Marine Biology (1999) 133: 571–582

S. H. D. Haddock · J. F. Case

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

Received: 27 April 1998 / Accepted: 27 October 1998

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

Communicated by M.F. Strathmann, Friday Harbor

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

Present address:
[1]Monterey 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 *Beroe* 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 m*M* Tris and 40 m*M* 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 m*M* $CaCl_2$.

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 m*M* Tris and 40 m*M* 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 nm ± 1.6 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 *Bathyctena chuni*, *Euplokamis stationis*, *Eurhamphaea vexilligera*, and a large red undescribed mertensiid (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 nm ± 2.9 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 nm ± 2.8 SE) and hydromedusae (473.7 ± 4.2 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)	(*n*)
Ctenophores					
Aulacoctena acuminata Mortensen	458	90	H	Deep, 1200	(2)
Bathocyroe fosteri Madin and Harbison	[459 to 492]	102	B	Deep, 609	(18)
Bathyctena chuni (Moser)	492 ± 5.6	91	P,H	Deep, 2167	(5)
Bathyctena sp. nov. A[a]	488	88	B	Deep, 684	(1)
Bathyctena sp. nov. B[a]	490 ± 5.3	94	H	Deep, 1200	(3)
Bathyctenidae, gen. nov., sp. nov.[a]	501 ± 4.6	121	B	Deep, 834	(3)
Beroe abyssicola Mortensen	491 ± 1.1	88	P,S	Deep, 489	(3)
Beroe cucumis Fabricius	489 ± 4.7	88	G,M,S	Shallow, 37	(11)
Beroe forskalii Milne Edwards	491 ± 5.0	89	S,M	Shallow, 14	(5)
Beroe gracilis Künne	495	89	S	Shallow, 12	(2)
Beroe ovata Bosc	493	89	B	Shallow, 18	(1)
Bolinopsis infundibulum Müller[b]	488 ± 4.2	88	G,M,S	Shallow, 100	(8)
Bolinopsis vitrea (L. Agassiz)	490	90	B	Shallow, 8	(2)
Cestum veneris Lesueur	493 ± 7.7	89	B,S	Shallow, 12	(3)
Charistephane fugiens Chun	468 ± 1.2	84	P	Deep, 300	(6)
Cydippida, unidentified species[a]	482	79	B	Deep, 662	(1)
Deiopea kaloktenota Chun	489 ± 4.3	95	S	Shallow, 19	(7)
Euplokamis sp.	483 ± 1.9	85	G	Deep, 243	(4)
Euplokamis stationis Chun	467 ± 4.5	82	M	Deep, 314	(3)
Eurhamphaea vexilligera Gegenbaur	496 ± 5.2	94	B,M	Shallow, 16	(8)
Haeckelia beehleri (Mayer)	500 ± 5.1	88	M,S	Shallow, 12	(10)
Haeckelia bimaculata Carré and Carré	490	98	S	Shallow, 17	(1)
Haeckelia rubra (Kölliker)	489 ± 1.2	98	S	Shallow, 17	(3)
Kiyohimea aurita Komai and Tokioka	491	103	B	Deep, 825	(2)
Lampea sp.	470	94	P	Deep, 2000	(1)
Lampea lactea (Mayer)	469	85	B	Shallow, 18	(1)
Lampea pancerina (Chun)	473	81	M	Deep, 494	(1)
Leucothea multicornis (Quoy and Gaimard)	488	93	M	Shallow, 14	(2)
Leucothea pulchra (Matsumoto)	488 ± 0.6	92	S	Shallow, 15	(5)
Lobata, sp. nov. A[a,c]	492 ± 1.6	90	B	Deep, 830	(4)
Lobata, 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 ± 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 ± 5.1	85	B	Deep, 787	(3)
Mertensiidae, gen. nov. B, sp. nov. D[a,d]	472 ± 4.6	94	B	Deep, 880	(6)
Ocyropsis maculata immaculata Harbison and Miller	489 ± 1.2	90	B,M	Shallow, 9	(4)
Thalassocalyce inconstans Madin and Harbison	491 ± 4.6	92	B,H,P,S	Deep, 322	(7)
Thalassocalycidae, gen. nov., sp. nov.[a]	483 ± 6.2	89	B,M	Deep, 388	(6)
Velamen parallelum (Fol)	501 ± 3.2	90	S	Shallow, 7	(3)
Scyphomedusae					
Atolla parva Russell	468	89	B	Deep, 833	(1)
Atolla vanhoeffeni Russell	469 ± 9.1	84	B,P	Deep, 588	(4)
Atolla wyvillei Haeckel	470	98	B	Deep, 908	(1)
Coronatae, unidentified species[e]	468	89	B	Deep, 848	(1)
Nausithoe globifera Broch	494 ± 5.5	85	P	Deep, 1333	(3)
Nausithoe atlantica Broch	480	88	P	Deep, 1350	(1)
Paraphyllina ransoni Russell	465	85	P	Deep, 1500	(2)
Periphylla periphylla Péron and Lesueur	465 ± 1.3	83	B,P	Deep, 1082	(5)
Periphyllopsis braueri Vanhöffen	473	85	B	Deep, 882	(1)
Phacellophora camtschatica Brandt[b]	491	107	G	Shallow, 182	(1)
Poralia sp.	468 ± 2.9	84	B	Deep, 832	(4)
Hydromedusae					
Aegina citrea Eschscholtz	469 ± 2.5	92	B,P	Deep, 934	(4)
Aeginura grimaldii Maas	464 ± 10.9	88	B,P	Deep, 742	(7)
Aequorea forskalea Péron and Lesueur	503	[f]	S	Shallow, 12	(1)
Bythotiara depressa Naumov	488 ± 2.2	80	P	Deep, 408	(4)
Clytia (= *Phialidium*) *hemisphaericum* (L.)	504	37	M	Shallow, 20	(1)
Cunina globosa Eschscholtz	462	76	P	Deep, 400	(2)
Euphysora valdiviae Vanhöffen	464 ± 2.1	93	P	Deep, 2167	(4)
Halicreas minimum Fewkes	469 ± 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[e]	445	79	B	Deep, 851	(1)
Haliscera conica Vanhöffen	451	85	M	Deep, 448	(2)
Halitrephes maasi Bigelow	458	125	B	Deep, 711	(1)
Halitrephes valdiviae H.B. Bigelow	443	80	B	Deep, 767	(2)
Halopsis ocellata Agassiz	458	99	G	Shallow, 20	(1)
Mitrocoma cellularia (Agassiz)	505	55	S	Shallow, 5	(1)
Mitrocomella sp.	500	62	S	Shallow, 8	(1)
Obelia sp.	502	[f]	S	Shallow, 3	(1)
Octophialucium funerarium (Quoy and Gaimard)	487	72	M	Deep, 391	(1)
Pandea conica (Quoy and Gaimard)	470	98	P	Deep, 450	(2)
Pegantha laevis H.B. Bigelow	460 ± 1.4	75	P	Deep, 300	(4)
Solmissus albescens (Gegenbaur)	478 ± 0.0	76	M	Deep, 485	(3)
Solmissus incisa (Fewkes)	465	76	B,P	Deep, 742	(2)
Solmissus marshalli Agassiz and Mayer	477	75	P	Deep, 250	(2)
Solmundella bitentaculata (Quoy and Gaimard)	477 ± 7.1	83	B,P	Deep, 492	(6)
Siphonophores					
Calycophorae					
Abylopsis tetragona (Otto)	489 [450]	61	M	15	(4)
Chuniphyes multidentata Lens and van Riemsdijk	481 ± 1.4	61	P	1500	(2)
Craseoa lathetica Pugh and Harbison	489	90	B	719	(1)
Hippopodius hippopus (Forskål)	450	83	P	150	(1)
Maresearsia praeclara Totton	486	[f]	P	800	(1)
Muggiaea sp.	500	76	S	3	(1)
Nectadamas diomedeae (Bigelow)	443 ± 2.1	83	B,P	1329	(5)
Nectopyramis natans (Bigelow)	447 ± 1.4	81	H,P	750	(4)
Praya dubia (Quoy and Gaimard)	477 ± 0.8	86	P	560	(5)
Prayidae, unidentified species[e]	448	80	P	300	(1)
Rosacea plicata sensu Bigelow	491 ± 2.1	77	B,P	431	(2)
Vogtia glabra Bigelow	448	79	B	537	(1)
Vogtia serrata (Moser)	451 ± 0.6	86	P	650	(3)
Physonectae					
Agalma okeni Eschscholtz	447 ± 1.2	90	H	550	(3)
Apolemia sp. 1	445	82	H	1500	(1)
Apolemia sp. 2	442	111	H	1500	(1)
Bargmannia sp.	[443 to 499]	92	B	512	(7)
Bargmannia sp. nov.[a]	480	100	P	1350	(1)
Erenna sp. nov.	455	109	P	1150	(1)
Frillagalma vityazi Daniel	[455 to 492]	91	B	363	(10)
Halistemma amphytridis (Lesueur and Petit)	451	88	B	894	(1)
Halistemma sp.	446	81	H	1500	(1)
Halistemma sp. nov.[a]	460	83	B	787	(2)
Nanomia bijuga (delle Chiaje)	457	87	S	9	(1)
Nanomia cara Agassiz	454 ± 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 *Lychnagalma 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 ± 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

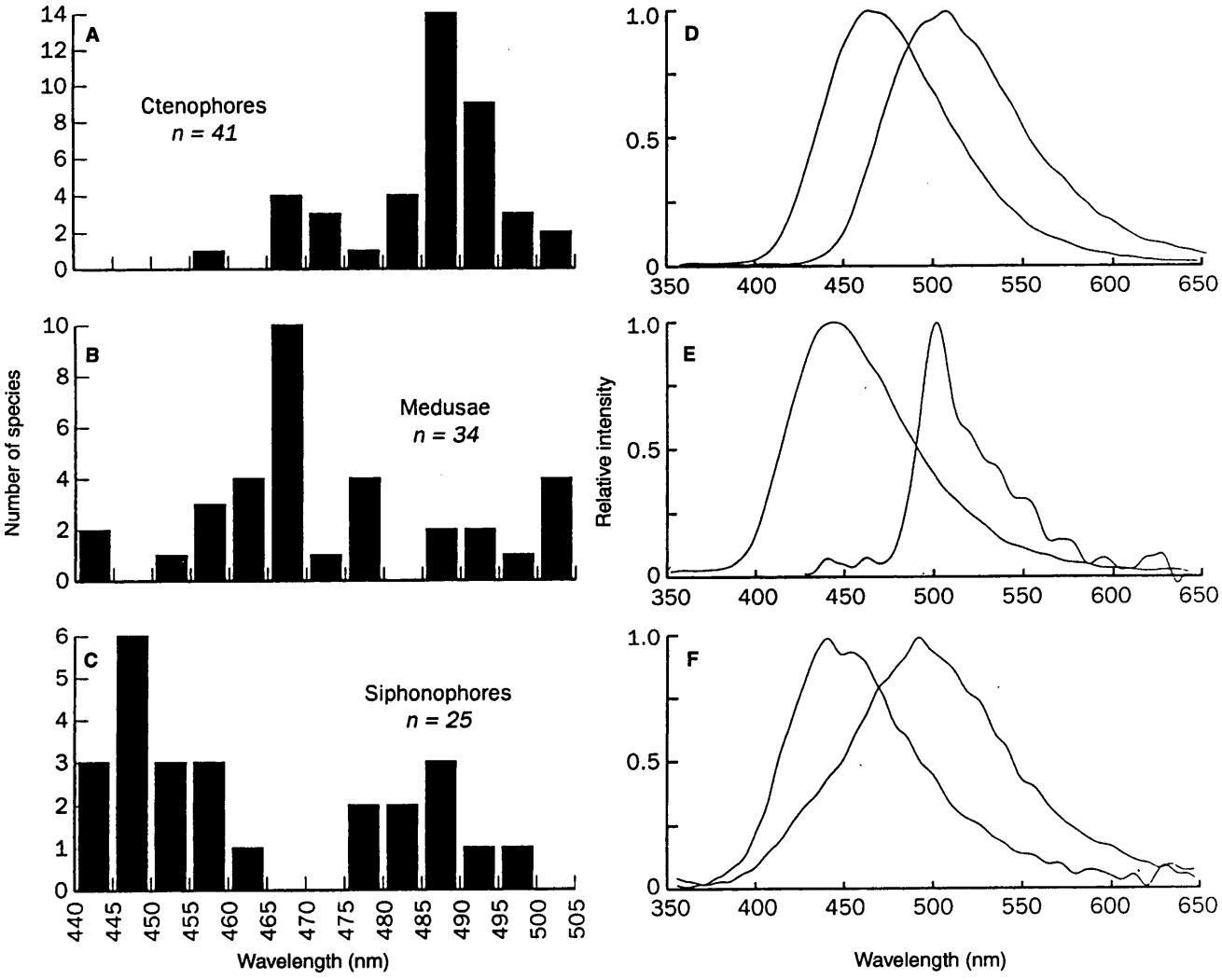

Fig. 1A–C Distribution of mean species wavelength for three groups of gelatinous zooplankton examined: ctenophores (**A**, $n = 41$) and medusae (**B**, $n = 34$) were evenly distributed about overall group mean, but siphonophore species (**C**, $n = 25$) produced bimodal distribution. **D–F** Representative spectra from extremes of each group (individual spectra shown here may have different values than species' averages in Table 1). **D** ctenophores: *Euplokamis stationis* ($\lambda_{max} = 464$ nm) and mertensiid gen. nov. A, sp. nov. A ($\lambda_{max} = 504$ nm). **E** medusae: *Halitrephes valdiviae* ($\lambda_{max} = 441$ nm) and *Clytia* (= *Phialidium*) *hemisphaericum* ($\lambda_{max} = 502$ 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 ($\lambda_{max} = 446$ nm) and green light ($\lambda_{max} = 493$ 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 ± SE	(n)	p
Ctenophores			
Shallow	490.8 ± 1.6	(18)	0.010
Deep	482.3 ± 2.2	(23)	
Total	486.1 ± 1.6	(41)	
Medusae			
Shallow	494.7 ± 6.4	(7)	0.003
Deep	468.4 ± 2.3	(27)	
Total	473.8 ± 2.8	(34)	
Siphonophores			
Green	486.3 ± 2.3	(9)	
Blue	450.0 ± 1.3	(16)	
Total		(25)	

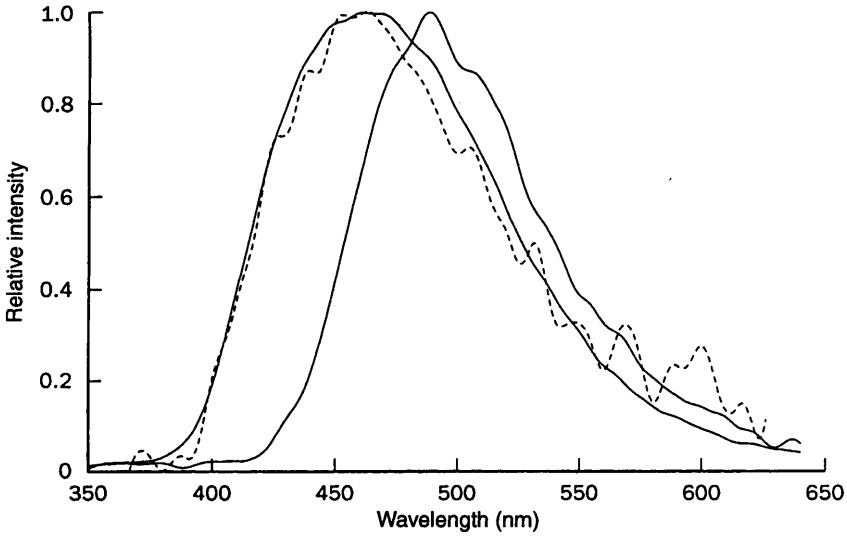

Fig. 2 *Bathocyroe fosteri*. Luminescence spectra of live specimens and extracts. Natural luminescence of this mesopelagic ctenophore may vary by >30 nm even within same individual [*continuous lines* (two different specimens)]. Extracts of *B. fosteri* produce blue light (λ_{max} = 457 nm; *dashed line*) 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

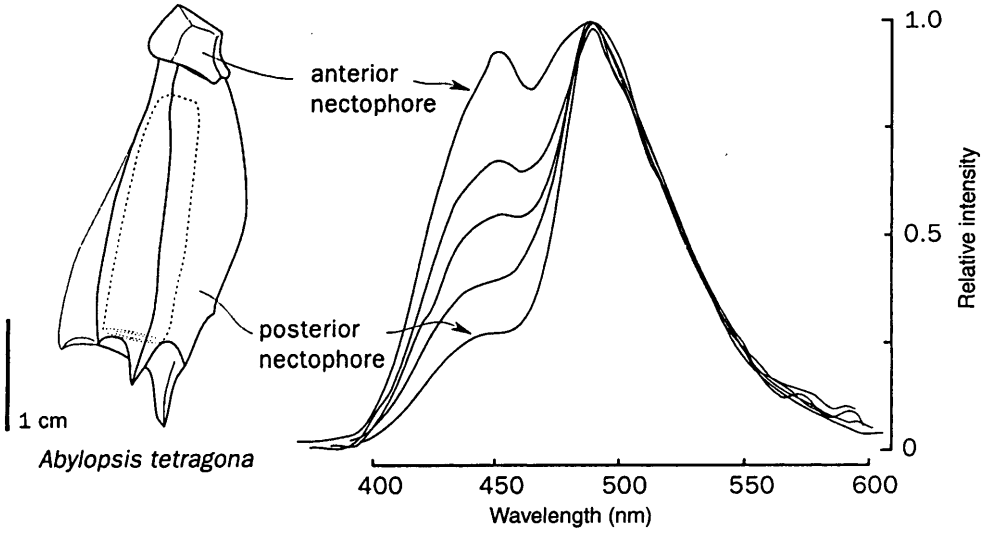

Fig. 3 *Abylopsis tetragona*. Luminescence spectra in relation to location along body. This calycophoran siphonophore produces two colors of luminescence, with short-wavelength emission generally more predominant near anterior nectophore. Spectra from three specimens are shown, although the most extreme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

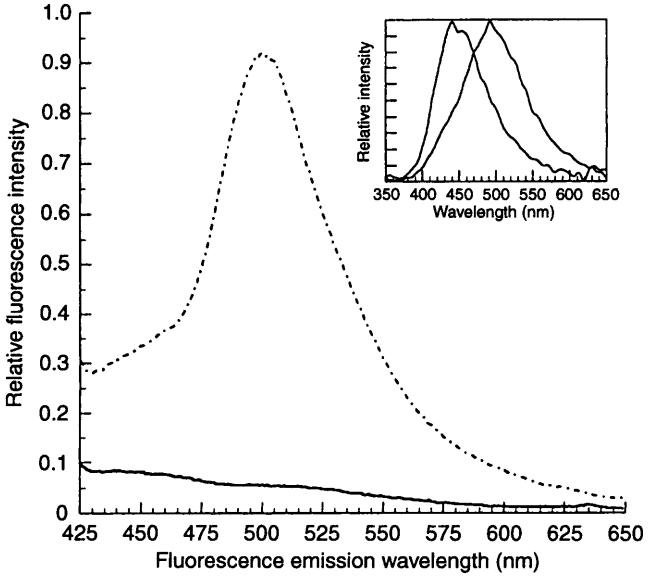

Fig. 4 *Mitrocoma cellularia* and *Bargmannia* sp. Fluorescence spectra of extracts. Hydromedusa *M. cellularia* is known to contain green-fluorescent protein (GFP), and spectrofluorometric assay ($\lambda_{excitation}$ = 400 nm, detection bandwidth = 5 nm) shows appropriate peak (*dashed line*). However, 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)

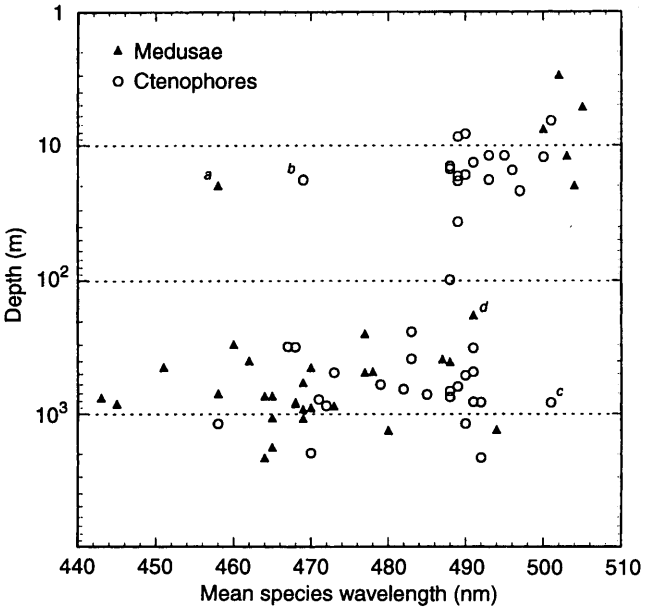

Fig. 5 Ctenophores and medusae. Relationship between wavelength of maximum emission and depth for each species. Outlier species include *Halopsis ocellata* (*a*), *Lampea lactea* (*b*), and a new species of ctenophore (*c*). *Phacellophora camtschatica* (*d*) was deepest species considered "shallow";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

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 (λ_{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 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 $\lambda_{max.}$

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,

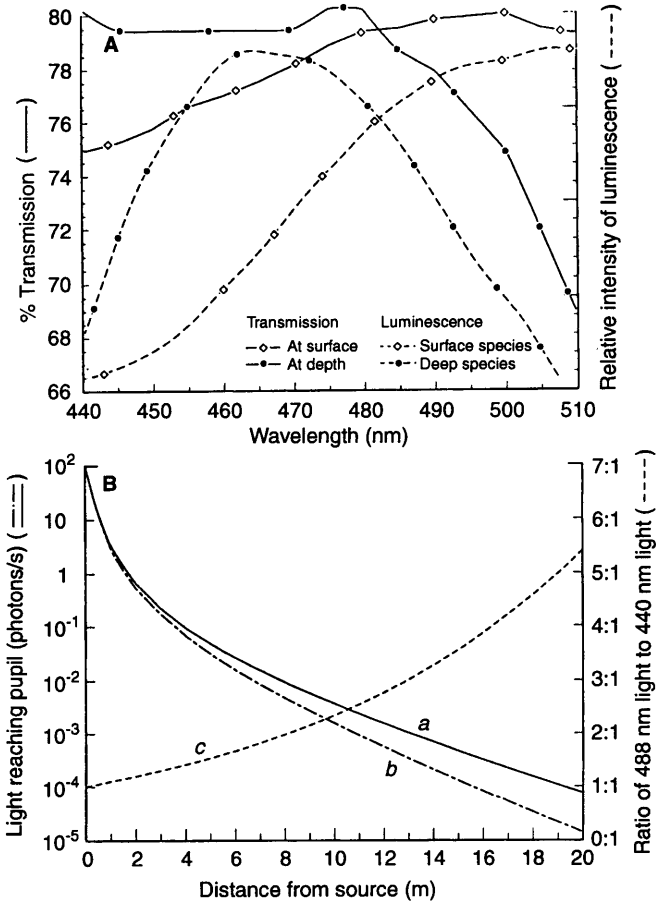

Fig. 6 **A** Relationship between spectra of light source and absorption of seawater at different depths (*continuous lines* fraction of light transmitted per distance in each water type; *dashed lines* extreme examples of spectra from ctenophores of each depth). In 440 to 510 nm range, shorter wavelengths 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 ± 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 ($\lambda_{max} \simeq 440$ 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.

Acknowledgements We are very indebted to E.A. Widder for several cruises with the Johnson-Sea-Link submersibles (Grants: ONR N00014-90-J1819, NOARL N00014-91-C6007, and NOAA UCAP-8667), and to J.J. Childress (NSF Grants OCE-9115551 and OCE-9415543) for opportunities to use his midwater trawl. The captains and crews of the R.V. ''Seward Johnson'', the R.V. ''Ed Link'', the R.V. ''Point Sur'' and the R.V. ''New Horizon'' provided expert support throughout. For consultation and taxonomic assistance, we are especially grateful to G.R. Harbison, C.E. Mills, P.R. Pugh, as well as G.I. Matsumoto, and E.V. Thuesen. Assistance in the field and with blue-water dives was provided by S. Groves, S. Anderson, J. McCullagh, K. Fleisher and G.R. Harbison. G. Chang kindly provided the data for the beam attenuation coefficients in Fig. 6B. The manuscript benefitted from review by V.M. Franck, E.A. Widder, C.E. Mills, R.H. Douglas, J.C. Partridge, and J. Morin's ''Wolfpack'' seminar at UCLA. This project was supported by an ONR AASERT fellowship (N00014-94-0751) to SHDH, ONR grants (N00014-97-1-0424; N00014-89-J1736) to JFC, the FBN Fund, and University of California Faculty Research Funds.

References

Ainley DG, Fraser WR, Sullivan CW, Torres JJ, Hopkins TL, Smith WO (1986) Antarctic mesopelagic micronekton: evidence from seabirds that pack ice affects community structure. Science, NY 232: 847–849

Arai MN (1988) Interactions of fish and pelagic coelenterates. Can J Zool 66: 1913–1927

Bailey TG, Torres JJ, Youngbluth MJ, Owen GP (1994) Effect of decompression on mesopelagic gelatinous zooplankton: a comparison of *in situ* and shipboard measurements of metabolism. Mar Ecol Prog Ser 113: 13–27

Bailey TG, Youngbluth MJ, Owen GP (1995) Chemical composition and metabolic rates of gelatinous zooplankton from midwater and benthic boundary layer environments off Cape Hatteras, North Carolina, USA. Mar Ecol Prog Ser 122: 121–134

Campbell AK, Herring PJ (1990) Imidazolopyrazine bioluminescence in copepods and other marine organisms. Mar Biol 104: 219–225

Case JF, Haddock SHD, Harper RD (1994) The ecology of bioluminescence. In: Campbell AK, Kricka LJ, Stanley PE (eds) Bioluminescence and chemiluminescence: fundamentals and applied aspects. John Wiley & Sons, New York, pp 115–122

Childress JJ, Barnes AT, Quetin LB, Robison BH (1978) Thermally protecting cod ends for the recovery of living deep-sea animals. Deep-Sea Res 25: 419–422

Denton EJ, Gilpin-Brown JB, Wright PG (1970) On the 'filters' in the photophores of mesopelagic fish and on a fish emitting red light and especially sensitive to red light. J Physiol, Lond 284: 72–73

Dickey TD, Chang GC, Agrawal YC, Willliams 3rd AJ, Hill PS (1998) Sediment resuspension in the wakes of Hurricanes Edouard and Hortense. Geophys Res Lett 25: 3533–3536

Douglas RH, Partridge JC, Hope AJ (1995) Visual and lenticular pigments in the eyes of demersal deepsea fishes. J comp Physiol (Sect A) 177: 111–122

Dunlap K, Takeda K, Brehm PH (1987) Activation of a calcium-dependent photoprotein by chemical signalling through gap junctions. Nature, Lond 325: 60–63

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125: 1–15

Fosså JH (1992) Mass occurrence of *Periphylla periphylla* (Scyphozoa, Coronatae) in a Norwegian fjord. Sarsia 77: 237–251

Frank TM, Case JF (1988) Visual spectral sensitivities of bioluminescent deep-sea crustaceans. Biol Bull mar biol Lab, Woods Hole 175: 261–273

Freeman G, Reynolds GT (1973) The development of bioluminescence in the ctenophore *Mnemiopsis leidyi*. Devl Biol 31: 61–100

Freeman G, Ridgway EB (1991) Endogenous photoproteins as calcium indicators in hydrozoan eggs and larvae. Zool Sci 8: 225–233

Gorry PA (1990) General least-squares smoothing and differentiation by the convolution (Savitzky–Golay) method. Analyt Chem 62: 570–573

Goto T (1980) Bioluminescence of marine organisms. In: Scheuer P (ed) Marine natural products. Academic Press, New York, pp 179–222

Haddock SHD, Case JF (1994) A bioluminescent chaetognath. Nature, Lond 367: 225–226

Haddock SHD, Case JF (1995) Not all ctenophores are bioluminescent: *Pleurobrachia*. Biol Bull mar biol Lab, Woods Hole 189: 356–362

Hamner WM (1975) Underwater observations of blue-water plankton: logistics, techniques, and safety procedures for divers at sea. Limnol Oceanogr 1045–1051

Harvey EN (1952) Bioluminescence. Academic Press, New York

Hastings JW (1983) Biological diversity, chemical mechanisms, and the evolutionary origins of bioluminescent systems. J molec Evolut 19: 309–321

Hastings JW, Morin JG (1991) Bioluminescence. In: Prosser CL (ed) Neural and integrative animal physiology. Wiley-Liss, Inc., New York, pp 131–170

Herring PJ (1983) The spectral characteristics of luminous marine organisms. Proc R Soc (Ser B) 220: 183–217

Herring PJ (1987) Systematic distribution of bioluminescence in living organisms. J Biolum Chemilum 1: 147–163

Herring PJ (1990) Bioluminescent responses of the deep-sea scyphozoan *Atolla wyvillei*. Mar Biol 106: 413–417

Hori H, Wampler JE, Matthews JC, Cormier MJ (1973) Identification of the product excited states during the chemiluminescent and bioluminescent oxidation of *Renilla* (sea pansy) luciferin and certain of its analogs. Biochemistry 12: 4463–4468

Jarms G (1991) Taxonomic characters from the polyp tubes of coronate medusae (Scyphozoa, Coronatae). Hydrobiologia 216: 463–370

Kirkpatrick PA, Pugh PR (1984) Siphonophores and velellids: keys and notes for the identification of the species. Synopses Br Fauna 29

Kramp PL (1959) The hydromedusae of the Atlantic ocean and adjacent waters. Dana Rep 46: 1–283

Lall AB, Seliger HH, Biggley WH, Lloyd JE (1980) Ecology of colors of firefly bioluminescence. Science, NY 210: 560–562

Larson RJ (1986) Pelagic scyphomedusae (Scyphozoa: Coronatae and Semaeostomeae) of the Southern Ocean. Biology of the Antarctic Seas XVI. Antarctic Res Ser 41: 59–165

Latz MI, Case JF (1982) Light organ and eyestalk compensation to body tilt in the luminescent midwater shrimp, *Sergestes similis*. J exp Biol 98: 83–104

Latz MI, Frank TM, Case JF (1988) Spectral composition of bioluminescence of epipelagic organisms from the Sargasso Sea. Mar Biol 98: 441–446

Mayer AG (1912) Ctenophores of the Atlantic coast of North America. Carnegie Institution, Washington, DC

Moeller JF, Case JF (1994) Properties of visual interneurons in a deep-sea mysid, *Gnathophausia ingens*. Mar Biol 119: 211–219

Morin JG (1983) Coastal bioluminescence: patterns and functions. Bull mar Sci 33: 787–817

Morin JG, Hastings JW (1971) Energy transfer in a bioluminescent system. J cell Physiol 77: 313–318
Nicol JAC (1958) Observations on luminescence in pelagic animals. J mar biol Ass UK 37: 705–752
Ohmiya Y, Ohashi M, Tsuji FI (1992) Two excited states in aequorin bioluminescence induced by tryptophan modification. Fedn eur biochem Soc (FEBS) Lett 301: 197–201
Partridge JC (1989) The visual pigments of deep-sea fishes: ecophysiology and molecular biology. Prog Underwat Sci 14: 17–31
Partridge JC, Archer SN, Lythgoe JN (1988) Visual pigments in the individual rods of deep-sea fishes. J comp Physiol (Sect A) 162: 543–550
Qi CF, Gomi Y, Hirano T, Ohashi M, Ohmiya Y, Tsuji FI (1992) Chemi- and bio-luminescence of coelenterazine analogues with phenyl homologues at the C-2 position. J chem Soc Perkins Trans (2: Phys org Chem) 13: 1607–1611
Seliger HH, Lall AB, Lloyd JE, Biggley WH (1982) The colors of firefly bioluminescence. II. Experimental evidence for the optimization model. Photochem Photobiol 36: 681–688
Seliger HH, McElroy WD (1964) The colors of firefly bioluminescence: enzyme configuration and species specificity. Proc natn Acad Sci USA 52: 75–81
Shimomura O (1985) Bioluminescence in the sea: photoprotein systems. In: Laverack MS (ed) Society for experimental biology Symposia. Vol. 39. Cambridge University Press, Cambridge, pp 351–372
Shimomura O, Inoue S, Johnson FH, Haneda Y (1980) Widespread occurrence of coelenterazine in marine bioluminescence. Comp Biochem Physiol 65B: 435–437
Shimomura O, Johnson FH, Saiga Y (1962) Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, *Aequorea*. J cell comp Physiol 59: 223–239
Smith RC, Baker KS (1978) Optical classification of natural waters. Limnol Oceanogr 23: 260–267
Totton AK (1954) Siphonophora of the Indian Ocean; together with systematic and biological notes on related specimens from other oceans. 'Discovery' Rep 27: 1–162
Totton AK (1965) A synopsis of the Siphonophora. British Museum, London
Van der Spoel S, Pierrot-Bults AC (1979) The zoogeography of the Pacific Ocean. In: Van der Spoel S, Pierrot-Bults AC (eds) Zoogeography and diversity of plankton. Edward Arnold, London, pp 293–327
Wampler JE, Jamieson BGM (1980) Earthworm bioluminescence: comparative physiology and biochemistry. Comp Biochem Physiol 66B: 43–50
Ward WW, Cormier MJ (1975) Extraction of *Renilla*-type luciferin from the calcium-activated photoproteins aequorin, mnemiopsin, and berovin. Proc natn Acad Sci USA 72: 2530–2534
Ward WW, Cormier MJ (1978) Energy transfer via protein–protein interaction in *Renilla* bioluminescence. Photochem Photobiol 27: 389–396
Ward WW, Seliger HH (1976) Action spectrum and quantum yield for the photoinactivation of mnemiopsin, a bioluminescent photoprotein from the ctenophore *Mnemiopsis* sp. Photochem Photobiol 23: 351–363
Widder EA, Latz MI, Case JF (1983) Marine bioluminescence spectra measured with an optical multichannel detection system. Biol Bull mar biol Lab, Woods Hole 165: 791–810
Wood KV (1990) *Luc* genes: introduction of color into bioluminescence assays. J Biolum Chemilum 5: 107–114
Young RE (1981) Color of bioluminescence in pelagic organisms. In: Nealson KH (ed) Bioluminescence, current perspectives. CEPCO Division, Burgess Publishing Co, Minneapolis, pp 72–81
Young RE (1983) Oceanic bioluminescence: an overview of general functions. Bull mar Sci 33: 829–845
Young RE, Mencher FM (1980) Bioluminescence in mesopelagic squids: diel color change during counterillumination. Science, NY 208: 1286–1288
Youngbluth MJ (1984) Manned submersibles and sophisticated instrumentation: tools for oceanographic research. In: SUBTECH '83 Proceedings. Society for Underwater Technology, London, pp 335–344