

S. H. D. Haddock · J. F. Case

Bioluminescence spectra of shallow and deep-sea gelatinous zooplankton: ctenophores, medusae and siphonophores

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Abstract We have examined the variability and potential adaptive significance of the wavelengths of light produced by gelatinous zooplankton. Bioluminescence spectra were measured from 100 species of planktonic cnidarians and ctenophores collected between 1 and 3500 m depth. Species averages of maximal wavelengths for all groups ranged from 440 to 506 nm. Ctenophores (41 species) had characteristically longer wavelengths than medusae (34 species), and the wavelengths from siphonophores (25 species) had a bimodal distribution across species. Four species each produced two different wavelengths of light, and in the siphonophore *Abylopsis tetragona* these differences were associated with specific body regions. Light from deep-dwelling species had significantly shorter wavelengths than light from shallow species in both ctenophores ($p = 0.010$) and medusae ($p = 0.009$). Although light production in these organisms was limited to the blue-green wavelengths, it appears that within this range, colors are well-adapted to the particular environment which the species inhabit.

Introduction

Bioluminescence is sometimes regarded as an oddity in the natural world, more curious than functional. It clearly must serve important roles in the sea, however, because it has evolved in nearly every group of marine organisms (Harvey 1952; Hastings 1983; Herring 1987; Case et al. 1994; Haddock and Case 1994). Sophisticated physiological and behavioral mechanisms for controlling

luminescence are also suggestive of its importance. Fishes, squid, and shrimp are able to modify intensity, kinetics, wavelength, and angular distribution of their light (Denton et al. 1970; Young and Mencher 1980; Latz and Case 1982), and even cnidarians and ctenophores, among the simplest of invertebrates, have developed ways to refine their expression of bioluminescence (Shimomura et al. 1962; Morin and Hastings 1971; Dunlap et al. 1987; Herring 1990).

Wavelength is an aspect of luminescence that is subject to great variation. It is important because it can determine how far light travels through the water and how well organisms can perceive a luminous signal. Although most marine luminescence has a maximal emission between 440 and 505 nm (Nicol 1958; Young 1981; Herring 1983; Widder et al. 1983; Latz et al. 1988), there is no widely accepted rationale explaining why some organisms emit light at one end of this range and others emit light at the other end. It has been suggested that the light of coastal species is generally green, while oceanic species tend to produce blue light (Young 1981; Morin 1983; Hastings and Morin 1991). Other investigators have speculated that the distinction lies between benthic (green-emitting) and pelagic (blue-emitting) organisms (Herring 1983; Young 1983). The published values of spectra support either of these assertions (Nicol 1958; Young 1981; Herring 1983; Widder et al. 1983; Latz et al. 1988).

In the systems which have been investigated, spectral differences can be traced to changes in the chemistry of the reaction. In luciferin–luciferase reactions, light is emitted from the luciferin molecule, but in many organisms the spectral differences are due to the luciferase, the enzyme which catalyzes the oxidation of the luciferin (Seliger and McElroy 1964; Wampler and Jamieson 1980; Wood 1990). The hydrozoans and ctenophores that have been examined use calcium-activated photoproteins in a role analogous to that of a luciferase (Shimomura 1985), with coelenterazine as the light-emitting molecule (Ward and Cormier 1975; Shimomura et al. 1980; Campbell and Herring 1990). Because differences in the amino acid sequences of photoproteins

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S.H.D. Haddock (✉)¹ · J.F. Case
Marine Science Institute,
University of California,
Santa Barbara, CA 93106, USA

Present address:

¹Monterey Bay Aquarium Research Institute,
P.O. Box 628, Moss Landing, California 95039, USA
Fax: 001 (0)831-775-1620
e-mail: haddock@mbari.org

can cause changes in the wavelengths of emission spectra (Ohmiya et al. 1992), the wavelength of luminescence could potentially be affected by selective pressure acting via the genes encoding photoproteins. In some species, the spectrum of light can be further modified by a green-fluorescent protein (GFP). GFP is a chromophore-bearing accessory protein which receives energy from a bioluminescent reaction and re-emits it as longer wavelength light, generally with an increase in quantum yield (Morin and Hastings 1971; Ward and Cormier 1978).

Although it appears intuitively likely that the spectrum of bioluminescence is an adaptive biological character, it is also possible that for some species the spectral detail is determined solely by chemical constraints and that variations have small ecological significance. As Herring (1983) observed, one cannot confidently associate a particular color with a function or a chemical system without spectral measurements from a variety of organisms and habitats.

Gelatinous organisms are extremely fragile, and are generally not suitable for analysis of their luminescence because of the trauma associated with collection by conventional means. In this study, use of specialized sampling techniques allowed the collection of many species of gelatinous zooplankton in good physiological condition from a variety of habitats. The assembled data set is sufficient for statistical analysis of the correlation of ecological factors such as depth and location with particular bioluminescence spectra.

Materials and methods

Collection

Deep-water species of gelatinous zooplankton were collected on cruises in the Bahamas (October 1989, December 1990, February 1991), the Alborán Sea (western Mediterranean; April 1991), and the Gulf of Maine (August 1992) from the Johnson-Sea-Link submersibles equipped with samplers described in Youngbluth (1984). Blue-water SCUBA divers, using techniques modified from those of Hamner (1975), hand-collected shallow-dwelling animals (< 25 m) at the above locations and in the Santa Barbara Channel, south of Santa Barbara, California (January 1990 to July 1992). Additional deep-water specimens were obtained from the Pacific Ocean 200 km west of Point Conception, California (March 1993) and near Oahu, Hawaii (April 1993) with a midwater trawl equipped with a thermally-insulated, closing cod-end (described in Childress et al. 1978). Each of these collection techniques recovers fragile specimens in excellent condition, making it possible to examine bioluminescence in species that are usually too damaged, or not obtainable at all, by typical net tows.

Specimens were identified based on descriptions by Kramp (1959) for hydromedusae, Totton (1954, 1965) and Kirkpatrick and Pugh (1984) for siphonophores, and Larson (1986) for scyphomedusae. Personal communications of G.R. Harbison (ctenophores), C.E. Mills (medusae and ctenophores), and P.R. Pugh (siphonophores) were extremely helpful. The taxonomy of the ctenophore genus *Beroë* is filled with synonymies and uncertainties, and we have followed the example of Mayer (1912) in assigning species' names, especially with regard to *B. cucumis* and *B. ovata*. Other ctenophores that are known to be new species (G.R. Harbison unpublished data) have been listed under the most specific taxonomic group to which they could be assigned.

After collection, specimens were transferred to filtered water and maintained at natural temperatures (depending on depth and location of collection) in the dark until testing, usually within a few hours of reaching the surface. Because chemical stimulation typically produces a steady glow rather than a bright flash, we evoked luminescence by physical stimulation, generally with a mesh screen or gentle prodding. This has the additional advantage of more closely approximating natural conditions that would induce a luminescent response.

Spectral measurements

Spectra were measured with an EG&G/PARC Model 1460 Optical Multichannel Analyzer, which simultaneously records light between 350 to 700 nm across a 700-channel diode array (see Widder et al. 1983 for details). This instrument was sensitive enough to detect light even from dim sources. Fiber-optic input permitted localized measurements when needed. A program was written in C to smooth the spectra three times with a second-order smoothing algorithm (Gorry 1990), correct for the spectral sensitivity of the detector against a tungsten standard lamp, and measure the full width of the spectral curve at half-maximum intensity (FWHM).

If a specimen produced more than one measurable flash, the record with the highest signal-to-noise ratio was selected. The wavelength of maximal emission for this curve (λ_{\max}) was averaged with the maxima from other individuals of the same species to generate a mean λ_{\max} for each species. Species' averages were considered as the relevant division for statistical analysis of taxa: although measuring more individuals of a particular species contributes to a better *species'* average, it should not affect the power of a statistical analysis of how a particular *group* of species (e.g. deep-sea ctenophores) differs. The dangers of using species as data points have been considered (Felsenstein 1985), yet the phylogenies of these groups are insufficiently understood to do thorough comparative analyses.

Generally, the wavelength of maximum emission did not vary between measurements from an individual specimen or species, but in the few cases in which a species produced distinctive colors, distinguishable spectra were treated independently and are reported as multiple values.

Chemical extraction

To examine the spectra of isolated photoproteins and to test for the presence of GFP, active photoproteins were extracted by homogenization on ice in a calcium-chelating buffer consisting of 200 mM Tris and 40 mM EDTA at pH 8.75. The EDTA in these extracts prevented activation of the calcium-activated photoproteins found in ctenophores and hydromedusae. In vitro luminescence was triggered by the addition of an excess of 360 mM CaCl_2 .

To test whether the long-wavelength emission of the siphonophore *Bargmannia* sp. indicated the presence of green fluorescent protein, specimens which had been frozen in liquid nitrogen were extracted into a buffer of 100 mM Tris and 40 mM EDTA. After prefiltration with a Gelman A/E glass-fiber filter, samples were concentrated by ultrafiltration with Millipore Ultrafree-PFL low-binding cellulose filters. Spectrofluorometry was conducted on an SLM Instruments SPF-500C fluorometer scanning both excitation and emission wavelengths.

Depth assignment

Species of ctenophores and medusae were separated into two depth categories to test whether λ_{\max} varied with vertical distribution. The mean depth of collection was used to separate each species into either a "shallow" or "deep" (= mesopelagic) group for statistical analysis. Independent of bioluminescence information, a depth of 200 m was chosen as the division below which organisms

were considered to be “deep” species. This threshold is similar to the depth (“roughly 150 m”) considered to be the lower limit of epipelagic plankton distribution by Van der Spoel and Pierrot-Bults (1979). Many researchers use minimum depth of occurrence to characterize vertical distribution of plankton, so in cases when one or two deep collections of a typically shallow species skewed the depth distribution, the species were placed in the shallow category. For example, *Beroe forskalii* and *Bolinopsis infundibulum* each had one occurrence at >300 m that was considered atypical because nearly all other specimens were caught near the surface. One problematic species, the ctenophore *Thalassocalyce inconstans*, was considered deep-living because adults were recovered only from deep waters, although juvenile specimens were found near the surface and produced the same spectrum. Only one species, the scyphomedusa *Phacellophora camtschatica*, had a mean collection depth which fell between 100 and 200 m, so the establishment of two depth categories appears sound. Although hydromedusae are more closely related to siphonophores than scyphomedusae, siphonophores were kept out of the grouped analysis because we obtained so few spectra from shallow siphonophores, and because there were several occurrences of multiple colors produced by one species, making the choice of a representative wavelength difficult.

Because a given species may be found at a wide range of depths, it may seem arbitrary to divide the data set into two groups based on a numeric depth standard. The properties of the water column do not change in a linear fashion, however, especially with regard to optical properties: the waters of the mixed layer above the thermocline are distinctly separable from those below. Furthermore, the sampling systems used, with SCUBA dives to collect shallow specimens and submersibles and midwater trawls to gather deep species, operationally divided the organisms into two groups. Therefore, a dichotomous treatment of depths of occurrence was deemed more appropriate than regression analysis. Because the shallow subsets of each phylum were distributed non-normally (Shapiro–Wilks test: ctenophores: $p = 0.008$; medusae: $p = 0.002$), nonparametric statistics were used for the analyses, although the parameters associated with each distribution are still reported.

There was not sufficient information to make statements about diel vertical migration for most of the species in this study, but shallow species were collected mainly during the day, and approximately two-thirds of the deep specimens were captured during the day. Although some gelatinous species are known to migrate vertically, there is no evidence to indicate that vertical migration would greatly affect the distribution categories.

Results

Luminescence spectra were obtained from 321 specimens representing 41 species of ctenophores, 11 species of scyphomedusae, 23 species of hydromedusae, and 25 species of siphonophores (Table 1). Light was produced by nearly every species of gelatinous plankton examined, but with some unexplained exceptions. Because luminescent ability is so dependent on the condition of the organisms, we are reluctant to make any assertions about which of the species gathered are not bioluminescent. Most of the species presented here have not been measured before, but in those cases where species have been investigated by modern instrumentation, our values match closely. Spectra of all gelatinous individuals (before species averaging) spanned 66 nm, from 440 to 506 nm. Since these numbers are individual values, they differ slightly from the ranges of species’ averages presented in Table 1.

Spectra by taxonomic group

Over 92% of the ctenophore genera examined in this study were bioluminescent, the only exceptions being *Pleurobrachia* spp. and *Hormiphora californensis*, which were found to be non-luminous by Haddock and Case (1995). Ctenophore species were generally “greener” (= longer wavelength: $486.1 \text{ nm} \pm 1.6 \text{ SE}$; range = 458 to 501 nm) than either medusae or siphonophores (Fig. 1A; Table 2). The lobates (488.2 ± 1.0) and cydippids (482.5 ± 2.7) were not significantly different from each other ($p = 0.073$; Scheffé contrast to selectively test two orders of five examined). The shortest wavelengths were in the deep-living species *Aulacoctena acuminata* and *Euplokamis stationis*, while the longest wavelengths were produced by *Haeckelia beehleri* and *Velamen parallelum* (Fig 1; Table 1). None had the characteristically shaped spectra or bright fluorescent structures which might indicate the presence of green-fluorescent proteins. As with the ctenophore *Mnemiopsis* sp. (Ward and Seliger 1976), the shallow lobate *Ocyropsis maculata immaculata* reversibly lost its bioluminescence after exposure to light. In contrast, some deep-sea species did not appear to require dark adaptation to be capable of luminescence. Generally, light was produced from the meridional canals and their extensions, but it was not limited to the regions immediately underlying the comb plates. Several ctenophores, including *Bathocyctena chumi*, *Euplokamis stationis*, *Eurhamphaea vexilligera*, and a large red undescribed merteniid (Species D in Table 1), produced luminous secretions when disturbed. In the cases we examined, the spectra of extracellular luminescence did not differ from bioluminescence originating within the body. Unlike previous investigators (Latz et al. 1988), we did not detect a slightly bimodal spectrum in any of the *Beroe* species.

The mean λ_{max} for all medusa species was $473.8 \text{ nm} \pm 2.9 \text{ SE}$ (Table 2), spanning a 62 nm range from 443 to 505 nm (Fig. 1B). Because there was no significant difference between scyphomedusae ($474.0 \text{ nm} \pm 2.8 \text{ SE}$) and hydromedusae ($473.7 \pm 4.2 \text{ SE}$) (Student’s t -test: $p = 0.96$; Mann–Whitney U -test: $p = 0.58$; F -test: $p = 0.054$), and because of the small sample size of shallow medusa species ($n = 7$), these classes were combined for considerations of spectral trends.

Among the medusae, the four shortest-wavelength species were Trachymedusae from the family Halicreidae (Table 1, Fig. 1E). The longest-wavelength medusae were Leptomedusae such as the well-known *Aequorea forskalea* and *Clytia* (= *Phialidium*) *hemisphaericum*, which bear GFP. Narcomedusae such as *Aegina citrea* and *Solmissus* spp. produced intermediate luminescence spectra, with λ_{max} between 460 and 478 nm. Family groupings did not give perfect correlation with spectra, however: *Halopsis ocellata*, which is in the same family as the GFP-bearing *Mitrocoma cellularia*, had a λ_{max} shorter even than would *M. cellularia* without GFP. It is

Table 1 Mean species' wavelengths and depth distributions [λ averaged maximum wavelength for each species and standard deviation of shown in square brackets next to the main peak]; *FWHM* average full width at half maximum intensity, an indication of width of curve; Pacific Ocean); S Santa Barbara Channel, off California; *Depth* depth category used for analysis, and mean depth of collection; (*n*) number

| Species | λ (nm) | FWHM (nm) | Collection area | Depth (m) | (<i>n</i>) |
|---|-------------------|--------------|--------------------|--------------|--------------|
| Ctenophores | | | | | |
| <i>Aulacoceta acuminata</i> Mortensen | 458 | 90 | H | Deep, 1200 | (2) |
| <i>Bathocyroe fosteri</i> Madin and Harbison | [459 to 492] | 102 | B | Deep, 609 | (18) |
| <i>Bathycytena chumi</i> (Moser) | 492 \pm 5.6 | 91 | P,H | Deep, 2167 | (5) |
| <i>Bathycytena</i> sp. nov. A ^a | 488 | 88 | B | Deep, 684 | (1) |
| <i>Bathycytena</i> sp. nov. B ^a | 490 \pm 5.3 | 94 | H | Deep, 1200 | (3) |
| <i>Bathycytenidae</i> , gen. nov., sp. nov. ^a | 501 \pm 4.6 | 121 | B | Deep, 834 | (3) |
| <i>Beroe abyssicola</i> Mortensen | 491 \pm 1.1 | 88 | P,S | Deep, 489 | (3) |
| <i>Beroe cucumis</i> Fabricius | 489 \pm 4.7 | 88 | G,M,S | Shallow, 37 | (11) |
| <i>Beroe forskalii</i> Milne Edwards | 491 \pm 5.0 | 89 | S,M | Shallow, 14 | (5) |
| <i>Beroe gracilis</i> Künne | 495 | 89 | S | Shallow, 12 | (2) |
| <i>Beroe ovata</i> Bosc | 493 | 89 | B | Shallow, 18 | (1) |
| <i>Bolinopsis infundibulum</i> Müller ^b | 488 \pm 4.2 | 88 | G,M,S | Shallow, 100 | (8) |
| <i>Bolinopsis vitrea</i> (L. Agassiz) | 490 | 90 | B | Shallow, 8 | (2) |
| <i>Cestum veneris</i> Lesueur | 493 \pm 7.7 | 89 | B,S | Shallow, 12 | (3) |
| <i>Charistephane fugiens</i> Chun | 468 \pm 1.2 | 84 | P | Deep, 300 | (6) |
| Cydippida, unidentified species ^a | 482 | 79 | B | Deep, 662 | (1) |
| <i>Deiopea kaloktenota</i> Chun | 489 \pm 4.3 | 95 | S | Shallow, 19 | (7) |
| <i>Euplokamis</i> sp. | 483 \pm 1.9 | 85 | G | Deep, 243 | (4) |
| <i>Euplokamis stationis</i> Chun | 467 \pm 4.5 | 82 | M | Deep, 314 | (3) |
| <i>Eurhamphaea vexilligera</i> Gegenbaur | 496 \pm 5.2 | 94 | B,M | Shallow, 16 | (8) |
| <i>Haeckelia beehleri</i> (Mayer) | 500 \pm 5.1 | 88 | M,S | Shallow, 12 | (10) |
| <i>Haeckelia bimaculata</i> Carré and Carré | 490 | 98 | S | Shallow, 17 | (1) |
| <i>Haeckelia rubra</i> (Kölliker) | 489 \pm 1.2 | 98 | S | Shallow, 17 | (3) |
| <i>Kiyohimea aurita</i> Komai and Tokioka | 491 | 103 | B | Deep, 825 | (2) |
| <i>Lampea</i> sp. | 470 | 94 | P | Deep, 2000 | (1) |
| <i>Lampea lactea</i> (Mayer) | 469 | 85 | B | Shallow, 18 | (1) |
| <i>Lampea pancarina</i> (Chun) | 473 | 81 | M | Deep, 494 | (1) |
| <i>Leucothea multicornis</i> (Quoy and Gaimard) | 488 | 93 | M | Shallow, 14 | (2) |
| <i>Leucothea pulchra</i> (Matsumoto) | 488 \pm 0.6 | 92 | S | Shallow, 15 | (5) |
| <i>Lobata</i> , sp. nov. A ^{a,c} | 492 \pm 1.6 | 90 | B | Deep, 830 | (4) |
| <i>Lobata</i> , sp. nov. B ^a | 488 | 88 | B | Deep, 758 | (1) |
| New family, gen. nov., sp. nov. A ^a | 490 | 89 | B | Deep, 520 | (1) |
| New family, gen. nov., sp. nov. B ^a | 485 | 88 | B | Deep, 726 | (1) |
| Mertensiidae, gen. nov. A, sp. nov. A ^a | 497 \pm 5.4 | 87 | M,S | Shallow, 20 | (6) |
| Mertensiidae, gen. nov. A, sp. nov. B ^a | 489 | 86 | M | Deep, 634 | (1) |
| Mertensiidae, gen. nov. B, sp. nov. C ^a | 471 \pm 5.1 | 85 | B | Deep, 787 | (3) |
| Mertensiidae, gen. nov. B, sp. nov. D ^{a,d} | 472 \pm 4.6 | 94 | B | Deep, 880 | (6) |
| <i>Ocyropsis maculata immaculata</i> Harbison and Miller | 489 \pm 1.2 | 90 | B,M | Shallow, 9 | (4) |
| <i>Thalassocalyce inconstans</i> Madin and Harbison | 491 \pm 4.6 | 92 | B,H,P,S | Deep, 322 | (7) |
| Thalassocalycidae, gen. nov., sp. nov. ^a | 483 \pm 6.2 | 89 | B,M | Deep, 388 | (6) |
| <i>Velamen parallelum</i> (Fol) | 501 \pm 3.2 | 90 | S | Shallow, 7 | (3) |
| Scyphomedusae | | | | | |
| <i>Atolla parva</i> Russell | 468 | 89 | B | Deep, 833 | (1) |
| <i>Atolla vanhoeffeni</i> Russell | 469 \pm 9.1 | 84 | B,P | Deep, 588 | (4) |
| <i>Atolla wyvillei</i> Haeckel | 470 | 98 | B | Deep, 908 | (1) |
| Coronatae, unidentified species ^c | 468 | 89 | B | Deep, 848 | (1) |
| <i>Nausithoe globifera</i> Broch | 494 \pm 5.5 | 85 | P | Deep, 1333 | (3) |
| <i>Nausithoe atlantica</i> Broch | 480 | 88 | P | Deep, 1350 | (1) |
| <i>Paraphyllina ransoni</i> Russell | 465 | 85 | P | Deep, 1500 | (2) |
| <i>Periphylla periphylla</i> Péron and Lesueur | 465 \pm 1.3 | 83 | B,P | Deep, 1082 | (5) |
| <i>Periphyllopsis braueri</i> Vanhöffen | 473 | 85 | B | Deep, 882 | (1) |
| <i>Phacellophora camtschatica</i> Brandt ^b | 491 | 107 | G | Shallow, 182 | (1) |
| <i>Poralia</i> sp. | 468 \pm 2.9 | 84 | B | Deep, 832 | (4) |
| Hydromedusae | | | | | |
| <i>Aegina citrea</i> Eschscholtz | 469 \pm 2.5 | 92 | B,P | Deep, 934 | (4) |
| <i>Aeginura grimaldii</i> Maas | 464 \pm 10.9 | 88 | B,P | Deep, 742 | (7) |
| <i>Aequorea forskalea</i> Péron and Lesueur | 503 | f | S | Shallow, 12 | (1) |
| <i>Bythotia depressa</i> Naumov | 488 \pm 2.2 | 80 | P | Deep, 408 | (4) |
| <i>Clytia</i> (= <i>Phialidium</i>) <i>hemisphaericum</i> (L.) | 504 | 37 | M | Shallow, 20 | (1) |
| <i>Cunina globosa</i> Eschscholtz | 462 | 76 | P | Deep, 400 | (2) |
| <i>Euphysora valdiviae</i> Vanhöffen | 464 \pm 2.1 | 93 | P | Deep, 2167 | (4) |
| <i>Halicreas minimum</i> Fewkes | 469 \pm 11.6 | 88 | B,H,P | Deep, 1093 | (10) |

these maxima when >2 specimens of same species were measured (where an organism emitted two wavelengths, the secondary peak is B Bahamas; G Gulf of Maine; H Hawaii; M western Mediterranean (Alborán Sea); P 100 miles west of Point Conception (eastern temperate of specimens examined])

| Species | λ (nm) | FWHM (nm) | Collection area | Depth (m) | (n) |
|--|-------------------|--------------|--------------------|--------------|------|
| Halicreidae, unidentified species ^c | 445 | 79 | B | Deep, 851 | (1) |
| <i>Haliscera conica</i> Vanhöffen | 451 | 85 | M | Deep, 448 | (2) |
| <i>Halitrephes maasi</i> Bigelow | 458 | 125 | B | Deep, 711 | (1) |
| <i>Halitrephes valdiviae</i> H.B. Bigelow | 443 | 80 | B | Deep, 767 | (2) |
| <i>Halopsis ocellata</i> Agassiz | 458 | 99 | G | Shallow, 20 | (1) |
| <i>Mitrocoma cellularia</i> (Agassiz) | 505 | 55 | S | Shallow, 5 | (1) |
| <i>Mitrocomella</i> sp. | 500 | 62 | S | Shallow, 8 | (1) |
| <i>Obelia</i> sp. | 502 | ^f | S | Shallow, 3 | (1) |
| <i>Octophalucium funerarium</i> (Quoy and Gaimard) | 487 | 72 | M | Deep, 391 | (1) |
| <i>Pandea conica</i> (Quoy and Gaimard) | 470 | 98 | P | Deep, 450 | (2) |
| <i>Pegantha laevis</i> H.B. Bigelow | 460 \pm 1.4 | 75 | P | Deep, 300 | (4) |
| <i>Solmissus albescens</i> (Gegenbaur) | 478 \pm 0.0 | 76 | M | Deep, 485 | (3) |
| <i>Solmissus incisa</i> (Fewkes) | 465 | 76 | B,P | Deep, 742 | (2) |
| <i>Solmissus marshalli</i> Agassiz and Mayer | 477 | 75 | P | Deep, 250 | (2) |
| <i>Solmundella bitentaculata</i> (Quoy and Gaimard) | 477 \pm 7.1 | 83 | B,P | Deep, 492 | (6) |
| Siphonophores | | | | | |
| Calycophorae | | | | | |
| <i>Abylopsis tetragona</i> (Otto) | 489 [450] | 61 | M | 15 | (4) |
| <i>Chumiphys multidentata</i> Lens and van Riemsdijk | 481 \pm 1.4 | 61 | P | 1500 | (2) |
| <i>Craseoa lathetica</i> Pugh and Harbison | 489 | 90 | B | 719 | (1) |
| <i>Hippopodius hippopus</i> (Forskål) | 450 | 83 | P | 150 | (1) |
| <i>Maresearsia praeclara</i> Totton | 486 | ^f | P | 800 | (1) |
| <i>Muggiaea</i> sp. | 500 | 76 | S | 3 | (1) |
| <i>Nectadamas diomedae</i> (Bigelow) | 443 \pm 2.1 | 83 | B,P | 1329 | (5) |
| <i>Nectopyramis natans</i> (Bigelow) | 447 \pm 1.4 | 81 | H,P | 750 | (4) |
| <i>Praya dubia</i> (Quoy and Gaimard) | 477 \pm 0.8 | 86 | P | 560 | (5) |
| Prayidae, unidentified species ^c | 448 | 80 | P | 300 | (1) |
| <i>Rosacea plicata</i> sensu Bigelow | 491 \pm 2.1 | 77 | B,P | 431 | (2) |
| <i>Vogtia glabra</i> Bigelow | 448 | 79 | B | 537 | (1) |
| <i>Vogtia serrata</i> (Moser) | 451 \pm 0.6 | 86 | P | 650 | (3) |
| Physonectae | | | | | |
| <i>Agalma okeni</i> Eschscholtz | 447 \pm 1.2 | 90 | H | 550 | (3) |
| <i>Apolemia</i> sp. 1 | 445 | 82 | H | 1500 | (1) |
| <i>Apolemia</i> sp. 2 | 442 | 111 | H | 1500 | (1) |
| <i>Bargmannia</i> sp. | [443 to 499] | 92 | B | 512 | (7) |
| <i>Bargmannia</i> sp. nov. ^a | 480 | 100 | P | 1350 | (1) |
| <i>Erenna</i> sp. nov. | 455 | 109 | P | 1150 | (1) |
| <i>Frillagalma vityazi</i> Daniel | [455 to 492] | 91 | B | 363 | (10) |
| <i>Halistemma amphitridis</i> (Lesueur and Petit) | 451 | 88 | B | 894 | (1) |
| <i>Halistemma</i> sp. | 446 | 81 | H | 1500 | (1) |
| <i>Halistemma</i> sp. nov. ^a | 460 | 83 | B | 787 | (2) |
| <i>Nanomia bijuga</i> (delle Chiaje) | 457 | 87 | S | 9 | (1) |
| <i>Nanomia cara</i> Agassiz | 454 \pm 2.0 | 92 | G | 9 | (4) |

^a Undescribed species, identified to nearest taxonomic level

^b Species generally found at shallow depths, but rare deep collection skewed depth-averaging

^c Undescribed species referred to as "UC-I" by Bailey et al. (1994)

^d Undescribed species referred to as "Agmayeria tortugensis" by Bailey et al. (1994, 1995)

^e Species which we were unable to identify, but which were used for statistical analyses

^f Not accurately discernible due to low signal

not clear whether this reflects more inconsistencies of taxonomy or of bioluminescence spectra. Contrary to Nicol (1958), we did not observe a secondary long wavelength peak in *Atolla wyvillei*.

All the mesopelagic siphonophores tested in good condition were luminous, except for *Lychmagalma utricularia* and the uncommon cystonect *Rhizophysa eysenhardti*, although some calycophorans were too dim to be measured in this study. Siphonophore spectra were distributed bimodally (Fig. 1C) with modes (Table 2)

centered at 450.5 ± 1.3 and at 486 ± 2.3 (mean \pm SE; range = 442 to 500 nm). Surprisingly, no siphonophores were found to emit between 462 and 476 nm, although the statistical mean for the whole group lies in that range. The shortest wavelengths were obtained from species of *Apolemia* and *Bargmannia*, (Fig. 1F), whereas the longest was from the epipelagic *Muggiaea* sp. In the siphonophores, there was no obvious relation between luminescence and taxon: calycophorans and physonects were equally well represented by short- and

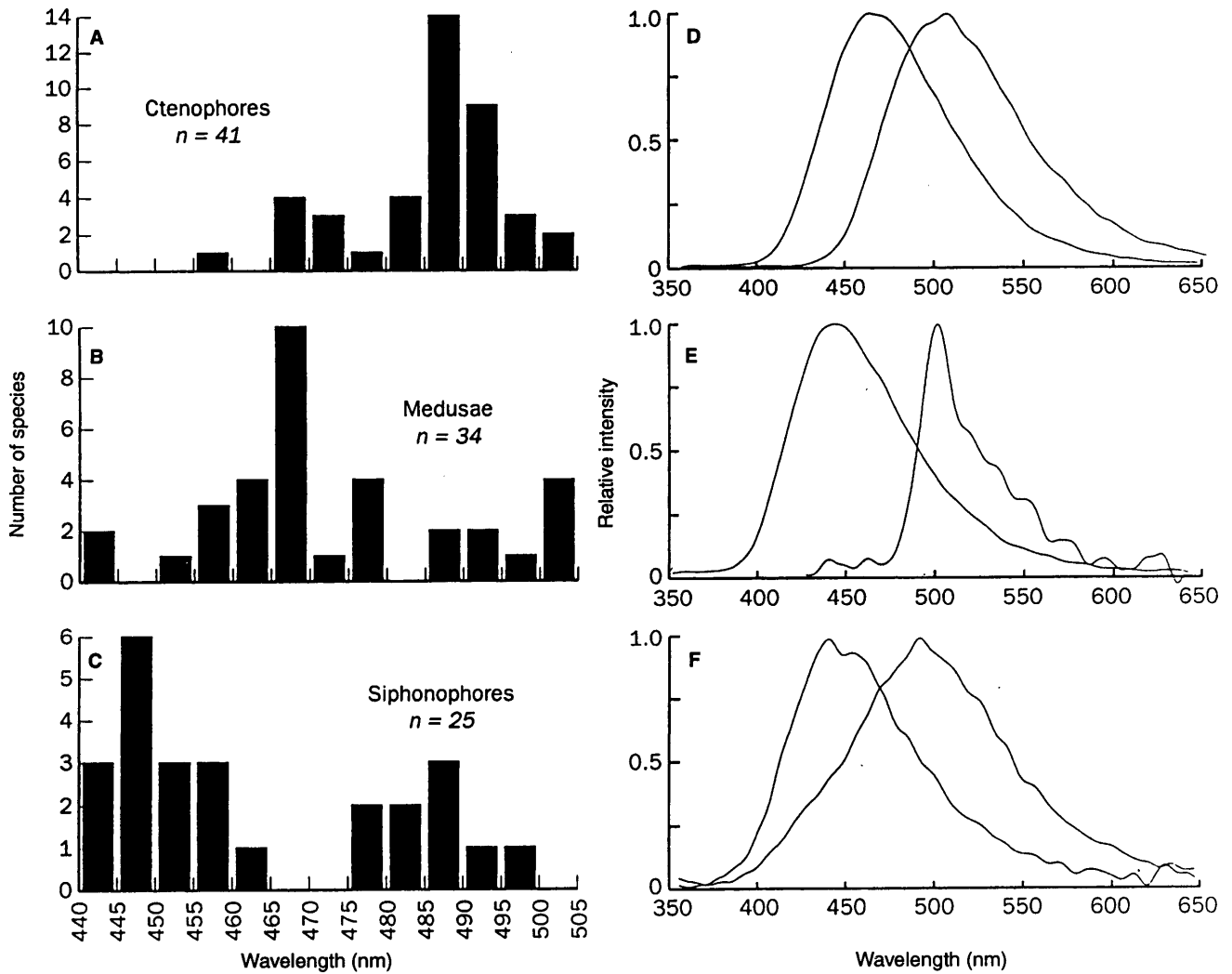


Fig. 1A–C Distribution of mean species wavelength for three groups of gelatinous zooplankton examined: ctenophores (**A**, $n = 41$) and medusae (**B**, $n = 34$) were evenly distributed about overall group mean, but siphonophore species (**C**, $n = 25$) produced bimodal distribution. **D–F** Representative spectra from extremes of each group (individual spectra shown here may have different values than species' averages in Table 1). **D** ctenophores: *Euplokamis stationis* ($\lambda_{\text{max}} = 464$ nm) and mertensiid gen. nov. A, sp. nov. A ($\lambda_{\text{max}} = 504$ nm). **E** medusae: *Halitrephes valdiviae* ($\lambda_{\text{max}} = 441$ nm) and *Clytia* (= *Phialidium*) *hemisphaericum* ($\lambda_{\text{max}} = 502$ nm); irregularity in the *C. hemisphaericum* spectrum is due to smoothing of relatively noisy signal. **F** Siphonophores: in this group, several species produced two colors of luminescence; surprisingly, *Bargmannia elongata* which emitted blue light ($\lambda_{\text{max}} = 446$ nm) and green light ($\lambda_{\text{max}} = 493$ nm) provided best examples of both extremes for siphonophores

long-wavelength species (Table 1). Contrary to an earlier report of a secondary peak in *Vogtia glabra* (Nicol 1958), we found that this species produced a unimodal emission with a λ_{max} near 450 nm.

Multiple wavelengths of light emission

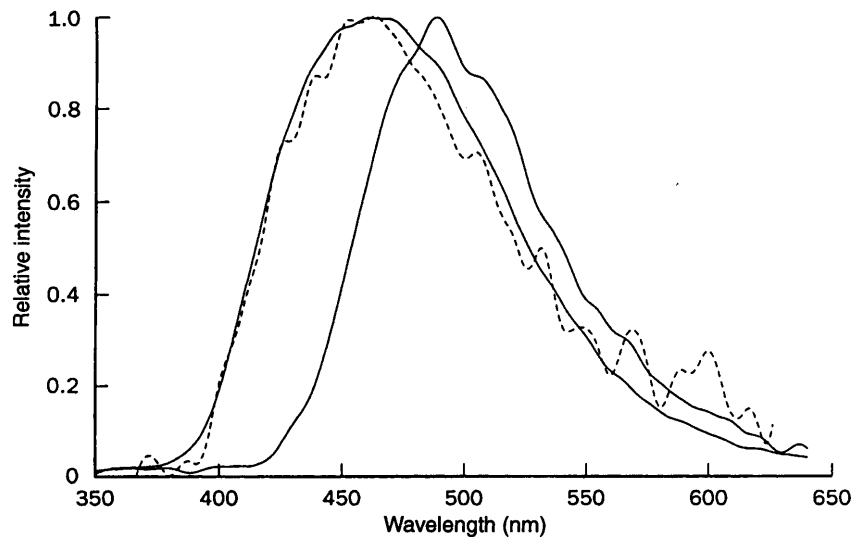
In most cases, variation within a species was small (SD < 6 nm), even when the specimens were taken from

different locations; however, four species showed substantial intraspecific differences in λ_{max} . The lobate ctenophore *Bathocyroe fosteri* was highly variable, with λ_{max} separated by up to 32.2 nm in the most extreme examples (Fig. 2). For depth analysis of this species, we

Table 2 Mean wavelengths of luminescence for groups of organisms tested. Because siphonophores were distributed bimodally (Fig. 2), means of each mode are presented here, rather than overall mean. Significance levels (p) are for Mann–Whitney U -tests of shallow vs deep species in ctenophores and in medusae

| Group | Mean \pm SE | (n) | p |
|---------------|-----------------|---------|-------|
| Ctenophores | | | |
| Shallow | 490.8 \pm 1.6 | (18) | 0.010 |
| Deep | 482.3 \pm 2.2 | (23) | |
| Total | 486.1 \pm 1.6 | (41) | |
| Medusae | | | |
| Shallow | 494.7 \pm 6.4 | (7) | 0.003 |
| Deep | 468.4 \pm 2.3 | (27) | |
| Total | 473.8 \pm 2.8 | (34) | |
| Siphonophores | | | |
| Green | 486.3 \pm 2.3 | (9) | |
| Blue | 450.0 \pm 1.3 | (16) | |
| Total | | (25) | |

Fig. 2 *Bathocyroe fosteri*. Luminescence spectra of live specimens and extracts. Natural luminescence of this mesopelagic ctenophore may vary by > 30 nm even within same individual [continuous lines (two different specimens)]. Extracts of *B. fosteri* produce blue light ($\lambda_{\max} = 457$ nm; dashed line) upon addition of CaCl_2 , similar to the shorter in vivo λ_{\max} .



used the mean and most common measure of 479 nm. Buffered extracts (pH 8.75, ~ 4 °C) when triggered with CaCl_2 produced light which most closely matched the short-wavelength in vivo emission (dashed line in Fig. 2). These differences were not associated with different anatomical regions.

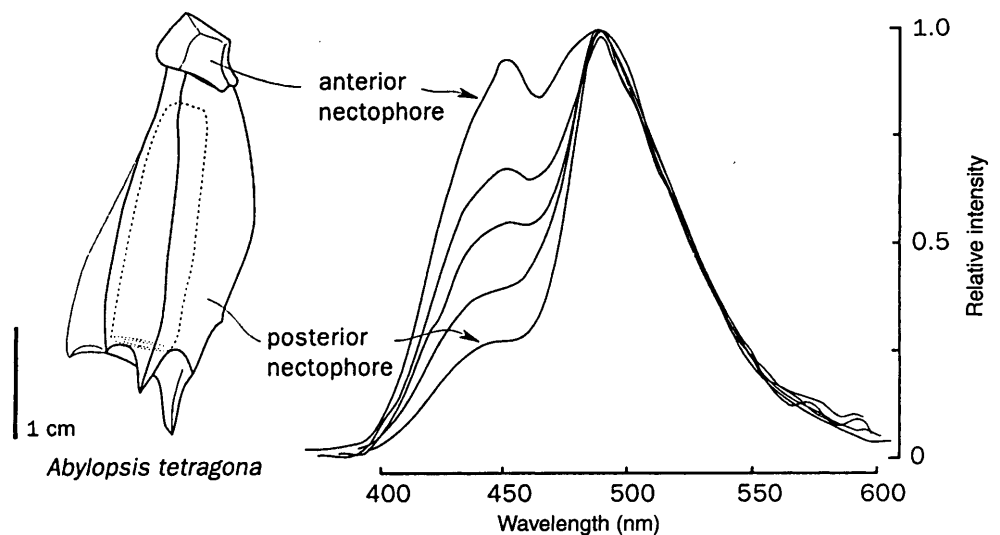
Three species of siphonophores, *Abylopsis tetragona*, *Bargmannia elongata*, and *Frillagalma vityazi*, also produced multiple colors of luminescence. In *A. tetragona* there was a major peak at 489 nm, and a secondary peak of variable intensity at 450 nm (Fig. 3). The colors were correlated with the region of the siphonophore from which the measurement was taken: the posterior end produced predominantly green light while the anterior end produced more blue light. Fluorescence microscopy of the entire siphonophore (366 and 490 nm excitation wavelengths) revealed no indication of GFP, but only the faint blue fluorescence (typical of bioluminescence systems) which was also seen in *Vogtia spinosa*, a siphonophore which emits short wavelength luminescence (SHDH personal observations). In contrast, *B. elongata*

did not display two distinct peaks, but a broad spectrum whose λ_{\max} varied by up to 56 nm (443 to 499 nm), which is 30% more than the entire range of the phylum Ctenophora. This variability is extreme enough to enable this species to represent both the bluest and greenest siphonophore spectra (Fig. 1F). Longer wavelengths were associated with a broader bandwidth, but not with a particular part of the siphonophore. A fluorometric assay for fluorescent proteins did not reveal any fluorescent material in *B. elongata*, in contrast to the control species, the hydromedusa *Mitrocoma cellularia*, which is known to have GFP (Fig. 4). No medusa species with multiple wavelengths of in vivo light emission was found.

Trends with depth

The mean emission wavelength for deep ctenophores was 8.4 nm shorter than that of shallow ctenophores, and the mean for deep medusae was 26.3 nm shorter

Fig. 3 *Abylopsis tetragona*. Luminescence spectra in relation to location along body. This calyphoran siphonophore produces two colors of luminescence, with short-wavelength emission generally more predominant near anterior nectophore. Spectra from three specimens are shown, although the most extreme examples were from same individual



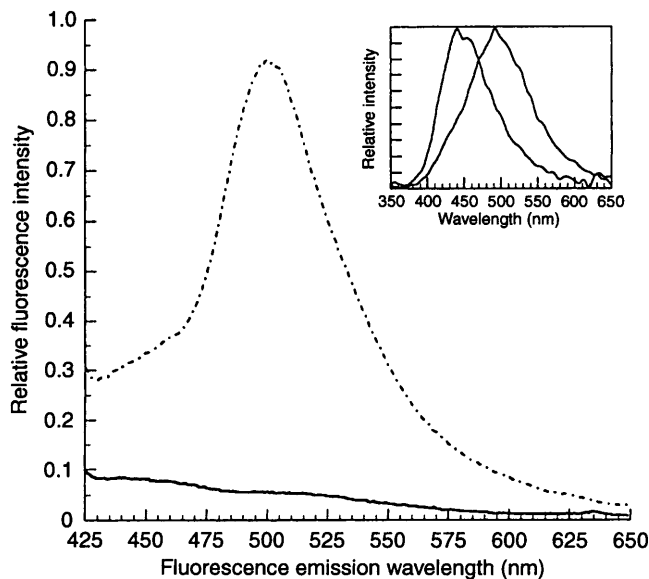


Fig. 4 *Mitrocoma cellularia* and *Bargmannia* sp. Fluorescence spectra of extracts. Hydromedusa *M. cellularia* is known to contain green-fluorescent protein (GFP), and spectrofluorometric assay ($\lambda_{\text{excitation}} = 400$ nm, detection bandwidth = 5 nm) shows appropriate peak (dashed line). However, this assay showed no evidence of green-fluorescent protein in the siphonophore *Bargmannia* sp. (continuous line) (Inset bioluminescence of *Bargmannia* sp., showing wide range of spectra which can be produced by this single species; also note absence of a narrow peak around 500 nm that is usually found in GFP-bearing organisms)

than for shallow species. These differences between shallow and deep species were significant within the ctenophores (Mann–Whitney U -test: $p = 0.010$) and the medusae (U -test: $p = 0.003$). There was significant interaction between phylum and depth ($p = 0.008$), suggesting that while depth operated in the same general way for both ctenophores and medusae (shorter wavelength with increasing depth), ctenophores were not affected as strongly.

The overall relationship between wavelength and depth was evident in the data before depth categorization (Fig. 5). Several outliers were interesting exceptions to the general trends between depths. The hydromedusa *Halopsis ocellata*, for example, had a short λ_{max} of 458 nm but was found in the surface waters of the Gulf of Maine (a in Fig. 5). *Lampea lactea*, which was collected on a blue-water dive, produced luminescence that was noticeably shorter ($\lambda_{\text{max}} = 469$) than other shallow ctenophores (b in Fig. 5): its congeners emit near the same wavelength but are found much deeper. The final outlier was a small red bathyctenid ctenophore that had a λ_{max} of 501 nm despite occurring at nearly 1000 m (c in Fig. 5).

Discussion and conclusions

Although there is variety in the spectra produced, the composite data set is perhaps more remarkable for the

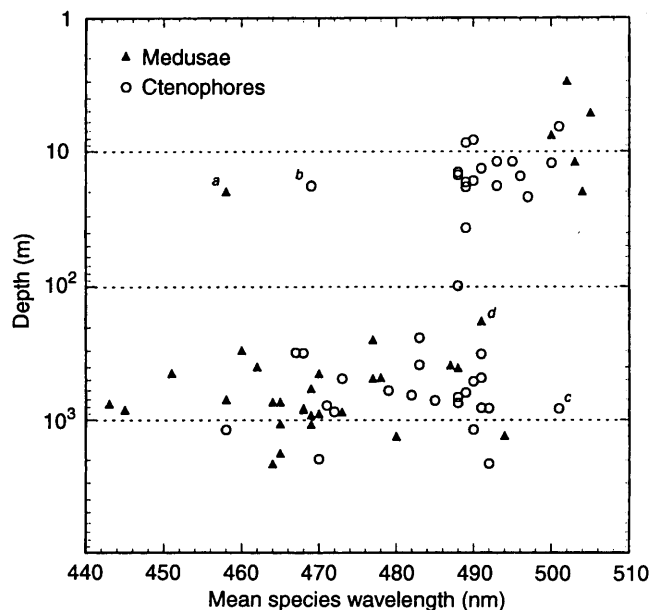


Fig. 5 Ctenophores and medusae. Relationship between wavelength of maximum emission and depth for each species. Outlier species include *Halopsis ocellata* (a), *Lampea lactea* (b), and a new species of ctenophore (c). *Phacellophora camtschatica* (d) was deepest species considered “shallow”; it was typically captured on blue-water dives, but specimen whose spectrum was measured happened to be collected by submersible

relatively limited range of wavelengths produced. Very few marine organisms have yet been found which produce light significantly shorter than 440 nm or beyond 510 nm. Unfortunately, detailed studies of cnidarian photoproteins and luminescence chemistry to date have focused on easily obtainable shallow medusae whose luminescence centers around 470 nm. This has led to the perception that the majority of bioluminescent coelenterates emit “green light” (e.g. Shimomura 1985). In addition to clarifying the true range of bioluminescent emission from cnidarians and ctenophores, perhaps a useful outcome of the present results will be to point out species of potential interest in molecular research, such as those with extreme wavelengths of luminescence.

Multiple colors of luminescence

There were two ways that multiple peak wavelengths of luminescence were expressed. The first was seen in the ctenophore *Bathocyroe fosteri* (Fig. 2), and the siphonophores *Frillagalma vityazi* and *Bargmannia elongata* (Fig. 1F), where the peak of a broad unimodal spectrum moved from one end of the range to the other. The shift in peak λ_{max} was not correlated with a particular body region in these organisms. In systems with GFP, extraction typically dissociates the photoproteins from the fluorescent molecule, so that the emission shifts to a shorter wavelength, as found in the extracts of *Bathocyroe fosteri* (Fig. 2). It is not clear whether the changes in λ_{max} are related to an energy-transfer system, how-

ever, because GFP characteristically emits light with a narrow peak at a fixed wavelength, and this was not observed in *B. fosteri*.

In contrast to the broad spectra described above, the spectrum of *Abylopsis tetragona* was bimodal, with two distinct narrow peaks (Fig. 3). In each measurement, both wavelengths were present but in varying amounts, depending on which end of the siphonophore was monitored. A similar situation has been found in the sea pen *Umbellula magniflora*, where a narrow 500 nm spectrum was obtained from the base of the sea pen, while a broader 470 nm emission was produced at the top of the stalk (Herring 1983; Widder et al. 1983); but in that organism there appears to be a different degree of association with GFP along the length of the rachis, so that at the top the spectrum matches the *in vitro* unassociated wavelength (blue), but nearer to the peduncle the energy has been shifted to a longer wavelength by GFP. In *A. tetragona*, preliminary examinations with fluorescence microscopy showed no GFP-like sources.

The siphonophores are unique not only in having several species which emit two wavelengths, but also because their spectra are distributed bimodally (Fig 1 C). The chemical basis for the distribution of siphonophore spectra might be found in the quantal nature of light emission, as investigated by Hori et al. (1973). The excited state of the luciferin and the spectrum shift as a function of proton concentration: the singlet excited state emits light at 460 nm, and in acidic conditions with the emitter in the neutral excited state the λ_{\max} is 410 nm; while the dianion, produced under alkaline conditions, emits at 530 nm (Qi et al. 1992). Partridge (1989) found similar "clumping" in the photopigment absorption wavelengths from deep-sea fish, and concluded that molecular differences in the opsins were probably responsible for the discontinuous distribution.

Depth-related differences in spectra

The most interesting overall trend was the clear distinction between shallow and deep populations of each phylum. Although we have few samples of shallow medusae, the addition of more shallow species would continue to support the trend, since GFP, which shifts luminescence to wavelengths around 500 nm, has been predominantly found in shallow or benthic cnidarians. The perception that "most coelenterates contain GFP" (Goto 1980) is not in accord with our current data; although it is relatively common in shallow leptomedusae, it is known to be present in <10% (5 of 59 species) of cnidarians we examined.

The differences with depth are clear, but there are still several prominent outliers. Depth distributions, however, are not rigidly restricted. Bathypelagic species may be found in the meso- or epipelagic zones in cold water or high latitudes (Fosså 1992; Van der Spoel and Pierrot-Bults 1979), and non-vertically-migrating mesopelagic

species of zooplankton are found in shallow Antarctic waters (Ainley et al. 1986). Therefore, it should not be surprising that spectra and vertical distributions vary considerably. Unfortunately, the distributions of these organisms have not been extensively documented, especially with regard to vertical migration.

In considering why these differences have arisen, one might conclude that because bioluminescence must be detected to be effective, anything which increases the visibility of the light, even slightly, would be advantageous. Biologically-significant differences, however, do not necessarily follow from statistically-significant differences, and it is difficult to determine whether an evolutionarily relevant effect requires an 8 or a 25 nm difference in λ_{\max} .

Because absorption and scattering are wavelength-dependent, one way the spectrum affects detection is by changing attenuation in seawater. Water containing chlorophyll will attenuate more in the blue end of the spectrum than in the green, whereas "deeper" water, containing no chlorophyll, transmits blue light slightly more effectively (continuous lines in Fig. 6A). Comparing two examples of luminescence, one blue and one green (dashed lines in Fig. 6A), it is clear that some difference in the transmission will occur. An example at wavelengths representative of medusae (Fig. 6B) shows that detectable light can vary by a factor of 5 at a distance of 20 m. Young (1981), however, suggested that in marine systems bioluminescence usually acts at distances of <1 m, whereas these two wavelengths have similar intensities at distances as far as several meters from the source (Fig. 6B). Although Young concluded that slight variations in transmission due to differences in color do not lead to selective advantages, in a terrestrial example, firefly spectra are "optimized" for visibility against different background light and a 5 to 10 nm difference has been found functionally important (Lall et al. 1980; Seliger et al. 1982).

In addition to affecting transmission, there is at least one other mechanism through which color affects the detection of bioluminescence by a "target" organism. Vision in deep-sea organisms has adapted to both the kinetic (Moeller and Case 1994) and spectral aspects of the local light environment. The peak visual sensitivities for deep-sea fish (Partridge et al. 1988) and shrimp (Frank and Case 1988) generally fall between 470 and 490 nm. Although the wavelengths of luminescence appear to co-vary with the colors of downwelling light, gelatinous zooplankton are not known to counterilluminate; they do not need to match the color of downwelling light except to the extent that this helps them match the visual sensitivities of organisms which have adapted to that light. The general depth trend seen in bioluminescence wavelengths (Fig. 5) has also been seen in the distribution of visual sensitivities of fish (Fig. 7 in Douglas et al. 1995).

Depth is only one of the several factors which might correlate with bioluminescence spectra, and it has been proposed that the distinction is between *coastal* (benthic,

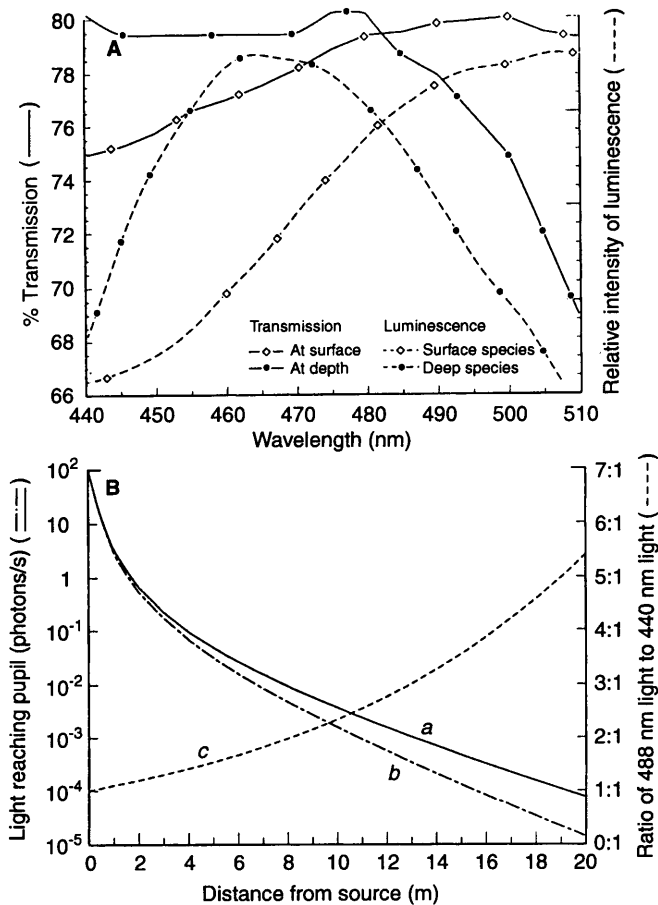


Fig. 6 **A** Relationship between spectra of light source and absorption of seawater at different depths (*continuous lines* fraction of light transmitted per distance in each water type; *dashed lines* extreme examples of spectra from ctenophores of each depth). In 440 to 510 nm range, shorter wavelengths of light transmit further in deep water, and longer wavelengths are favored near surface. For comparison, path length was adjusted to make maximum transmission values equal. Increased dissolved organic matter would decrease blue transmission (surface water, 0.5 mg chlorophyll *a* m⁻³, path length = 2.7 m; deep water, no chlorophyll, path length = 10 m) (model after Smith and Baker 1978). **B** Brightness of light sources calculated using wavelength-specific attenuation coefficients and inverse-square law. Visibility of short-wavelength source decreases more rapidly than that of longer wavelength source due to differences in beam attenuation coefficients. Wavelengths are representative of range that might be produced by different species of hydromedusae [*a* green light (488 nm; beam attenuation coefficient = 0.248 m⁻¹); *b* blue light (440 nm; beam attenuation coefficient = 0.333 m⁻¹); *c* ratio of amount of green light to blue light entering observer's pupil]. Radiant flux of the isotropic point-source in model was adjusted so that light emitted from one hemisphere was 100 photons s⁻¹. Attenuation coefficients are averages of WetLabs AC-9 data from 30 m depth off Cape Cod, Massachusetts (Dickey et al. 1998)

demersal, neritic) species which are mostly green, and *oceanic* (pelagic) species, which are mostly blue (Herring 1983; Morin 1983; Hastings and Morin 1991). Our data, which are restricted to a relatively small group of planktonic species, support a depth hypothesis more compellingly than a coastal/oceanic or latitudinal distinction. To analyze these last hypotheses, it is easiest to use a subset consisting of the shallow ctenophores (deep

species are difficult to divide into coastal and oceanic groups, and there are too few species of shallow medusae). Some of the predominant shallow species of ctenophores, such as *Beroe ovata*, *Cestum veneris*, *Eurhamphaea vexilligera*, *Ocyropsis maculata* and *Velamen parallelum*, are found at tropical latitudes in the open ocean, yet their luminescence is among the greenest of the ctenophores. If the shallow ctenophores are divided into two groups – species occurring coastally ($n = 11$; mean \pm SE: 491.3 ± 1.3) and those more typical of an open-ocean habitat ($n = 7$; 490.1 ± 3.8) – there is no significant difference between the populations (Mann-Whitney *U*-test: $p = 0.4376$). In the data presented here, which are limited to planktonic organisms, depth has a greater effect than whether an organism is coastal or oceanic, tropical or temperate.

Functions of luminescence

There has been no clear demonstration of the functions of luminescence in gelatinous plankton. Considering the limited photosensing ability of the cnidarians and ctenophores in question (Cubozoa, which happen to be non-luminous, notwithstanding), it is not likely that their bioluminescence is employed for intraspecific communication. In our experience, bioluminescence is elicited under physical duress, as might arise when interacting with predators, or with parasites such as amphipods. Photosensitive responses such as chromatophore contraction in species of *Beroe* (SHDH personal observations) or vertical migration occur at relatively high light levels and over integration times much larger than those characteristic of bioluminescent flashes. Therefore, the photosensing systems driving the evolution of bioluminescence are most likely those of fishes and crustaceans (such as shrimp, copepods, and hyperiid amphipods) with which these gelatinous organisms interact.

The short-wavelength violet bioluminescence ($\lambda_{\max} \approx 440$ nm) produced by several species of medusae and siphonophores is below the typical range of maximum visual sensitivity for deep-sea organisms (470 to 490 nm) (Frank and Case 1988; Partridge et al. 1988). There are, however, examples of photopigments and of behavioral responses which operate at or below these wavelengths. *Bathylagus bericoides* and *Alepocephalus bairdii*, fishes which are unique for having visual pigments with $\lambda_{\max} < 470$ nm (Partridge et al. 1988), are both thought to eat gelatinous plankton (Arai 1988), so that evolution of the wavelength of bioluminescence may be related to very particular interactions which await better elucidation.

An important element in considering function is the point in the life-cycle of an organism when luminescence is most critical. Young stages of ctenophores, for example, can luminesce more brightly, in relation to body size, than mature individuals, whose luminescence is often diffusely distributed (SHDH personal observations). Furthermore the ability to make light appears

very early in the development of both cnidarians (Freeman and Ridgway 1991) and ctenophores (Freeman and Reynolds 1973). Bioluminescence may serve a more important function for the shallow coastal hydroid stage of a hydromedusa or for the polyp stage of a deep-sea scyphozoan (Jarms 1991) than for the oceanic or mesopelagic adult medusa. These complications point to the importance of understanding the natural history of a species before advancing explanations of functions and adaptations of bioluminescence.

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