra of the M and R pigments in the rhabdom were determined by the method of Cronin and Goldsmith (1982) as further developed by Kent (1997). In brief, visual pigment templates (Stavenga *et al.,* 1993; Palacios *et al.,* 1996) were fitted to the measured difference spectra to provide estimates of the λ_{max} of R and M pigments (Partridge and De Grip, 1991; Hart *et al.,* 1998). The fraction of R in the photo-steady state resulting from the red light exposure was estimated by comparing the integrated absorbances of the two templates to the red light. This fraction was then subtracted from the R/M mixture in the photo-steady state and a new template fitted to provide a better estimate of the λ_{max} of the M pigment. This traditional analytical method assumes that all the visual pigment in the initial measurements made from the rhabdom was in the form of R. The use of iterative template fitting methods developed by Kent (1997) attempted to avoid the need for this assumption, and provided estimates of the proportion of M in the initial measurements and the λ_{max} values of R and M.

Electroretinogram experiments

Specimens used for electrophysiological recordings were mounted under dim red light in a holder and suspended in a chamber filled with chilled (10°C) seawater. This arrangement allowed for enough pleopod movement to maintain respiratory currents across the gills. The electroretinogram (ERG), which is the summed mass responses from a large number of photoreceptor cells, was recorded with a 10 μm tip metal microelectrode (F. Haer & Co.) placed subcorneally under dim red light. The reference electrode was placed in the other eye, which was covered with black Vaseline to block out light, and a silver-chloride electrode grounded the water bath. Signals were amplified with an Xcell-3 Microelectrode amplifier (F. Haer & Co.), equipped with a high impedance probe to eliminate electrode polarization problems. Low frequency filters were set to minimal filtering (0.01–1 Hz) to minimize distortion due to AC amplification. Monochromatic test flashes were provided by a tungsten light source, coupled to a grating monochromator (Instruments SA) with 1 mm slits in place, and delivered to the eye through one branch of a bifurcated light pipe. The light pipe was placed 3 mm away from the eye, providing illumination which covered the whole eye. Flash duration of 100 ms was controlled by a Uniblitz shutter (Model VS14S) under computer control using LabView software (National Instruments, Inc.). Irradiance was controlled with a neutral density wheel under computer control, calibrated in units of photons $cm^{-2}\,s^{-1}$ with a UDT Optometer (Model S370) and a radiometric probe placed 3 mm from the tip of the light guide. The adapting light source for chromatic adaptation experiments was an incandescent light filtered with a 400 nm or 480 nm broadband filter (Melles Griot). The adapting light was delivered to the eye through the other branch of the bifurcated light guide, ensuring that both the adapting and test lights were acting on the same group of photoreceptor cells. Data were instantaneously analyzed for

peak to peak response height using a program written in LabView, digitized and stored to disk for later analysis.

The eye was stimulated with 100 ms test flashes of monochromatic light adjusted for irradiance until either a 100 or 200 μV criterion response was obtained at each wavelength tested. To ensure that the eye remained in the same state of dark adaptation during the experiment, the response to a flash of standard wavelength and irradiance was tested periodically throughout the experiment. Spectral sensitivity curves were generated based on the inverse of the irradiance required to produce the criterion response at each wavelength. Absorptance spectra were constructed from visual pigment templates (Stavenga *et al.,* 1993).

Behavioral sensitivity experiments

Because *Sergestes similis* produces bioluminescence only in the presence of a light stimulus, a chopped light source was used to avoid detection of the stimulus illumination by the light detector (see below). Bioluminescence was measured in the brief dark intervals when the stimulus was off, similar to the methods of Warner *et al.* (1979) and Latz and Case (1992). Illumination from a Dolan-Jenner model 180 tungsten-halogen source was conducted through an optical fiber bundle to an Optometrics model DMC1 monochromator and then through another optical fiber bundle to a JML Optical electro-mechanical shutter that controlled the timing of the "on" and "off" transitions of the stimulus. The stimulus light was chopped at 80 Hz using an Oriel variable frequency chopper, attenuated by neutral density filters to control light intensity, and diffused by a single layer of glassine paper before entering the test chamber. Because the critical flicker-fusion rate at such low irradiances is <60 Hz for marine crustaceans (Waterman, 1961; Frank, 1999), the test animal perceived the chopped stimulus light as continuous.

For testing, specimens were loosely restrained by a clear acrylic clamp around the cephalothorax and placed in a sealed, clear acrylic chamber (1.75 × 2.5 × 10 cm) filled with 10–11°C seawater. The clamp allowed free movement of pleopods and other appendages, yet prevented the specimen from shifting position. Throughout an experiment, seawater chilled to 11–12°C by a Fisher Scientific model 1016S recirculating chiller was recirculated through the chamber at a rate of 100 ml min^{-1} using a Masterflex peristaltic pump. The specimen chamber was placed in the center of the light collection chamber, which consisted of a 25 cm diameter Labsphere integrating sphere. The advantage of an integrating chamber is that the measurement of emitted light is minimally affected by photophore or animal position. Bioluminescence was detected by a Burle model 8850 photon-counting photomultiplier operating at −1790 V, after passing through a second optical chopper operating at 80 Hz but synchronized 180° out of phase with the stimulus light chopper by means of a Scitec Instruments synchronizer. Thus, the photomultiplier measured only the bioluminescence produced by the test specimen and not the stimulus light. The photomultiplier signal was processed by a Pacific Instruments amplifier/discriminator; square wave pulses were sent to a Newport Instruments model P6000A frequency to voltage converter. The resulting voltage was measured using a Data Translation model 2801 data acquisition board mounted in a personal computer. Data acquisition, real-time display, and storage were controlled by a data collection program programmed using Data Translation DTVEE software. Bioluminescence and photodiode levels were continuously acquired at 2 Hz. Voltages were subsequently converted to photon values based on a photometric calibration of the photomultiplier using 1 ml of Cyalume® chemiluminescent liquid and a calibrated Quantalum 2000 photometer. Bioluminescence in units of photons $m^{-2} s^{-1}$ was obtained by dividing photon flux (photons s^{-1}) by the cross-sectional area of the ventral surface of the organs of Pesta for an adult shrimp of size 14 mm carapace length (Latz, 1983).

To measure stimulus intensity, a Graseby Optronics model 260 calibrated sensor head was mounted in the integrating sphere in the same position as the specimen chamber. Light levels measured in watts with a Graseby Optronics model S370 Optometer were converted to irradiance units of photons $m^{-2} s^{-1}$.

Quantum sensitivity. To determine the threshold level of light that prompts luminescent countershading by *Sergestes similis,* bioluminescence produced by induced animals in response to various intensities of light was measured. To induce luminescent countershading, dark-adapted animals were exposed to a standard light stimulus (490 nm, 2.24×10^{13} photons $m^{-2} s^{-1}$) for 25 min (Latz and Case, 1992); wavelength and irradiance settings were chosen based on preliminary experiments. After induction, shrimp were exposed to a series of test stimuli as follows: 60 s darkness, 60 s test stimulus, 60 s darkness, 5 or 10 min standard stimulus (490 nm, 2.24×10^{13} photons $m^{-2} s^{-1}$), repeating this pattern with new test stimuli until all irradiances were tested, typically within 2 h. The purpose of the standard stimulus was to maintain shrimp in the light-adapted induced state for luminescent countershading so that their bioluminescence responses to the test stimuli would show fast kinetics representative of luminescent countershading (Latz and Case, 1992). In addition, the responses to the standard stimulus were used to monitor the condition of the animal, and, if necessary, correct for changes in animals' responsiveness over time (see data correction below). The duration of the standard stimulus was determined by the return to the level of bioluminescence measured during the initial induction. Typically, this occurred within 5 min, but occasionally, after the dimmest irradiance test stimuli, 10 min of the standard stimulus was required. Test stimuli of

490 nm light were used at the following irradiance levels (in units of photons $m^{-2} s^{-1}$): 2.22×10^{11}, 3.45×10^{11}, 6.41×10^{11}, 3.95×10^{12}, 1.01×10^{13}, 2.24×10^{13}, 3.40×10^{13}, 3.87×10^{13}, 4.98×10^{13}, 7.65×10^{13}, 9.72×10^{13}.

Spectral sensitivity. To measure the spectral efficiency of luminescent countershading, dark-adapted animals were first exposed to a 490-nm stimulus (at 2.24×10^{13} photons $m^{-2} s^{-1}$) for 25 min to induce counterillumination. Animals were then exposed to light stimuli at 20-nm increments from 400 to 640 nm, following the general protocol described above: 60 s darkness, 60 s test wavelength stimulus, 60 s darkness, 5 or 10 min of the standard illumination to maintain the induced countershading state. This process was repeated for each wavelength tested. The average duration of a complete trial was 3 h. The order of test wavelengths was randomized. Because shrimp eyes operate as photon counters, and the total number of photons in a given stimulus is a function of both light intensity and wavelength, stimulus irradiance was adjusted at each wavelength using neutral density filters to obtain equal photon irradiance levels at each wavelength. Even so, there were slight variations in stimulus irradiance levels, which ranged from 1.16×10^{13} photons $m^{-2} s^{-1}$ at 400 nm to 2.52×10^{13} photons $m^{-2} s^{-1}$ at 620 nm. Bioluminescence data were subsequently corrected as detailed below to reflect a standard irradiance of 1.20×10^{13} photons $m^{-2} s^{-1}$. Mean bioluminescence was based on the last 20 s of each 60-s test stimulus, and the last 4 min for the standard 5–10-min illumination.

Data correction. Because the test stimuli in the spectral sensitivity experiments varied slightly in intensity, all data were corrected to reflect the intensity of bioluminescence at each wavelength based on a stimulus irradiance of 1.20×10^{13} photons $m^{-2} s^{-1}$. Data collected in the quantum sensitivity experiments were used to model the correlation between bioluminescence intensity and stimulus intensity at 490 nm. One of two regression equations (for either San Diego or Santa Barbara collected specimens) was used to calculate (1) the predicted bioluminescence at 1.20×10^{13} photons $m^{-2} s^{-1}$, and (2) the predicted bioluminescence at the irradiance level measured for each test wavelength stimulus. Dividing (1) by (2) gave a proportional correction factor that was multiplied by the bioluminescence value at each test wavelength. Because not enough specimens were available to empirically derive the relationship between stimulus intensity and bioluminescence intensity at every wavelength tested, the assumption was made that quantum sensitivity did not change with stimulus wavelength, so that the relationship observed at 490 nm holds for the other wavelengths. This assumption of univariance is supported by the visual sensitivity and MSP experiments which demonstrated that only a single visual pigment is present.

The correction for variable stimulus intensity also assumes that the observed relationship between bioluminescence intensity and stimulus intensity holds at all levels of light adaptation or for changes in specimen responsiveness due to fatigue. This is an important assumption because in 4 of 9 spectral efficiency experiments, bioluminescence intensity showed slight but significant decreases over the course of the experiment. Nevertheless, in all cases the bioluminescent responses to test stimuli showed the characteristic fast kinetics indicative of the induced counterillumination condition (Latz and Case, 1992). In those 4 specimens demonstrating a decrease in the standard response at 490 nm, a correction factor for each data point for each of these individuals was determined based on a linear regression describing the intensity of bioluminescence in response to the standard stimuli as a function of time.

The effects of these corrections are shown for a single individual (Fig. 1). Responses were corrected for a standard stimulus irradiance of 1.20×10^{13} photons $m^{-2} s^{-1}$ as shown in the following example. For this individual, the relationship between bioluminescence irradiance (in original units of volts s^{-1}) and stimulus irradiance at 490 nm (the standard stimulus wavelength) is best described by the equation: bioluminescence $= 3.66 \times 10^{-6} *$ irradiance$^{0.391}$ ($r^2 = 0.40$, F = 20.03, d.f. = 1,31, $P = 0.0001$). Using this regression equation, the predicted bioluminescence at an irradiance of 1.20×10^{13} photons $m^{-2} s^{-1}$ is 0.4805 volts s^{-1}. For a test wavelength of 400 nm, the measured stimulus irradiance was 1.28×10^{13} photons $m^{-2} s^{-1}$. The predicted bioluminescence (using the regression equation) for a 490 nm stimulus at this irradiance level is 0.4924 volts s^{-1}, giving a correction factor of 0.976 (0.4805/0.4924). Thus, for a 490-nm stimulus, bioluminescence intensity at 1.20×10^{13} photons $m^{-2} s^{-1}$ is 0.976 times that at 1.28×10^{13} photons $m^{-2} s^{-1}$. Making the important assumption that quantum sensitivity does not change with stimulus wavelength, the bioluminescence value measured at 400 nm is multiplied by 0.976 to reflect the response to a "standard"

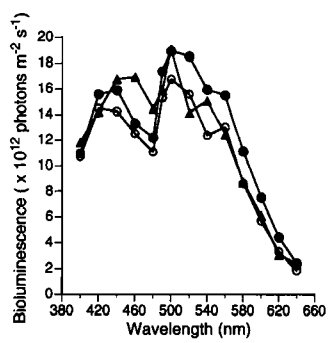

Figure 1. Correction of spectral efficiency data for a single specimen of *Sergestes similis*. Uncorrected data (solid circles) were corrected for variations in stimulus intensity (open circles) and for both stimulus intensity and temporal decrease in responsiveness (closed triangles). Refer to Materials and Methods for details on data correction.

stimulus of 1.20×10^{13} photons $m^{-2}\ s^{-1}$. This correction was made in turn for each stimulus wavelength.

Data from this individual were also corrected for a decrease in responsiveness, because the least-squares linear regression between bioluminescence intensity (in volts s^{-1}) and the order of presentation of the 490 nm standard stimulus showed a slight but significant decay in response according to the following equation: bioluminescence = -0.022 * (order of presentation) + 0.887 ($r^2 = 0.72$, F = 36.35, d.f. = 1,15, $P < 0.0001$). Because the response to 490 nm standard stimuli was somewhat variable over time, this regression equation is the best description of the general decay in response. The decay correction factor was calculated based on time of stimulus presentation by first calculating the predicted bioluminescence at a given stimulus time using the previous equation, and then dividing that result by the bioluminescence measured at the first 490 nm standard stimulus. For example, bioluminescence in response to the fifth 490 nm standard stimulus was calculated to be 0.7771 using this regression equation. Dividing this value by the value for the first standard stimulus (0.7857) gives a decay correction factor of 0.989. Making similar calculations for the order of presentation of each test wavelength stimulus, the bioluminescence value for each test stimulus was divided by the appropriate decay correction factor. The decay correction assumes that the effects were equivalent at all wavelengths tested. Following corrections for stimulus intensity and response decay, all bioluminescence data were converted from units of volts s^{-1} to photons $m^{-2}\ s^{-1}$ as previously described, based on the photometric calibration of the photomultiplier and the cross-sectional area of the ventral surface of the organs of Pesta for an adult shrimp of size 14 mm carapace length (Latz, 1983). To directly compare visual and behavioral spectral sensitivity, irradiance values from the electrophysiological experiments and bioluminescence values from the behavioral experiments were normalized for each individual.

Results

Microspectrophotometry (MSP)

Spectral absorbance, based on MSP measurements of five sections of rhabdomeric tissue from a single individual, was unimodal with maximum absorbance in the blue-green (Fig. 2A). Assuming that the initial scans were uncontaminated with metarhodopsin (M) pigment, template fitting to the difference spectra (Fig. 2B) indicated a rhodopsin (R) pigment with a λ_{max} of 492 nm. The template best-fitting the R/M mixture after red light exposure yields a λ_{max} of 485 nm. This value was corrected to allow for the residue of R in the R/M mixture to give a best estimate of the M pigment λ_{max} of 484 nm, and an M/R extinction ratio (at the respective λ_{max} values) of 1.333. Iterative template-fitting methods (Kent, 1997) suggest, however, that the fraction of M in the initial measurements may have been as high as 15% and, after correction for this contamination, the R and M λ_{max} values can be revised to 495 nm and 484 nm, respectively, with a M/R extinction ratio of 1.406.

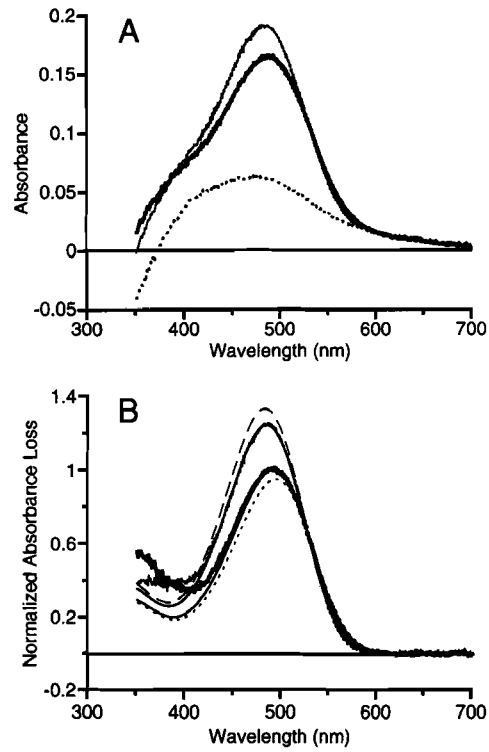

Figure 2. Spectral absorbance based on microspectrophotometry of five sections of rhabdomeric tissue from the retina of *Sergestes similis*. (A) Averaged absorbance spectra; bold trace shows the initial absorbance, light trace shows the absorbance following saturating red light illumination, dashed trace shows the absorbance following photobleaching with bright white light. For display, spectra have been standardized to an absorbance of zero at 730 nm, the limit of the spectral scan. (B) Averaged difference spectra for photobleaching of the rhabdom from its initial state [bold; derived from bold trace minus dotted trace in (A)] and for the photobleaching of the rhabdom from its steady state R/M mixture following saturating red light [light; derived from light trace minus dotted trace in (A)]. Specific absorbances at the λ_{max} for these absorbance spectra were 0.0078 μm^{-1} and 0.0094 μm^{-1}, respectively. Smooth solid traces are best-fit templates (Stavenga *et al.*, 1993) with λ_{max} values of 492 nm and 485 nm, respectively. The dashed line is the estimated metarhodopsin absorbance spectrum (λ_{max} = 484 nm) resulting from the correction for the residual M in the R/M mixture difference spectrum (light trace), and the absorbance spectrum of the rhodopsin (dotted; λ_{max} = 495 nm), after correcting for contaminating M in the initial scan (bold).

Visual sensitivity of the eye (ERG)

The electrophysiologically determined visual spectral sensitivity of dark-adapted specimens of *Sergestes similis* indicated that the sensitivity maximum was centered at approximately 500 nm in the blue-green region of the spectrum (Fig. 3). Both blue (480 nm) and near-UV (400 nm) chromatic adaptation uniformly depressed the sensitivity

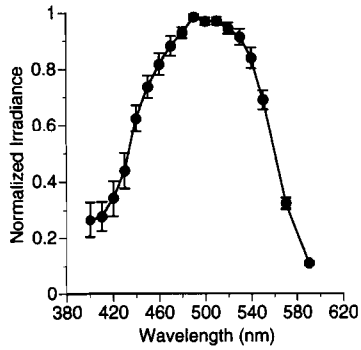

Figure 3. Visual spectral sensitivity of *Sergestes similis* based on electroretinogram (ERG) measurements. Sensitivity, based on the inverse of the irradiance required to elicit a 100 or 200 μV response, showed a broad maximum centered around 500 nm. Symbols represent means ± SE for 6 specimens.

curve across the spectrum (Fig. 4), and had no effect on ERG waveform (Fig. 5). These results are consistent with those of the MSP study indicating that only a single visual pigment is present.

Behavioral sensitivity

The magnitude of luminescent countershading by *S. similis* depended on the level of stimulus irradiance (Fig. 6).

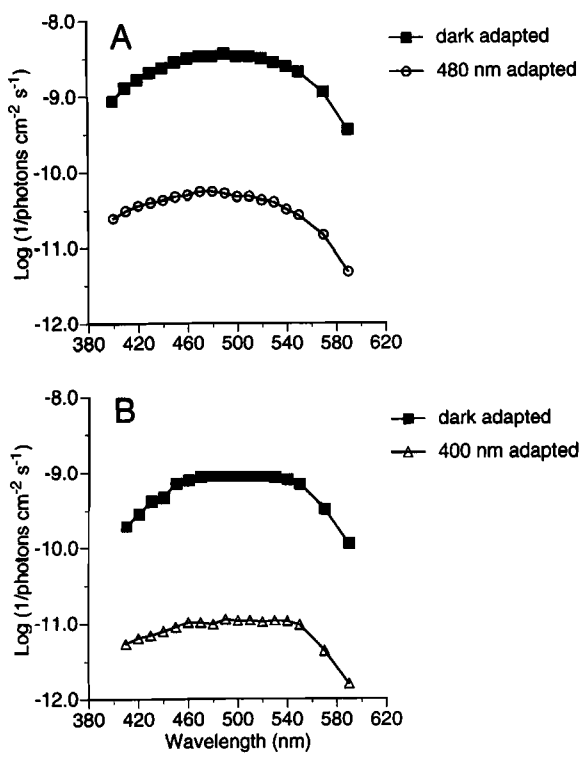

Figure 4. Effect of chromatic adaptation on the spectral sensitivity of *Sergestes similis*. Illumination of the eyes with (A) 480 nm, and (B) 400 nm light uniformly depressed spectral sensitivity across the spectrum compared to the response of dark-adapted eyes.

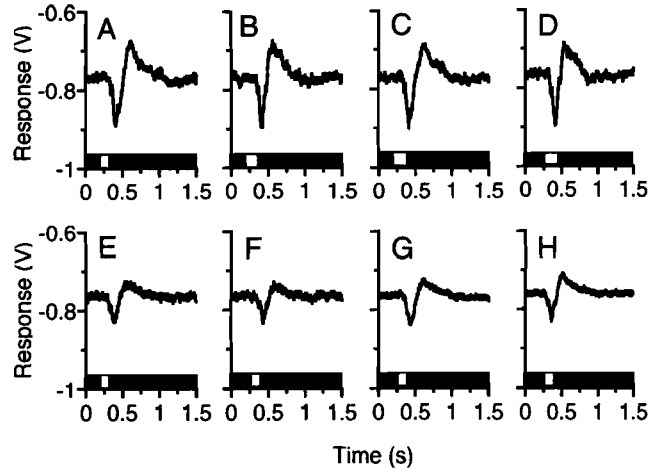

Figure 5. Effect of chromatic adaptation on the ERG waveform of *Sergestes similis*. Dark bars indicate light stimulus off; white bars indicate light stimulus on. (A–D) 200 μV responses measured in one specimen. (E–H) 100 μV responses measured in another specimen. Response to a 430 nm stimulus for the (A) dark-adapted condition and (B) after 400 nm light adaptation. Response to a 530 nm stimulus for the (C) dark-adapted condition and (D) after 400 nm light adaptation. Response to a 430 nm stimulus for the (E) dark-adapted condition and (F) after 480 nm light adaptation. Response to a 530 nm stimulus for the (G) dark-adapted eye and (H) after 480 nm light adaptation.

A behavioral threshold occurred at approximately 2–3 × 10^{12} photons $m^{-2}\,s^{-1}$ (Fig. 7). At lower stimulus irradiances, light levels were near background as measured in an empty chamber. At higher stimulus irradiances, bioluminescence increased as the 0.35 power of irradiance according to a power law (log-log) regression (r^2 = 0.61). Thus the increase in bioluminescence by *S. similis* did not match the increase in stimulus irradiance.

The magnitude of bioluminescence varied according to stimulus wavelength (Fig. 8). After correcting the data to reflect a standard irradiance of 1.20 × 10^{13} photons $m^{-2}\,s^{-1}$, and when necessary, for temporal decreases in responsiveness, the mean spectral efficiency curve for luminescent countershading showed a broad peak between 480 and 540 nm (Fig. 9) in the blue-green region of the visible spectrum. The wavelength dependence of the spectral efficiency was not symmetrical. Bioluminescence decreased dramatically approaching the red wavelengths and was negligible above 600 nm, while an intermediate response still occurred in the near-UV at 400 nm. A comparison of the mean data for all 9 specimens tested showed that the corrected data fell within the 95% confidence limits of the original data.

Discussion

In the midwater shrimp, *Sergestes similis*, there is a close similarity between visual and behavioral photon and spectral sensitivities. The microspectrophotometry results indi-

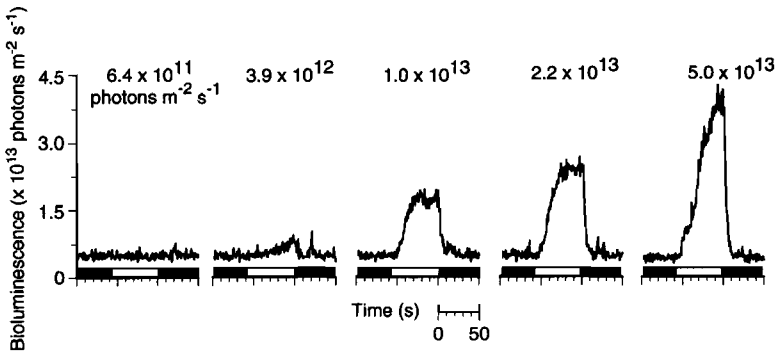

Figure 6. Representative bioluminescent responses by a single specimen of *Sergestes similis* to increasing stimulus irradiance. Bioluminescence was first induced using a 490 nm stimulus of intermediate intensity, then the specimen was presented with 60-s stimuli from a range of intensities, all at 490 nm. The line represents the level of bioluminescence versus time. Dark bars indicate stimulus light off; white bars indicate stimulus light on. Values above graphs are stimulus intensities.

cated that *S. similis* possesses a single visual pigment with maximum absorbance around 495 nm. Such data can be used to calculate the spectral sensitivity of a photoreceptor containing this visual pigment by first calculating the spectral absorptance of an axially illuminated rhabdom. To do this, two additional pieces of information are required: rhabdom length and the specific absorbance (*i.e.*, absorbance μm^{-1}) of the visual pigment in the photoreceptor. Rhabdom lengths vary somewhat with eye size, but by sectioning the aldehyde preserved eyes of *S. similis*, rhabdom lengths were found to range from 128 to 161 μm for shrimps with carapace lengths of 11.8 to 14.2 mm (T. Frank, unpublished data). For the specimens used in the ERG experiments, which ranged in size from 10.3 to 13.5 mm carapace length, the estimated upper and lower bounds for rhabdom lengths in these animals were approximately 120 to 150 μm, with a mean of approximately 135 μm. This value is similar to that reported by Hiller-Adams *et al.* (1988) for the sergestid *Sergia tenuiremis*. MSP measurements of specific absorbance for *S. similis* suggest a specific absorbance of 0.0074 μm^{-1} (Kent, 1997) although this is significantly lower that the value of 0.01 μm^{-1} reported by Cronin and Frank (1996) in *Systellaspis debilis*, but only slightly lower than the value of 0.008 μm^{-1} reported as being 'typical' of crustacean photoreceptors (*e.g.*, Cronin and Goldsmith, 1982). At wavelengths greater than the peak absorbance (495 nm), the spectral absorptance closely matches spectral sensitivity data from ERG measurements and the spectral efficiency curve of luminescent countershading (Fig. 10). Using maximum or minimum values instead of mean rhabdom length has little effect on spectral absorptance, while increasing specific absorbance to levels more typical of crustacean photoreceptors leads to a better fit between the different data sets at long wavelengths. At short wavelengths, however, there is significant divergence which probably cannot be attributed to the photosensitivity spectrum of the rhodopsin departing from the absorptance spectrum at short wavelengths, although data on this subject are limited (Dartnall, 1972). It is more likely that the effective spectral sensitivity of the eye is affected by intraocular, pre-retinal filters which selectively filter short wavelength light (Goldsmith, 1978; reviewed by Fein and Szuts, 1982).

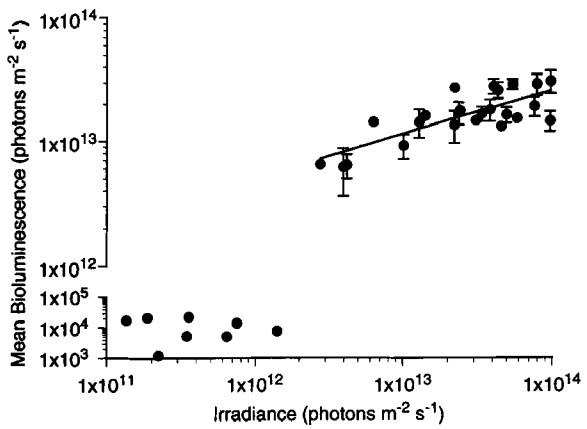

Figure 7. Effect of light intensity on average maximum bioluminescence produced by *Sergestes similis*. All stimuli were at a wavelength of 490 nm. The magnitude of bioluminescence measured during the last 20 s of each 60-s test stimulus was averaged for each individual. Ten specimens were tested; means ± SE are shown. Responses for stimulus irradiance > 2×10^{12} photons m^{-2} s^{-1} were best described by the power law (log-log) equation $y = (1.87 \times 10^6)x^{0.35}$ ($r^2 = 0.61$). At lower stimulus irradiance values, light levels were near background. Note the separate scale for above-threshold bioluminescence.

Electrophysiological measurements using the electroretinogram (ERG), corresponding to the summed mass response of a large number of photoreceptor cells to a light stimulus, were performed to determine the behaviorally relevant spectral sensitivity of *S. similis*. While this technique provides a more comprehensive assessment of the visual spectral sensitivity of an organism than do measure-

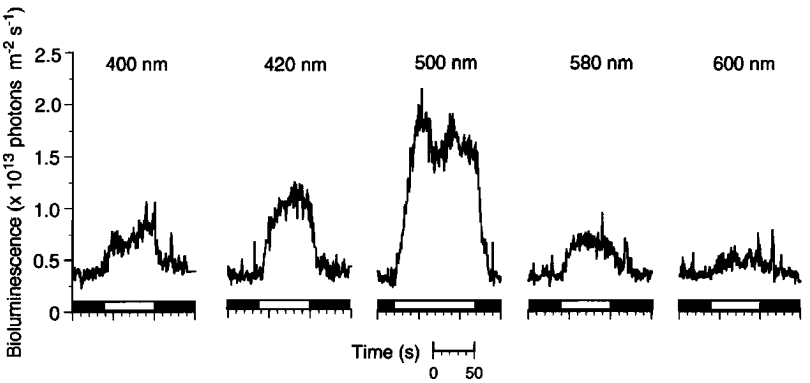

Figure 8. Representative bioluminescent responses by a single specimen of *Sergestes similis* to different wavelengths of light. Bioluminescence was first induced using a 490 nm stimulus of intermediate intensity, then the specimen was presented with 60 s stimuli at various wavelengths, at approximately equal irradiance of 1.5×10^{13} photons m^{-2} s^{-1}. Bars as for Figure 5.

ments from single photoreceptors (reviewed by Goldsmith, 1986), ERG results do not reflect the amount of higher order processing of visual input, nor the behavioral response to visual stimuli. Thus the behavioral studies of luminescent countershading extend the physiological assessment, resulting in a comprehensive description of the organism's sensory and behavioral response to ecologically relevant light stimulation.

Luminescent countershading by *S. similis* occurred over a relatively narrow range of irradiance. A behavioral threshold occurred at approximately 3×10^{12} photons m^{-2} s^{-1}, as lower irradiance levels resulted in minimal levels of bioluminescence which were not significantly different from background. This illumination level may represent the minimum irradiance causing light adaptation of the eye, which appears to be required for luminescent countershading (Latz and Case, 1992). Under ideal conditions, bioluminescence should exactly match stimulus irradiance. As discussed by Young *et al.* (1980), differences in geometry between stimulus and response as well as calibration assumptions make direct comparisons difficult, although relative changes should still be valid. In the present study, the range of stimulus irradiance tested was less than two orders of magnitude. Within this range, bioluminescence increased with stimulus irradiance according to a power law (log-log) regression as found by Young *et al.* (1980) for midwater squid and fish. However, the magnitude of the increase in bioluminescence did not match the magnitude of the increase in stimulus irradiance. Other counterilluminating animals may not precisely match changes in the stimulus irradiance (Young *et al.*, 1980), yet they exhibit a better match over a larger dynamic illumination range for luminescent countershading than did *S. similis* in the present study.

In *Sergestes similis,* behavioral spectral efficiency was similar to visual spectral sensitivity. A survey of species for which behavioral and physiological spectral sensitivity data are available (Table I) suggests that behavioral spectral

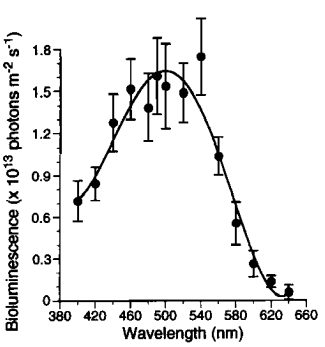

Figure 9. Behavioral spectral sensitivity of *Sergestes similis* based on the bioluminescence spectral efficiency, corrected for a stimulus irradiance of 1.2×10^{13} photons m^{-2} s^{-1} (see text). Symbols represent means ± SE for 9 specimens; the curve is a fourth degree polynomial function fitted to the data, where $y = 6.1 \times 10^{4}x^{4} - 1.2 \times 10^{8}x^{3} + 9.2 \times 10^{10}x^{2} - 3.0 \times 10^{13}x + 3.6 \times 10^{15}$.

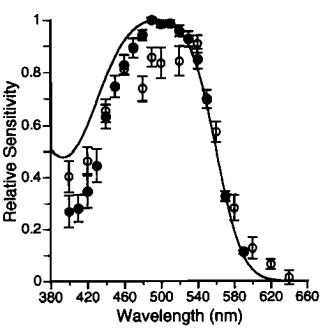

Figure 10. Comparison of relative visual and behavioral spectral sensitivity of *Sergestes similis.* Symbols represent means ± SE. Both the normalized ERG sensitivity (solid circles) and normalized bioluminescence spectral efficiency (open circles) coincide well at long wavelengths with the calculated spectral absorbance (solid line) of a rhodopsin with peak absorbance at 495 nm, a rhabdom axial length of 135 μm and a specific absorbance of 0.0074 μm^{-1}.

Table I

Comparison of behavioral and visual spectral sensitivities of several species

Species	Behavioral λ_{max} (nm)	Behavior assayed	Visual λ_{max} (nm)*	Photopigment absorption λ_{max} (nm)	References
Bioluminescent					
Photinus pyralis (Firefly)	565	Female BL flash in response to simulated male BL flash	565	Rhodopsin, 545; screening pigment, 517 (MSP)	Worthy and Lall, 1996
Photinus scintillans (Firefly)	575	Initiation of BL flashing, horizontal light stimulus	Approx. 435, approx. 575, possibly 360	2 receptors with near-UV and yellow peak sensitivity; screening pigment, 525 (MSP)	Lall, 1993 Lall *et al.*, 1988
	360, 425	Initiation of BL flashing, vertical light stimulus			
Meganyctiphanes norvegica (euphausiid crustacean)	Broad peak from 470–490	Number of individuals producing BL in response to photoflash	490	488 (MSP)	Kay, 1965 Denys and Brown, 1982 Frank, unpubl. data
Sergestes similis (penaeid shrimp)	Broad peak from 480–540	Magnitude of bioluminescence	500	Rhodopsin, 495 (MPS)	Present study
Non-bioluminescent					
Nautilus pompilius (cephalopod mollusc)	470	Positive phototaxis	NA	Rhodopsin based, 467 (PE)	Muntz, 1986, 1987
Clupea harengus (herring)	510–520	Feeding, phototaxis, barrier perception	NA	500 (PE)	Blaxter, 1964
Gadus morhua (cod)	460–520 and 550 (dark adapted) 400–470 (light adapted)	Heart rate, using cardiac conditioning protocol	490 (dark adapted) 490 and 550–590 (light adapted)	NA	Anthony and Hawkins, 1983
Hyalomma dromedarii (tick)	380 and 480–520	Positive phototaxis	470, weak secondary max in UV	NA	Kaltenrieder *et al.*, 1989
Amblyomma variegatum (tick)	480–520	Positive phototaxis	470	NA	Kaltenrieder *et al.*, 1989
Hyla cinerea (treefrog)	430 and 500	Optomotor response	NA	Two pigments: 435 and 503	King *et al.*, 1993

In most examples, data are drawn from several sources rather than from a single comprehensive study. Studies were limited to those that measured sensitivity in response to a broad spectrum of wavelength stimuli. Maximum absorbance by photopigments was determined by microspectrophotometry (MSP) or pigment extraction (PE). BL = bioluminescence/bioluminescent, ERG = electroretinogram, NA = not available or unknown.

* Visual λ_{max} based on ERG data except for Worthy and Lall (1996), where it is based on both ERG data and intracellular retinal recordings.

Table II

Relative behavioral and visual sensitivities to near-UV versus blue-green light for bioluminescent deep-sea crustaceans possessing a single visual pigment

Species	Visual Spectral Sensitivity Maximum (nm)	Relative Visual Sensitivity (400 nm/500 nm)	Relative Behavioral Sensitivity (400 nm/500 nm)
Acanthephyra curtirostris	510	0.24	0.18
Acanthephyra smithi	510	0.30	0.16
Notostomus gibbosus	490	0.24	0.10
Sergestes similis	500	0.27	0.48

For species except *Sergestes similis,* behavioral sensitivity was defined as the reciprocal of the irradiance required to elicit a simple movement behavior for 400 nm or 500 nm stimuli (Frank and Widder, 1996). For *S. similis,* behavioral sensitivity was based on the standardized magnitude of bioluminescence. For all species, visual sensitivity was based on electroretinogram (ERG) measurements (data from present study and Frank and Case, 1988).

sensitivity tends to be somewhat broader than that measured electrophysiologically, though the behavioral maxima are similar to maxima in visual sensitivity and photopigment absorption. Douglas and Hawryshyn (1990) noted that behavioral measurements of spectral sensitivity in fish varied with method, with some behaviors, such as the tail-flip response, being activated only by certain wavelength stimuli. Behavioral sensitivity also may depend on the type of stimulus applied. For example, the initiation of bioluminescent flashing by the firefly, *Photinus scintillans,* has different spectral sensitivity maxima for horizontal and vertical light stimuli (Table I; Lall, 1993). These different sensitivities appear to be associated with different classes of photoreceptors. Similarly, multiple peaks in behavioral sensitivity in the tree frog *Hyla cinerea* (King *et al.,* 1993) and the tick *Hyalomma dromedarii* (Kaltenrieder *et al.,* 1989) correspond to peak sensitivities of different classes of photoreceptors in these animals (Table I).

Sergestes similis shows somewhat greater behavioral sensitivity in the near-UV compared to several other species of deep-sea decapods that have single visual pigments and similar ERG-measured visual sensitivities (Table II; Frank and Widder, 1996). This difference may be a reflection of the different behaviors assayed (*i.e.,* luminescent countershading versus movement behaviors) and their ecological context. The ecological significance of sensitivity to near-UV and UV light remains unknown. The intensity of downwelling irradiance at mesopelagic depths, calculated utilizing attenuation coefficients measured in the epipelagic zone, may be sufficient to be visually detected (Frank and Widder, 1996). High UV visual sensitivity may aid in luminescent countershading where the strategy is to match the downwelling irradiance field. Alternatively, UV sensitivity may play a role in regulating the diurnal vertical migrations of *S. similis* and other vertically migrating crustaceans (Forward, 1988).

The present study is the first to measure the complete spectral efficiency of behavior in a deep-sea animal using ecologically relevant light stimuli. Previously, Kay (1965) measured peak behavioral spectral sensitivity of 470–490 nm in the euphausiid, *Meganyctiphanes norvegica,* based on the number of individuals responding to bright photoflash stimulation. The peak in spectral efficiency corresponds to the visual pigment absorbance maximum at 488 nm determined by MSP (Denys and Brown, 1982) and to the peak visual sensitivity of 490 nm measured by electroretinogram (Frank and Widder, in press). As with *S. similis,* both visual and behavioral peaks for *M. norvegica* lie in the blue-green region of the visible spectrum. Even though it has long been suggested that bioluminescence by euphausiid crustaceans may serve as camouflage (Herring and Locket, 1978), to date there has been no direct demonstration that dim bioluminescence appropriate for luminescent countershading is produced by euphausiids in response to environmentally relevant light cues.

The response thresholds for light-induced behaviors of three species of deep-sea caridean shrimp with single visual pigments occurs at approximately $0.4–4 \times 10^{11}$ photons $m^{-2}\ s^{-1}$ at 500 nm (Frank and Widder, 1994b). Because the eyes of the caridean shrimp were in the dark-adapted state, it is expected that the response threshold for *S. similis* bioluminescence would occur at higher illumination levels because of the apparent need for the eyes to be light-adapted for the initiation of luminescent countershading (Latz and Case, 1992). The *S. similis* response threshold of $2–3 \times 10^{12}$ photons $m^{-2}\ s^{-1}$ at 490 nm for the light-adapted eye suggests that the absolute visual sensitivity of *S. similis* is similar to that for the caridean shrimp.

Sergestes similis and other midwater animals inhabit daytime depths where dim downwelling light is sufficient to silhouette their opaque body structures, making them potentially more detectable by predators. Previous studies have demonstrated that the spectral emission (Widder *et al.,* 1983), angular distribution (Latz and Case, 1982) and irradiance of bioluminescence (Warner *et al.,* 1979) of *S. similis* are consistent with a camouflage function. The results of the present study show that the behavioral sensitivity of bioluminescence is also appropriate for camouflage, based on the daytime optical environment encountered by this species. In the northeast Pacific, *S. similis* inhabits daytime depths of approximately 200–700 m (*e.g.,* Clarke, 1966; Pearcy *et al.,* 1977). The apparent behavioral threshold for luminescent countershading at approximately 3×10^{12} photons $m^{-2}\ s^{-1}$ (for a 490 nm light stimulus) would be reached at a daytime

depth of approximately 350 m in the coastal waters off San Diego (Kampa, 1960). Therefore, ambient light levels at the depths inhabited by *S. similis* would be sufficient to induce and maintain luminescent countershading.

The control of luminescent countershading in *S. similis* represents a simple case, where the visual system, bioluminescence emission, and oceanic downwelling light all operate in the same spectral range. There are other cases where these simple conditions do not hold. All vertically migrating animals including *S. similis* experience diel changes in their optical environment. However, animals such as the squid *Abralia,* which is able to modify the spectral emission of bioluminescence (Young and Mencher, 1980), must coordinate the adjustable spectral emission of its bioluminescence with diel changes in the spectral distribution of downwelling light in order for luminescent countershading to be effective. Animals with multiple visual pigments, such as the oplophorid shrimp *Systellaspis debilis* (Frank and Case, 1988), may use only one of their photoreceptor classes to drive luminescent countershading. In these more complex cases, the relationship between visual sensitivity and behavioral spectral efficiency promises to offer an intriguing insight into the coordination of luminescent camouflage behavior in midwater animals.

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