Effects of Light Adaptation on the Temporal Resolution of Deep-sea Crustaceans[1]

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Synopsis. The effects of light adaptation on flicker fusion frequency were examined in the photoreceptors of 13 species of deep-sea crustaceans. Light adaptation produced a significant increase in the maximum critical flicker fusion frequency (CFF_{max}) in 7 species—all 6 species of euphausiids in the study, and 1 species of oplophorid (Group 1). This is the first example of an increase in temporal resolution due to light adaptation in a deep-sea species. In the other six species—2 oplophorids, 1 pandalid, 1 pasiphaeid, 1 penaeid and 1 sergestid (Group 2)—light adaptation had no effect, or resulted in a decrease in the flicker fusion frequency. The mean dark-adapted CFF_{max} of the Group 1 species was significantly higher, and the mean response latency significantly lower, than those of the Group 2 species. Possible explanations for these differences include the activity and bioluminescence mode of preferred prey items, as well as the retention of larval/juvenile adaptations in adult eyes.

INTRODUCTION

Autrum's classic studies on insects (1950, 1958) established that the retinal response dynamics of insect photoreceptors match their lifestyle and habitat. His work, utilizing extracellular recordings, and more recent work utilizing intracellular recordings (Howard *et al.*, 1984; deSouza and Ventura, 1989; Laughlin and Weckström, 1993), demonstrated that predominantly diurnal species have better temporal resolution than predominantly nocturnal species. Recent studies on the effects of light-adaptation on insect photoreceptors demonstrated that all species studied to date show an improvement in the frequency response under light-adaptation (Laughlin and Weckström, 1993), with the magnitude of the improvement again related to the visual ecology of the species. In fast moving, diurnally active Diptera, the improvement was dramatic, while in slow moving, nocturnal species, the improvement was slight.

The temporal resolutions of the dark-adapted photoreceptors of several species of mesopelagic crustaceans have been described in earlier studies (Frank, 1999, 2000). While the light environment of shallow water crustaceans may vary dramatically on a 24 hour cycle, deep-sea species live in a much more constant light environment, similar to that of nocturnally active insects. Therefore, the current study was undertaken to determine if these mesopelagic species, which live in a light regime similar to that of nocturnal insects, also exhibit only a slight improvement in temporal resolution responses when light-adapted.

METHODS

Animal collection and preparation

All the work reported here was conducted on shipboard. Animal collections and electrophysiological recordings were carried out as described in Frank (1999). Briefly, animals were collected with a midwater trawl net fitted with a light-tight, thermally insulated cod-end, brought to the surface in the closed cod-end, and sorted out under dim red light. Data were obtained from adult specimens of 13 species of mesopelagic (200–900 m depth) crustaceans distributed over six families. All these species have spherical eyes, with the exception of 4 species of euphausiids, which have bilobed eyes (Fig. 1, Table 1). Collections were made in the following locations:

Acanthephyra purpurea: Northwest Providence Channel, Bahamas; Oceanographer Canyon, on the south edge of Georges Bank

Funchalia villosa: off the south coast of Cuba; Gulf of Mexico (27–28°N, 86–88°W)

Janicella spinicauda: Cuba; Bahamas

Meganyctiphanes norvegica: Oceanographer Canyon

Nematobrachion sexspinosus: Bahamas; Cuba; off the Cape Verde Islands between 15–17°N, 20.17°–20.26°W

Nematoscelis megalops: Oceanographer Canyon

Pasiphaea multidentata: Wilkinson Basin, Gulf of Maine

Plesionika rossignoli: Cape Verde Islands

Sergestes arcticus: Oceanographer Canyon

Stylocheiron maximum: Bahamas; Cape Verde Islands; Cuba

Daytime depth ranges in Jerlov's Type 1 and 1A water (calculated where only data from less transparent water types were available) are given in Table 1.

Animals were maintained in chilled seawater in the dark, and set-up for experiments under dim red light. The recording chamber contained seawater chilled to 4–6°C (the water temperature at their daytime depths). Animals were restrained such that their pleopods were free to generate respiratory currents across the gills, and recordings of the electroretinogram were made from intact eyes of living animals. For the bilobed species, all data presented are from the upper lobe. Light from an Instruments SA monochromator was transmitted to the eye through a 4 mm light guide composed of random-
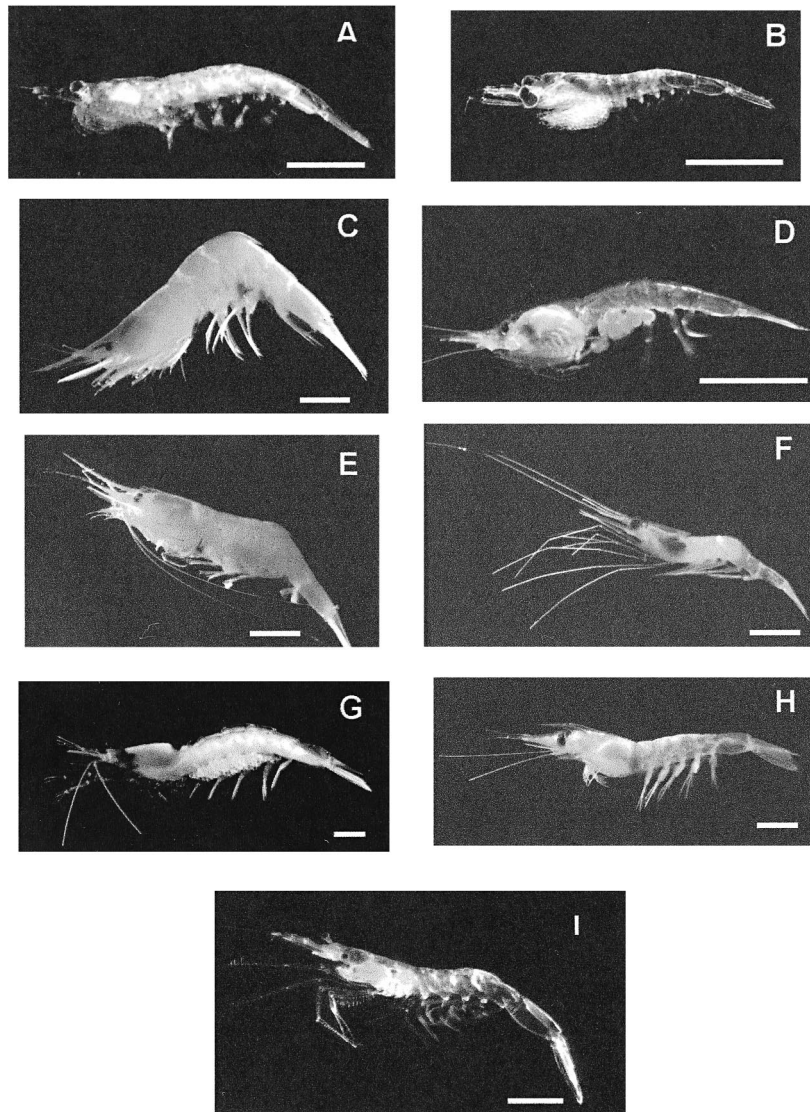

Fig. 1.   A. *Meganyctiphanes norvegica*, euphausiid with spherical eye. B. *Nematoscelis megalops*, euphausiid crustacean with bilobed eye. C. *Acanthephyra purpurea*. D. *Janicella spinicauda*. E. *Systellaspis debilis*. F. *Plesionika rossignoli*. G. *Pasiphaea multidentata*. H. *Funchalia villosa*. I. *Sergestes arcticus*. White bar on each photo indicates 1 cm.

ized fused silica fibers, placed 3 mm from the eye. Stimulus irradiance was controlled with neutral density filters. Stimulus duration was controlled via a Uniblitz shutter under computer control. Irradiance was measured with a UDT Optometer (United Detector Technology Model S370) and radiometric probe with point calibrations provided by UDT. Responses were displayed on the computer screen using a data acquisition program written in LabView (National Instruments, Inc.), and stored for later analysis.

Dark-adapted flicker fusion

Temporal resolution of the eye was examined by determining the critical flicker fusion frequency (CFF) in response to square pulses of light with a constant 50% duty cycle (50:50 light:dark ratio). CFF is the highest stimulus rate at which the eye can produce electrical responses that remains in phase with a flickering light of a certain intensity. As CFF depends on the intensity of the stimulating light (Bröcker, 1935; Crozier and Wolf, 1939; Crozier *et al.*, 1939), the maximum CFF (CFF_{max}), which is the highest flicker rate that the eye is capable of following at any intensity, was used to compare temporal resolution between different species. The response to a dim test flash that generated a 100 μV response in the dark-adapted eye was monitored between every flickering light stimulus, and subsequent flickering stimuli were not given until the response had recovered to 100 μV. At the brightest flickering stimuli, recovery took up to one hour.

Light-adapted flicker fusion

The eye was bathed with an adapting light transmitted through one leg of the bifurcated light guide

TABLE 1. *Approximate daytime depth ranges and CFF$_{max}$ of dark-adapted and light-adapted adult mesopelagic crustaceans.*

Family/species	Daytime depth(m)	CFF$_{max}$-DA (Hz) ± S.E.	CFF$_{max}$-LA (Hz) ± S.E.	Mean ↑ or ↓ in CFF$_{max}$	
Euphausiidae					
Monolobed species					
Euphausia gibboides	400–500[a]	24 (±0.9; n = 4)	32+		**1**
Meganyctiphanes norvegica	400–600	23 (±0.7; n = 6)	41 (±2.5; n = 6)	↑ 18*	**1**
Bilobed species					
Nematobrachion boopis	400–600	33 (±1.9; n = 5)	50+		**1**
Nematobrachion sexspinosus	400–600	36 (±2.5; n = 4)	54 (±1.6; n = 8)	↑ 18*	**1**
Nematocelis megalops	400–700[b]	28 (±2.0; n = 3)	48 (±2.3; n = 3)	↑ 20*	**1**
Stylocheiron maximum	250–500	34 (±1.8; n = 5)	44 (±1.0; n = 5)	↑ 10*	**1**
Oplophoridae					
Acanthephyra purpurea	700–900[c]	18 (±1.3; n = 4)	16 (±3.1; n = 3)	↓ 2	**2**
Janicella spinicauda	500–600	23 (±0.4; n = 7)	29 (±1.2; n = 6)	↑ 6*	**1**
Systellaspis debilis	600–900	21 (±0.3; n = 8)	15 (±2.3; n = 4)	↓ 7*	**2**
Pandalidae					
Plesionika rossignoli	up to 1,000[d]	14 (±1.0; n = 4)	13 (±1.5; n = 3)	↓ 1	**2**
Pasiphaeidae					
Pasiphaea multidentata	600–900	21 (±1.5; n = 6)	19 (±1.4; n = 4)	↓ 2	**2**
Penaeidae					
Funchalia villosa	300–550	21 (±0.9; n = 8)	21 (±1.2; n = 3)	0	**2**
Sergestidae					
Sergestes arcticus	600–960	21 (±1.1; n = 6)	16 (±3.4; n = 4)	↓ 5*	**2**

Depths are for Jerlov's Type 1 and 1A water. Equivalent optical depths in Type 1A water were calculated for those species where the only data available were for other water types. References for daytime depth data can be found in Frank and Widder, 1999, and Frank, 2000, unless otherwise indicated. Species are divided into two groups. Group 1: species in which light adaptation produced a statistically significant increase ($P \leq 0.05$) on CFF$_{max}$; Group 2: species in which there were no statistically significant increases. * denotes statistical significance.

[a] Baker, 1970.
[b] Anderson and Sardou, 1992.
[c] Hopkins *et al.*, 1994.
[d] Crosnier and Forest, 1973.

and flickering light stimuli were superimposed on the background light through the other leg of the light guide. In order to determine the intensity of adapting light to use, a stimulus/response curve (V/log I) was generated in the dark-adapted eye (see below), and the irradiance required to elicit a response that was 50% of V$_{max}$ was determined. The adapting light was then turned on and adjusted in intensity with neutral density filters such that the response to the 50% V$_{max}$ irradiance was decreased by half. Subsequent experiments demonstrated that increasing the adapting light above this level had no additional affect on the light-adapted flicker fusion frequency.

Sensitivity measurements

The eye was stimulated with 100 ms flashes of varying irradiances of 490 nm monochromatic light (FWHM = 2 nm). The eye was allowed to dark adapt between every light stimulus; return to the previous level of dark-adaptation was determined by the response to a dim test flash of set irradiance. At the lower irradiances, an interflash interval of 2 minutes was sufficient to ensure complete dark adaptation. An interflash interval of 60 minutes was required at the highest irradiances. At these short flash durations, only the transient response was visible, and the peak to peak amplitudes of these responses were quantified using a

program written in LabView. These data were normalized to the peak response (V$_{max}$) for each animal (after Glantz, 1968), and the mean stimulus/response data for each species 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*a*, *b*; Zettler, 1969):

$$\frac{V}{V_{max}} = \frac{I^m}{I^m + K^m}$$

where I = stimulus irradiance; V = response amplitude at irradiance I; V_{max} = maximum response amplitude; m = slope of the linear part of the V/logI curve; K = stimulus irradiance eliciting half the maximum response (V_{max}).

As an additional measure of sensitivity, response latencies were measured for the 50% V$_{max}$ responses. Response latency is defined as the time between stimulus onset and the beginning of the photoreceptor response.

The dynamic range was also determined for each species from the V/log I curves. The dynamic range is defined as the log irradiance range between response limits of 5–95% V$_{max}$, and corresponds to the voltage

bandwidth of the photoreceptor (Laughlin and Hardie, 1978).

Data analysis

Data were analyzed using the Tukey multiple comparison test with unequal sample sizes (Zar, 1999), which simultaneously examines differences between all possible pairs of means in the data set. The degree of correlation between two variables was determined with the Pearson's Correlation test (Zar, 1999).

RESULTS

Light adaptation increased the maximum critical flicker fusion frequencies of all the euphausiid species in the study (Table 1), and in those species for which enough data were available for statistical analysis, this increase was statistically significant (one factor Anova, $P < 0.004$). Light adaptation had no effect on flicker fusion frequency or led to a decrease in CFF_{max} in all the other species with the exception of *Janicella spinicauda*. The CFF_{max} in *J. spinicauda* increased by 6 Hz, which, although smaller than the increase in the euphausiid species, is a statistically significant increase ($P = 0.002$).

The species that showed a significant increase in CFF_{max} upon light adaptation were grouped together (Group 1) and the characteristics of their photoreceptors while dark-adapted were compared with those of the species (Group 2) that did not show an enhancement of CFF_{max} under light adaptation (Table 1). *Euphausia gibboides* and *Nematobrachion boopis* were added to Group 1 because light adaptation produced a substantial increase in CFF_{max} even though enough data were not available for statistical analysis.

The mean dark-adapted CFF_{max} of the Group 1 species (28.6 ± 2.11) was significantly higher than that of the Group 2 species (19.4 ± 1.19—single factor Anova, $P = 0.007$). A Tukey multiple range test was performed to look at the source of the difference, and the results demonstrated that CFF_{max} values of the four species of bilobed euphausiids in Group 1 were significantly higher than those of all the Group 2 crustaceans (Fig. 2A). The flicker fusion frequencies of other three Group 1 species—the two spherical eyed euphausiids and the oplophorid *Janicella spinicauda*—were only significantly higher than that of *P. rossignoli*, the Group 2 species with the lowest CFF_{max} (14.3 Hz).

Other characteristics of the dark-adapted photoreceptors were compared between the two groups by examining their respective V/log I curves. The V/log I curves for all species in the study were sigmoidal (Fig. 3), with dynamic ranges from 4.0 (*Systellaspis debilis*) to 5.5 (*Pasiphaea multidentata*). The dynamic range is the range of irradiances over which the photoreceptor will operate, and is directly related to the slope of the V/log I curve, with steeper slopes indicating smaller dynamic ranges. There were no significant differences between the dynamic ranges or slopes of Group 1 *vs.* Group 2 crustaceans (one factor Anova, $P > 0.8$).

Saturation of the photoreceptor response occurred at V_{max}, which is the largest response the eye is capable of generating at any irradiance. Although the V_{max} was not reached in some preparations, the V_{max} was calculated with the Naka-Rushton equation, and if the highest response recorded in the eye reached 90% of the calculated V_{max}, these data were used in the analysis. Acceptable V/log I curves were not obtained from *S. maximum*; therefore, *K* and latency data are also not available for this species. *K,* which is the irradiance required to generate a response that is 50% V_{max}, has been used as an indicator of absolute sensitivity in intracellular studies on insects (Laughlin, 1976; Autrum, 1981; Laughlin and Hardie, 1978). Utilizing extracellular techniques, the response characteristics of the eye depend strongly on electrode placement (Konishi, 1955; Frank and Case, 1988), and therefore *K* cannot be used as an indicator of the absolute sensitivity. However, Eguchi and Horikoshi (1984) demonstrated that *K* can be used in comparative studies utilizing the ERG to look at differences in **relative** sensitivity between species. Therefore, in the current study, responses were divided by the V_{max} before plotting and generating a V/log I curve using the Naka-Rushton equation. Technically, when using extracellular data, the Naka-Rushton equation should only be used for species that have a single pigment (Naka and Rushton, 1966*a*), because the ERG is the summed massed response from a large population of receptor cells. Two species in the present study, *Janicella spinicauda* and *Systellaspis debilis*, have two visual pigments, with absorption maxima at 410 and 500 nm (Frank and Case, 1988; Cronin and Frank, 1996). However, the polarity of the response as well as the low sensitivity to UV wavelengths of the preparations used in the current analysis indicate that the electrode was in a region where the 500 nm photoreceptors dominated the response (Frank and Case, 1988). In addition, utilizing a 490 nm monochromatic light minimized a significant contribution by any of the 410 nm receptors, as demonstrated by the smooth V log I curves for both these species (Fig. 3). Mean values of log *K* for each species are listed in Table 2.

The mean dark-adapted log *K* for the Group 1 crustaceans was significantly greater than that of the Groups 2 species (11.8 ± 0.12 *vs.* 10.8 ± 0.17; single factor Anova, $P < 0.001$). However, interspecies comparisons with the Tukey test demonstrated that the log *K* values of all the Group 1 crustaceans were significantly greater than only that of *P. rossignoli* in Group 2, the species with the smallest log *K*.

Response latencies of the 50% V_{max} response were determined for all the species except *Stylocheiron maximum*, and are given in Table 2. The mean response latency for the Group 1 species was significantly shorter than that of the Group 2 species (21.7 ± 2.71 *vs.* 44.4 ± 3.24, single factor Anova, $P = 0.0003$). The Tukey test demonstrated that all the euphausiid species in Group 1 had significantly shorter response latencies than all the group 2 species (Fig.
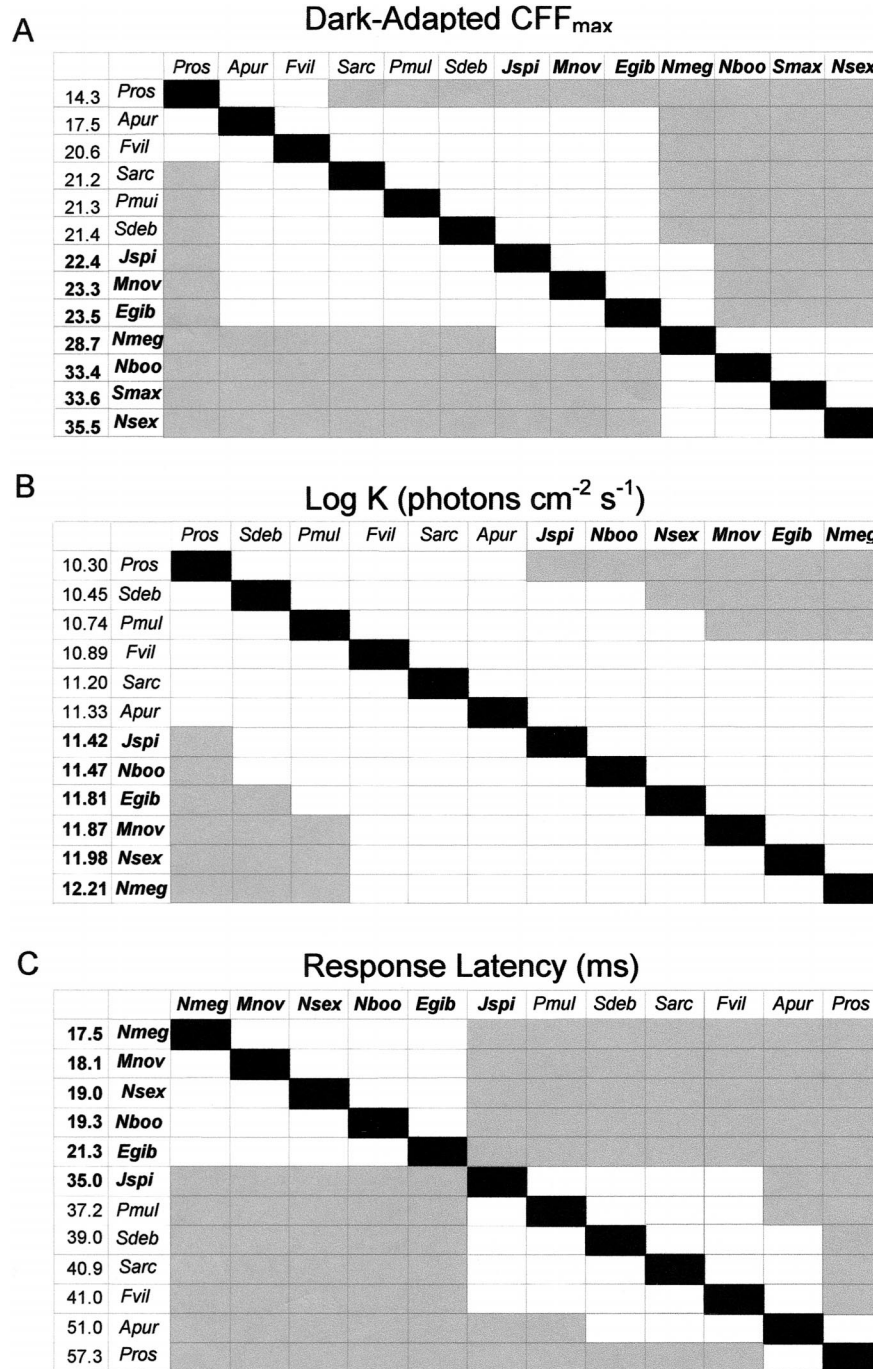
A. Dark-Adapted CFF$_{max}$

		Pros	Apur	Fvil	Sarc	Pmul	Sdeb	Jspi	Mnov	Egib	Nmeg	Nboo	Smax	Nsex
14.3	Pros													
17.5	Apur													
20.6	Fvil													
21.2	Sarc													
21.3	Pmui													
21.4	Sdeb													
22.4	Jspi													
23.3	Mnov													
23.5	Egib													
28.7	Nmeg													
33.4	Nboo													
33.6	Smax													
35.5	Nsex													

B. Log K (photons cm^{-2} s^{-1})

		Pros	Sdeb	Pmul	Fvil	Sarc	Apur	Jspi	Nboo	Nsex	Mnov	Egib	Nmeg
10.30	Pros												
10.45	Sdeb												
10.74	Pmul												
10.89	Fvil												
11.20	Sarc												
11.33	Apur												
11.42	Jspi												
11.47	Nboo												
11.81	Egib												
11.87	Mnov												
11.98	Nsex												
12.21	Nmeg												

C. Response Latency (ms)

		Nmeg	Mnov	Nsex	Nboo	Egib	Jspi	Pmul	Sdeb	Sarc	Fvil	Apur	Pros
17.5	Nmeg												
18.1	Mnov												
19.0	Nsex												
19.3	Nboo												
21.3	Egib												
35.0	Jspi												
37.2	Pmul												
39.0	Sdeb												
40.9	Sarc												
41.0	Fvil												
51.0	Apur												
57.3	Pros												

FIG. 2. Results of Tukey analysis. A. Comparison of dark-adapted maximum critical flicker fusion frequencies. Numbers at left are mean CFF$_{max}$. Names in bold are Group 1 crustaceans. Shaded bars indicate statistically significant difference between the species. For example, the CFF$_{max}$ of *S. arcticus* is significantly different from the CFF$_{max}$ of *P. rossignoli*, *N. megalops*, *N. boopis*, *S. maximum* and *N. sexspinosus*. B. Comparison of log *K,* the stimulus irradiance required to generate a response with an amplitude of 50% V$_{max}$. Numbers at left are mean log *K*. C. Comparison of response latencies of the 50% V$_{max}$ response. Numbers at left are mean response latencies.

2C). The response latency of *Janicella spinicauda*, the only non-euphausiid species in Group 1, was significantly shorter than only two of the Group 2 species—*A. purpurea* and *P. rossignoli*. In addition, although this species was placed in Group 1 because of the significant effect of light adaptation on its CFF$_{max}$, its response latency is significantly longer than all the other Group 1 species (Fig. 2C).

Since flicker fusion frequency and response latency are both temporal characteristics of the photoreceptors, and numerous studies in insects have demonstrated a relationship between sensitivity and the temporal char-
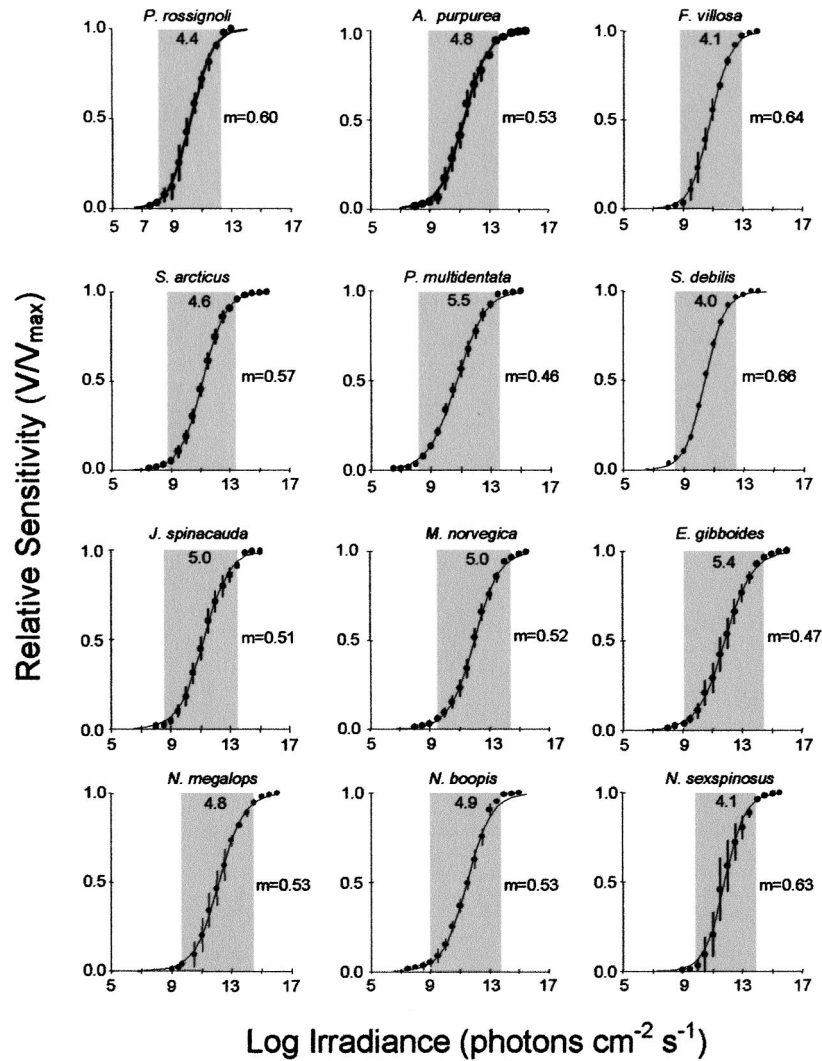
FIG. 3. V/log I curves for all the species in the study, in the same order as Table 2. Shaded boxes indicate dynamic range; numbers at the top indicate the size. m = slope of curve.

TABLE 2. *Dark-adapted CFF_max, log K and latency of 50% V_max response arranged from lowest to highest dark-adapted CFF_max rates.**

Species	CFF_{max}-DA (Hz)	Log K (photons cm^{-2} s^{-1})	Latency (ms)
Plesionika rossignoli	14.2 (±1.0; n = 4)	10.30 (±0.24; n = 3)	57.3 (±1.67; n = 4)
Acanthephyra purpurea	17.5 (±1.3; n = 4)	11.33 (±0.21; n = 3)	51.0 (±3.06; n = 3)
Funchalia villosa	20.6 (±0.9; n = 8)	10.89 (±0.17; n = 2)	41.0 (±3.0; n = 2)
Sergestes arcticus	21.2 (±1.1; n = 6)	11.20 (±0.15; n = 8)	40.9 (±2.19; n = 8)
Pasiphaea multidentata	21.3 (±1.5; n = 6)	10.74 (±0.15; n = 9)	37.2 (±1.69; n = 9)
Systellaspis debilis	21.4 (±0.3; n = 8)	10.45 (±0.04; n = 4)	39.0 (±0.41; n = 4)
Janicella spinicauda	22.5 (±0.4; n = 5)	11.42 (±0.20; n = 5)	35.0 (±4.32; n = 5)
Meganyctiphanes norvegica	23.3 (±0.7; n = 6)	11.98 (±0.18; n = 9)	18.1 (±0.82; n = 9)
Euphausia gibboides	23.5 (±0.9; n = 4)	11.81 (±0.31; n = 4)	23.8 (±2.93; n = 4)
Nematoscelis megalops	28.7 (2.4; n = 3)	12.21 (±0.28; n = 2)	17.5 (±5.5; n = 2)
Stylocheiron maximum	33.6 (±1.8; n = 5)	N.A.	N.A.
Nematobrachion boopis	33.4 (±1.9; n = 5)	11.47 (±0.14; n = 3)	19.3 (±2.3; n = 3)
Nematobrachion sexspinosus	35.5 (±2.5; n = 4)	11.88 (±0.24; n = 4)	19 (±2.5; n = 4)

* Numbers are mean values; numbers in parentheses are SE and sample size.
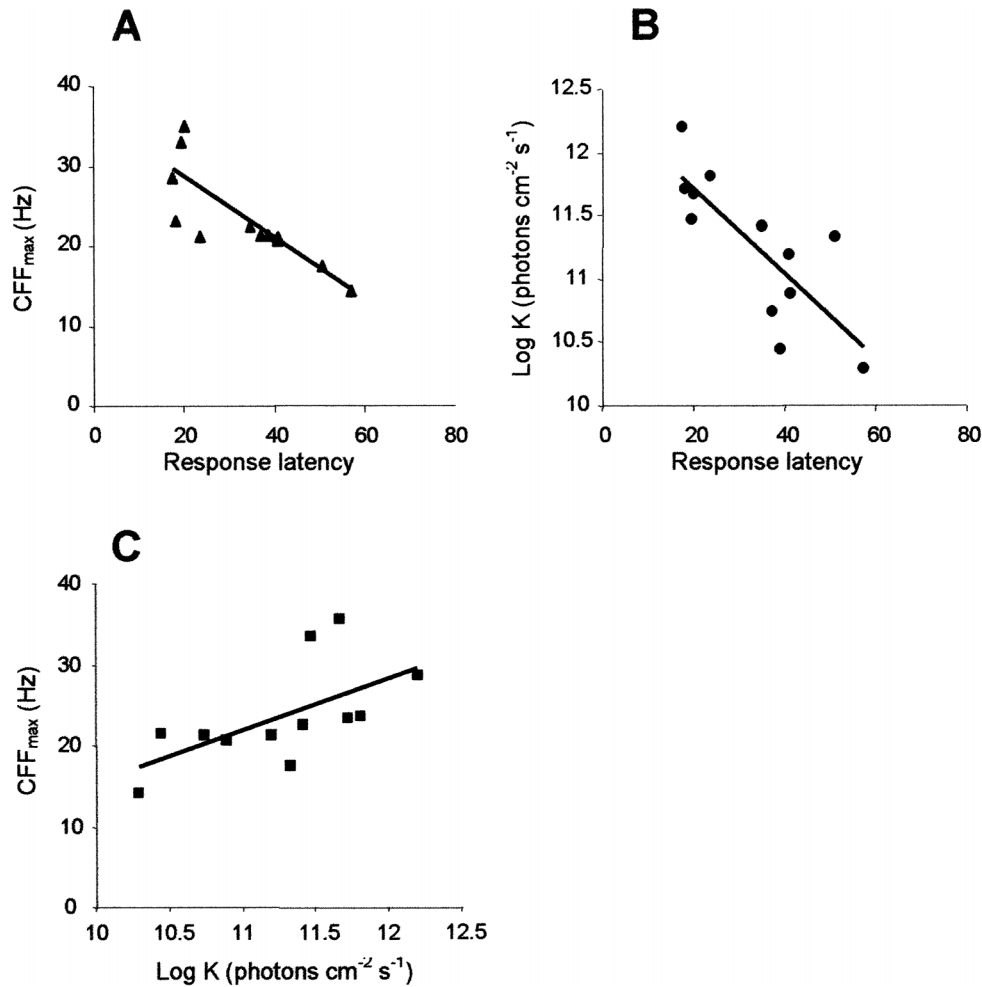
FIG. 4. A. Correlation between response latency and CFF_{max} of dark-adapted eyes for all species. Line is linear trend line fit to the data. Pearson's correlation coefficient $= -0.82$ ($P = 0.0012$). B. Correlation between response log K (irradiance required to generate 50% V_{max} amplitude response) and response latency. Pearson's correlation coefficient $= -0.81$ ($P = 0.0014$). C. Correlation between log K and CFF_{max}. Pearson's correlation coefficient $= 0.64$ ($P = 0.025$).

acteristics of the eye (see Laughlin and Weckström, 1993 for rev.), a relationship between log K and these two temporal parameters were analyzed with the Pearson correlation test. There is a good correlation between CFF_{max} and response latency. The correlation coefficient $R = -0.82$ indicates that as response latency increases, flicker fusion rate decreases ($P = 0.0012$; Fig. 4A). The correlation between log K and latency is almost as good, with an R of -0.81, and this correlation is significant as well ($P = 0.0014$; Fig. 4B), demonstrating that as response latency increases, log K decreases, meaning that sensitivity increases. The correlation between log K and flicker fusion frequency is weaker, with a correlation coefficient of 0.64, but is also statistically significant ($P = 0.025$; Fig. 4C).

DISCUSSION

Parameters of dark-adapted eyes

Dark-adapted flicker fusion frequencies have been previously published for 7 of the species presented in Table 1—*Nematobrachion sexspinosus*, *Stylocheiron*

maximum, *Janicella spinicauda*, *Systellaspis debilis*, *Pasiphaea multidentata*, *Funchalia villosa*, and *Sergestes arcticus* (Frank, 1999, 2000). For the non-euphausiid species, the data are similar to those that were published earlier, with minor changes due to the larger sample sizes now available. However, the data for the euphausiids (*N. sexspinosus*, *S. maximum*) have changed substantially. This is due to the significant effect of light adaptation on the flicker fusion frequencies of euphausiids (see below). In examining the earlier data, it became clear that the test flash, used to monitor the recovery from light-adaptation resulting from a bright stimulus train, was too bright, and therefore the response to the test flash did not change significantly under light adaptation that did affect the flicker fusion frequency. The dimmer test flash, which was used with the dark-adapted data presented here, provided a more accurate indication of recovery from light adaptation. The maximum amount of time required for dark-adaptation between stimulus trains using the brighter test flash was 30 minutes, while the

dimmer test flash demonstrated that as much as 60 minutes was required after the brightest stimulus trains. The new results presented here demonstrate that the CFF_{max} values of dark-adapted euphausiids are substantially lower than previously thought.

Light adaptation significantly increased the CFF_{max} in some species, which were grouped together into Group 1, while it had no effect, or actually reduced the frequency response in other species, which were placed in Group 2. In addition, the mean CFF_{max} of dark-adapted photoreceptors from Group 1 species was almost 10 Hz higher than that of the Group 2 species, and this difference was statistically significant. Other parameters of the dark-adapted eyes of Group 1 and Group 2 species were compared, to determine if there were other differences in the response dynamics of the photoreceptors that could be correlated with the higher flicker fusion frequencies in the Group 1 species. Response latency is another indication of transduction speed in photoreceptors, with longer response latencies associated with a lower frequency response and higher sensitivity (see Laughlin, 1990, for rev). There was a good correlation between response latency and CFF_{max}, with $r = -0.82$ (Fig. 4A), indicating, as stated above, that as response latency goes up, the frequency response goes down. The response latencies of all the Group 1 euphausiid species were significantly lower than the response latencies of all the Group 2 species, as well as that of *Janicella spinicauda*. Although *J. spinicauda* was placed in Group 1 because light adaptation significantly increased its flicker fusion frequency, the increase was not of the same magnitude as in the euphausiid species, and its significantly higher response latency indicates that this species is more closely aligned with the Group 2 crustaceans.

There was an equally good correlation between response latency and relative sensitivity (log K), with $r = -0.81$ (Fig. 4B), indicating that the shorter the response latency, the less sensitive the eye. However, in spite of this correlation and the fact that all the Group 1 euphausiid species had significantly shorter response latencies than the Group 2 species, they are not significantly less sensitive than all, or even most (in the case of *N. boöpis* and *E. gibbosus*) of the Group 2 species (Fig. 2B). Sensitivity of the eye depends on other factors than just the temporal characteristics of the eye, however, such as sizes of the eye, ommatidia, and rhabdoms, amount of visual pigment present, presence or absence of spatial summation, etc. (see Land and Nilsson, 2002 for review), so the lack of a clear difference in sensitivity between Group 1 and Group 2 crustaceans is not unexpected.

The dynamic ranges (4.0–5.5) and slopes (0.46–0.65) from the V/log I plots are comparable to those reported for other arthropod species (Glantz, 1968; Laughlin, 1976; Laughlin and Hardie, 1978; Eguchi and Horikoshi, 1984; Barth *et al.*, 1993; Laughlin and Weckström, 1993), and there is no significant difference with respect to this parameter between Group 1 and Group 2 crustaceans.

Effects of light adaptation

Light adaptation had no significant effect on the flicker fusion frequency of *Funchalia villosa*, *Acanthephyra purpurea*, *Plesionika rossignoli* or *Pasiphaea multidentata* and significantly reduced the frequency response in *Systellaspis debilis* and *Sergestes arcticus*. Deep-sea crustaceans are extremely sensitive to light (Frank and Case, 1988; Frank, 1999), and the adapting light was considerably brighter than any background light levels they would ever experience in their normal environments, so it is possible that the reduction of frequency response observed in *S. debilis* and *S. arcticus* was a result of light damage. However, in one experiment with *S. debilis*, the CFF_{max} was measured again after the eye had recovered from the effects of the adapting light. The dark-adapted CFF_{max} was 22 Hz, decreased to 14 Hz under light adaptation, and returned to 22 Hz thirty minutes after the adapting light was extinguished. This suggests that light damage was not responsible for the reduction in temporal resolution in this species. It is still possible that the decrease in the frequency response is an aberrant response to abnormal light levels, as demonstrated by Johnson *et al.* (2000) on several other deep-sea species. They examined the effects of light adaptation on band-pass characteristics of two species of deep-sea crabs, and found that low intensity light had no effect, while high intensity light completely abolished the response. In the only other study of the effects of light-adaptation on deep-sea species, Moeller and Case (1995) found that light-adaptation had no effect on flicker fusion frequencies of the oplophorid *Oplophorus spinosus* and the mysid *Gnathophausia ingens*, although there was a decrease in response latency, a parameter which was not examined in light-adapted eyes in the current study. The results of these studies together with the current study suggest that, with the exception of euphausiids, and perhaps *Janicella spinicauda* (see discussion of response latency below), photoreceptors of deep-sea crustaceans do not improve their temporal resolution with light adaptation.

In photoreceptors in general, light adaptation shortens the response time, resulting from a reduction in the duration of the quantum bumps, as well as a reduction in the spread of their latencies and the time to peak of the latency dispersion (Wong *et al.*, 1982). This reduction in response time has been shown to significantly enhance the frequency response of all arthropod photoreceptors that have been studied to date (Fuortes and Hodgkin, 1964; Zettler, 1969; Pinter, 1972; Wong, 1978; Howard *et al.*, 1984; Glantz, 1991; Laughlin and Weckström, 1993), with the exception of tipulid flies. Tipulid flies fail to significantly increase their frequency responses during light adaptation (Laughlin and Weckström, 1993). In these species, the quantum bump duration is reduced during light adaptation, but the latency dispersion is not affected, meaning that sensitivity is reduced without the usual increase in response speed (Laughlin, 1996). Tipulids are

slow-moving flies that would not be able to catch fast moving prey even if their photoreceptors could track it. Not only could they not take advantage of having a faster eye, but there is also a metabolic cost for faster response dynamics, as fast responses are metabolically more expensive (see Laughlin *et al.*, 1998 for rev.). Fast responses require a membrane with a short time constant and low resistance, characterized by a non-inactivating delayed rectifier. These low resistances draw current, and the non-inactivating delayed rectifier results in large ion fluxes, necessitating strong ion pumps to counteract these fluxes, which utilize substantial metabolic energy, energy that would be wasted in slow-moving species (Weckström *et al.*, 1991; Laughlin and Weckström, 1993). In addition, membranes of slow photoreceptors contain potassium conductances that inactivate rapidly, reducing current flow and resulting in a longer membrane time constant. Rapid fluctuations in membrane voltage are smoothed out, meaning that fast signals are destroyed. In nocturnal, slow moving species such as tipulid flies, a rapid change in membrane voltage is probably noise, and this smoothing is therefore beneficial (Laughlin, 1996).

The same case can be made for the Group 2 crustaceans in this study, as they are similar to tipulid flies both with respect to light environment and locomotory abilities. By 400 m depth in clearest ocean water (Jerlov's Type 1 or 1A water), the downwelling irradiance has been reduced to less than 0.0001% of surface irradiance (Jerlov, 1976), which is about as bright as moonlight seen by terrestrial species (Munz and McFarland, 1973; Land, 1981). The shallowest daytime depth of the Group 2 crustaceans (with the exception of *Funchalia villosa*) is 600 m, where the background light is similar to that experienced by nocturnal terrestrial species in the absence of moonlight. In addition, there is a reduction in metabolic rate with increasing depth in pelagic species, which is a reflection of reduced musculature and swimming effort resulting from reduced predator-prey detection distances (see Childress, 1995, for review). Therefore, living in a dark environment with reduced locomotor abilities, these deep-sea crustaceans would derive no significant benefits to counteract the metabolic costs and increased noise of a faster photoreceptor.

What is very unexpected is that light adaptation had such a large effect on the temporal resolution of the Group 1 crustaceans. The magnitude of the increase in *Janicella spinicauda* and *Stylocheiron maximum* was only 6–10 Hz, which is similar to the magnitude of the increase in the corner frequency of tipulid flies, which have only slow cells (Laughlin and Weckström, 1993). The increase in the other Group 1 species was almost two times as great, and reached light adapted CFF_{max} values of >50 Hz, similar in magnitude to the increase in corner frequencies seen in bibionid flies (Laughlin and Weckström, 1993) (*E. gibboides* is not included in this discussion, as CFF_{max} was not reached in the one light-adapted experiment conducted). Bibionid flies have faster cells with a non-inactivating

delayed rectifier, and the combination of short response latencies and large increase in CFF_{max} under light-adaptation in *Meganyctiphanes norvegica*, *Nematobrachion boöpis*, *Nematobrachion sexspinosus*, and *Nematoscelis megalops* indicates that these species may possess faster cells as well.

Ecological considerations

The daytime depth distributions of these species, while shallower than the Group 2 species (minimum depth of 400 m *vs.* 600 m), are still deep enough that their light environment is similar to that of nocturnal insects in moonlight. However, there is another source of light in the deep-sea, and that is bioluminescence. While relatively rare in the terrestrial environment, it has been estimated that 80–90% of the individuals living between 200–900 m are bioluminescent (Herring, 2002). Therefore, the bioluminescence and activity of the prey needs to be taken into consideration, in addition to downwelling illumination, when examining the light environment of marine organisms. Among the 4 species of euphausiids under consideration, three species have bilobed eyes, and all bilobed species possess elongated 2nd and/or 3rd thoracic legs, which are linked to carnivory (Mauchline and Fisher, 1969; Roger, 1973). The preferred prey of *N. sexspinosus* and *N. boopis* is the copepod *Pleuromamma* (Hu, 1978; Kinsey and Hopkins, 1994), which emits a bright luminescent secretion when harassed (Herring, 1985). *N. megalops* is also a carnivore (Gurney *et al.*, 2001), but its preferred copepod prey has not been identified to genus (Barange, 1991). If this species also specializes on a bioluminescent copepod, then it might be advantageous for these three bilobed species, with appendages clearly designed for grasping prey, to sacrifice sensitivity (contrast detection) for greater temporal resolution (tracking ability), due to the substantially greater contrast between a flashing or glowing prey item against a dim background *vs.* that of a dark prey item. However, the spherical eyed *M. norvegica*, which also appears to possess faster cells, does not have grasping appendages, and is an omnivore, preferring the non-luminescent copepod *Calanus finmarchicus* as well as phytoplankton (Beyer, 1992, and references therein). In addition, *Acanthephyra purpurea* and *Systellaspis debilis*, Group 2 crustaceans with slow eyes, are active carnivores, with bioluminescent euphausiids making up a substantial portion of their diets (Hopkins *et al.*, 1994). Therefore, the presence or absence of bioluminescence in the preferred prey item does not provide a sufficient explanation for the advantages of a faster frequency response in euphausiid eyes. However, in addition to the presence of bioluminescence, one must also consider the type of luminescence exhibited by the prey, as well as its activity level. An organism that specialized primarily on rapidly moving prey that produced flashes of bioluminescence (such as some species of copepods), would benefit from a photoreceptor with a faster temporal response, while one that fed primarily on slower moving species that

glowed (such as some species of gelatinous zooplankton), would not. Not much is known about the flash patterns of bioluminescent organisms in their natural environments, and this remains an intriguing area for future research.

Another part of the answer as to why some deep-sea species may possess metabolically expensive fast cells is that a fast frequency response may be important for the survival of juvenile stages, where the ecological benefits offset the metabolic disadvantages of its retention in adults. Juveniles tend to have shallower depth distributions than the adults for a number of species of crustaceans and fish, and would therefore experience a brighter ambient light field than adults. Based on studies of mesopelagic fish, Giske *et al.* (1990) suggested that juveniles or smaller species have shallower distributions than adults or larger species because they are less visible in more illuminated waters, and this pattern is seen in a number of crustacean species as well. For example, in the Firth of Clyde, off the coast of Scotland, the 3–4 mm size class of *Meganyctiphanes norvegica* came to the surface at night, and could be found at around 90 m during the day, while the 9–10 mm size class only came up to 40 m at night, and was found around 140 m during the day (Mauchline, 1960). Similarly, small ($\leq$11 mm) individuals of *Nematoscelis megalops* have a bimodal in distribution during the day, with population peaks between 50–150 m and 350–450 m, while the large (>11 mm) individuals were found between 350–700 m during the day in the Northwestern Mediterranean Sea (Andersen and Sardou, 1992). In the shallower, brighter waters occupied by the juvenile stages, the advantages of a faster eye for capturing prey may offset the metabolic costs as well as the resultant loss in sensitivity.

There are also structural differences between the adult and juvenile or larval eye that indicate the eye was originally adapted for the brighter light field. For example, the eyes of juvenile *Acanthephyra purpurea* possess large amounts of proximal screening pigment (Welsh and Chace, 1937), which is absent in the adult eye (Gaten *et al.*, 1992). Eye development in juvenile euphausiids (Nilsson *et al.*, 1986) and oplophorids (Gaten and Herring, 1995) also involves the gradual transition from apposition optics, which would be more efficient for viewing dark objects against the downwelling light field, to superposition optics, which are more efficient for detecting point sources against a dark background (Gaten and Herring, 1995).

A recent study indicates that there is another environmental factor that may affect transduction speed, and that is temperature. Tatler *et al.* (2000) showed that temperature has a dramatic effect on the temporal resolving power of fly photoreceptors, with the speed of the response more than doubling over the natural temperature range of the fly. For many mesopelagic species, this is not an issue, as they live at a relatively stable temperature, rarely differing by more than several °C. However, for those species that vertically migrate, leaving the cold dark depths at night to ascend into warmer surface waters to feed, temperature changes can be significant. For tropical species, the temperature can vary from 4°C at their daytime depths to 24–29°C in surface waters (Bahamas, unpublished data). Of the species in the present study, all have been reported to be vertical migrators with the exception of *N. sexspinosus* and *P. rossignoli*. Temperatures during this study were kept at low daytime depth temperatures (4°–7°C), both during maintenance and experiments, so any effect due to elevated temperature was not seen. Since these species migrate into warmer surface waters at night to feed, a temperature-induced increase in temporal resolution might greatly enhance their prey-tracking abilities if their prey are bioluminescent, and this will be examined in future experiments.

The deep-sea visual environment was once thought to be relatively simple, with organisms living at these depths possessing photoreceptors maximized for sensitivity to the ambient light field. However, bioluminescence, which is quite rare on land, is found at all depths in the sea, and ranges from a steady glow to flashes of varying duration, frequency and intensity (see Herring, 2002 for review). This substantially complicates the determination of what the visual task for a particular species is—is it imaging a dim object against a bright background, imaging a bright object against a dim background, imaging a point light source, imaging a diffuse light source, imaging a flashing light source, etc.? In addition, different life history stages may have different visual tasks, and some of the adaptations of larval or juvenile stages may be retained in the adult eye, adding to the complexity of the problem. Study of deep-sea visual systems should be a fruitful area of research for many years to come.

REFERENCES

Anderson, V. and J. Sardou. 1992. The diel migrations and vertical distributions of zooplankton and micronekton in the Northwestern Mediterranean Sea. 1. Euphausiids, mysids, decapods and fishes. J. Plankton Res. 14(8):1129–1152.

Autrum, H. 1950. Die Belichtungspotentiale und das Sehen der Insekten (Untersuchungen an *Calliphora* und *Dixippus*) Z. Vergleich. Physiol. 32:176–227.

Autrum, H. 1958. Electrophysiological analysis of the visual systems in insects. Exp. Cell Res. 5:426–439.

Autrum, H. 1981. Ch. 1. Light and dark adaptation in invertebrates. *In* H. Autrum (ed.), *Comparative physiology and evolution of vision in invertebrates,* pp. 2–91. Springer-Verlag, Berlin.

Baker, A. de C. 1970. The vertical distribution of euphausiids near

Fuerteventura, Canary Islands ('Discovery'' SOND cruise, 1965). J. Mar. Biol. Ass. U. K. 50:301–342.

Barange, M. 1991. Vertical migration and habitat partitioning of six euphausiid species in the northern Benguela upwelling system. J. Plankton Res. 12:1223–1237.

Baylor, D. A. and A. L. Hodgkin. 1974. Changes in timescale and sensitivity in turtle photoreceptors. J. Physiol. London 242:729–758.

Barth, F. G., T. Nakagawa, and E. Eguchi. 1993. Vision in the ctenid spider *Cupiennius salei*: spectral range and absolute sensitivity. J. Exp. Biol. 188:63–79.

Beyer, F. 1992. *Meganyctiphanes norvegica* (M. Sars) (Euphausiacea) a voracious predator on *Calanus*, other copepods, and ctenophores in Oslofjorden, Southern Norway. Sarsia 77:189–206.

Bröcker, H. 1935. Untersuchungen über das Sehvermögen der Einsiedlerkrebse. Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere 55:399–430.

Crosnier, A. and J. Forest. 1973. Les Crevettes Profondes De L'Atlantique Oriental Tropical. Faune Tropicale XIX:7–399.

Crozier, W. J. and E. Wolf. 1939. The flicker response contour for the crayfish. J. Gen. Physiol. 23:1–10.

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

Cartes, J. E. 1993. Day-night feeding by decapod crustaceans in a deep-water bottom community in the western Mediterranean. J. Mar. Biol. Ass. U. K. 73:795–811.

Childress, J. J. 1995. Are there physiological and biochemical adaptation of metabolism in deep-sea animals? Trends Ecol. Evol. 10(1):30–36.

Cronin, T. W. and T. M. Frank. 1996. A short-wavelength photoreceptor class in a deep-sea shrimp. Proc. R. Soc. London Ser. B 263:861–865.

de Souza, J. M. and D. F. Ventura. 1989. Comparative study of temporal summation and response form in hymenopteran photoreceptors. J. Comp. Physiol. 165:237–245.

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

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. Phil. Trans. R. Soc. London B 355:1195–1198.

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

Frank, T. M. and E. A. Widder. 1998. Comparative study of the spectral sensitivities of mesopelagic crustaceans. J. Comp. Physiol. 185:255–265.

Frank, T. M. and E. A. Widder. 2002. Effects of a decrease in downwelling irradiance on the daytime vertical distribution patterns of zooplankton and micronekton. Mar. Biol. 140:1181–1193.

Fuortes, M. G. F. and A. L. Hodgkin. 1964. Changes in time-scale and sensitivity in the ommatidia of *Limulus*. J. Physiol. London 172:239–263.

Gaten, E. and P. J. Herring. 1995. Morphology of the reflecting superposition eyes of larval Oplophorid shrimps. J. Morph. 225:29.

Gaten, E., P. M. J. Shelton, and P. J. Herring. 1992. Regional morphological variation in the compound eyes of certain mesopelagic shrimps in relation to their habitat. J. Mar. Biol. Ass. U. K. 72:61–75.

Giske, J., D. L. Aksnes, B. Baliño, S. Kaartvedt, U. Lie, J. T. Nordeide, A. GroVea Salvanes, S. M. Wakili, and A. Aadnesen. 1990. Vertical distribution and trophic interactions of zooplankton and fish in Masfjorden, Norway. Sarsia 75:65–81.

Glantz, R. M. 1968. Light adaptation in the photoreceptor of the crayfish, *Procambarus clarki*. Vision Res. 8:1407–1421.

Glantz, R. M. 1991. Motion detection and adaptation in crayfish photoreceptors—a spatio-temporal analysis of linear movement sensitivity. J. Gen. Physiol. 97:777–797.

Gurney, L. J., P. W. Froneman, E. A. Pakhomov, and C. D. McQuaid.

2001. Trophic positions of three euphausiid species from the Prince Edward Islands (Southern Ocean): Implications for the pelagic food web structure. Mar. Ecol. Prog. Ser. 217:167–174.

Herring, P. J. 1985. Bioluminescence in the Crustacea. J. Crust. Biol. 5(4):557–573.

Herring, P. J. 2002. *The biology of the deep ocean.* Oxford University Press, New York.

Hopkins, T. L., M. E. Flock, J. V. Gartner, Jr., and J. J. Torres. 1994. Structure and trophic ecology of a low latitude midwater decapod and mysid assemblage. Mar. Ecol. Prog. Ser. 109:143–156.

Howard, J., A. Dubs, and R. Payne. 1984. The dynamics of phototransduction in insects: A comparative study. J. Comp. Physiol. A 154:707–718.

Hu, V. J. H. 1978. Relationships between vertical migration and diet in four species of euphausiids. Limnol. Oceanog. 23(2):296–306.

Jerlov, N. G. 1976. *Marine optics*. Elsevier, Amsterdam.

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

Kinsey, S. T. and T. L. Hopkins. 1994. Trophic strategies of euphausiids in a low-latitude system. Mar. Biol. 118:651–661.

Konishi, J. 1955. Retinal and optic nerve response of the compound eye of spiny lobster, *Panulirus japonicus* von Siebold. Rep. Fac. Fish. Univ. Mie 2(1):138–144.

Land, M. F. 1981. Optics and vision in invertebrates. *In* H. Autrum (ed.), *Handbook of sensory physiology,* Vol. VII/6B, pp. 471–592. Springer-Verlag, Berlin.

Land, M. F. and D.-E. Nilsson. 2002. *Animal Eyes.* Oxford University Press, New York.

Laughlin, S. B. 1976. The sensitivities of dragonfly photoreceptors and the voltage gain of transduction. J. Comp. Physiol. 111:214–247.

Laughlin, S. B. 1996. Matched filtering by a photoreceptor membrane. Vis. Res. 36(11):1529–1541.

Laughlin, S. B. 1990. Invertebrate vision at low luminances. *In* R. F. Hess, L. T. Sharpe, and K. Nordby (eds.), *Night vision,* pp. 223–250. Cambridge Univ. Press, Cambridge.

Laughlin, S. B., R. R. de Ruyter van Steveninck, and J. C. Anderson. 1998. The metabolic cost of neural information. Nature Neurosci. 1(1):36–42.

Laughlin, S. B. and R. C. Hardie. 1978. Common strategies for light adaptation in the peripheral visual systems of fly and dragonfly. J. Comp. Physiol. 128:319–340.

Laughlin, S. B. and M. Weckström. 1993. Fast and slow photoreceptors—a comparative study of the functional diversity of coding and conductances in the Diptera. J. Comp. Physiol. 172:593–609.

Mauchline, J. 1960. The biology of the Euphausiid crustacean, *Meganyctiphanes norvegica* (M. Sars). Proc. Roy. Soc. B 67:141–179.

Mauchline, J. and L. R. Fisher. 1969. The biology of euphausiids. *In* F. S. Russell and M. Yonge (eds.), *Advances in marine biology,* vol. 7, pp.1–454. Academic Press, New York.

Moeller, J. F. and J. F. Case. 1995. Temporal adaptation in visual systems of deep-sea crustaceans. Mar. Biol. 123:47–54.

Munz, F. W. and W. N. McFarland. 1973. The significance of spectral position in the rhodopsins of tropical marine fishes. Vision Res. 13:829–1874.

Naka, K. I. and W. A. H. Rushton. 1966a. S-Potentials from colour units in the retina of fish (cyprinidae). J. Physiol. 185:536–555.

Naka, K. I. and W. A. H. Rushton. 1966b. S-potentials from luminosity units in the retina of fish (Cyprinidae). J. Physiol. 185:587–599.

Nilsson, D.-E., E. Hallberg, and R. Elofsson. 1986. The ontogenetic development of refracting superposition eyes in crustaceans: Transformation of optical design. Tiss. Cell 18(4):509–519.

Pinter, R. B. 1972. Frequency and time domain properties of retinula cells of the desert locust (*Schistocerca gregaria*) and house cricket (*Acheta domesticus*). J. Comp. Physiol. 77:383–397.

Roger, C. 1973. Recherches sur la situation torphique d'un groupe

d'organismes pélagiques (Euphausiacea). I. Niveaux trophiques des espéces. Mar. Biol. 18:312–316.

Tatler, B., D. C. O'Carroll, and S. B. Laughlin. 2000. Temperature and the temporal resolving power of fly photoreceptors. J. Comp. Physiol. A 186:399–407.

Weckström, M., R. C. Hardie, and S. B. Laughlin. 1991. Voltage-activated potassium channels in blowfly potoreceptors and their role in light adaptation. J. Physiol. London 440:635–657.

Welsh, J. H. and F. A. Chace, Jr. 1937. Eyes of deep-sea crustaceans. I. Acanthephyridae. Biol. Bull. 72:57–74.

Wong, F. 1978. Nature of light induced conductance changes in ventral photoreceptors of *Limulus*. Nature 276:76–79.

Wong, F., B. W. Knight, and F. A. Dodge. 1982. Adapting bump model for ventral photoreceptors of *Limulus*. J. Gen. Physiol. 79:1089–1113.

Zar, J. H. 1999. *Biostatistical analysis*. Prentice-Hall, Inc., New Jersey.

Zettler, F. 1969. Die Abhängigkeit des Ubertragungsverhaltens von Frequenz und Apatationszustand, gemessen am einzelnen Lichtrezeptor von *Calliphora erythrocephala*. Z. vergl. Physiol. 64: 432–449.