17. Feasibility of using in situ measurements of bioluminescence spectra to determine the vertical distribution of plankton.

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ABSTRACT

Vertical profiles of stimulated bioluminescence spectra were obtained using an *in situ* spectrometer deployed on a cruise in the Gulf of Maine. By comparing the bulk spectral signal with spectra of individual species determined by shipboard measurements, we examined the possibility of reconstructing the assemblage of bioluminescent species present. The spectrometer measured an integrated signal, so fine structure of the original spectra could not be resolved, and the ability to discriminate between species was limited. Although this technique can not determine the exact composition of the planktonic community, the system could be improved by increasing the sensitivity and resolution of the spectrometer.

INTRODUCTION

The vertical distribution of organisms affects our picture of their overall biogeography. In the case of the scyphomedusa *Periphylla periphylla*, samples from shallow waters, even at a global scale, would give the impression that this species occurs mainly in boreal regions. In contrast, a sampling program that included the deep-sea would show that its true distribution spans equatorial latitudes, despite mass occurrences in cold shallow water (e.g. Fosså, 1992). Unfortunately, mapping vertical distributions requires sampling at several depths over a short time interval, a difficult and labour-intensive process.

Light generated by a marine organism, whether by fluorescence or bioluminescence, often has a colour which is characteristic of a particular taxonomic group. For example, the fluorescence spectra of phytoplankton indicate which photopigments are present, and these pigments in turn vary between taxa. This relationship has been used to infer relative vertical abundance from *in situ* measurements. In one field study, Cowles et al. (1993) reconstructed the gross phytoplankton assemblage using measurements of *in vivo* fluorescence. The authors were able to distinguish three taxonomic groups (phycoerythrin-containing cryptomonads and cyanobacteria vs. other autotrophs) by detecting shifts as small as 5 nm in the bulk fluorescence signal.

Similarly, bioluminescence emission spectra could potentially indicate which species are producing the light. In a laboratory study, discriminant analyses of the spectra and kinetics of bioluminescent flashes from ten species were sufficient to allow successful 'back prediction' of the source organisms (Nealson et al., 1986)

Here we examine the feasibility of using *in situ* measurements of bioluminescence spectra to determine the vertical distribution of the most abundant bioluminescent species present in the Gulf of Maine.

METHODS

Shipboard spectral measurements

The study was conducted from August 11-28, 1992 in the Wilkinson Basin of the Gulf of Maine (42°45'N 69°39'W). Organisms were collected by blue-water divers and with plankton tows. Luminescence spectra, elicited by mechanical stimulation, were measured using an EG&G spectrometer with a diode-array detector. (see Widder et al., 1983 for details of the instrumentation.) Spectra were described by their wavelength of maximum emission and by the width of the spectrum at half the maximum intensity (FWHM).

Plankton abundance

In lieu of plankton tows, abundance information for the most commonly found species was obtained by the Johnson-Sea-Link submersible. At discrete depths during each dive, a known volume of water was pumped through a 64 µm screen, using the pump samplers described by Youngbluth (1984). The concentrated samples were subsampled and counted using conventional techniques.

In situ spectrometry

A HIDEX bathyphotometer (Case et al., 1993; Widder et al., 1993) was equipped with a multichannel diode array spectrometer designed by Casey Moore and Glen Schiferl of the Quantum Institute at U.C. Santa Barbara. With the spectrometer and bathyphotometer, we recorded the intensity of bioluminescence down to ~200 m, along with the bulk integrated bioluminescence spectrum, and physical parameters such as conductivity, *in vivo* fluorescence, transmission, and temperature. At the typical flow rate of 16 l/s, a particle was observed by the spectrometer for approximately 20 ms. The spectral signal was conducted through a 0.64 mm slit, recorded by the diode array, binned into 5 nm divisions, and stored every 3 seconds. Samples were analyzed by identifying peaks in the spectra, noting the maximum wavelength, and comparing this to the table of values obtained from shipboard spectral measurements. Because the spectrometer was designed to measure the bulk bioluminescence spectrum, the main goal of this experiment was to determine whether this information can be used to determine the individual organisms which are responsible for that integrated signal.

RESULTS

Plankton abundances obtained from pumped samples in the Gulf of Maine varied between dives, but several species were consistently the most abundant from each taxonomic group (Table 1). The dinoflagellates *Protoperidinium depressum* (mean ± SD: 992

Table 1. The most common bioluminescent organisms in the Gulf of Maine. Planktonic phyla are well represented by this subsample of species. Phytoplankton and small zooplankton dominate the numbers and are likely the main producers of light above 200 m. FWHM = Full width of the spectrum at half maximum intensity.

Species	Type	Wavelength (nm)	FWHM	Max. abundance ind.m^{-3}	Depth (m)
Nanomia cara	Siphonophore	455 nm	92	3	25
Meganyctiphanes norvegica	Euphausiid	467 nm	48	--	-
Conchoecia elegans	Ostracod	475 nm†	95	255	250
Protoperidinium depressum	Dinoflagellate	479 nm†	37	8388	75
Euplokamis sp.	Ctenophore	480 nm	85	--	-
Metridia lucens	Copepod	482 nm	78	393	175
Tomopteris sp.	Polychaete	560 nm	34	--	-

†Spectra collected at locations other than Gulf of Maine
Omitted abundance values - Not in pump samples (but noted on blue-water and submersible dives)

± 2462 ind.m^{-3}) and *Ceratium longipes* (2138 ± 4019 ind.m^{-3}) and the copepods *Oithona* spp. (726 ± 589 ind.m^{-3}), *Calanus finmarchicus* (307 ± 386 ind.m^{-3}), and *Metridia lucens* (111 ± 131 ind.m^{-3}) were the most numerous overall. Gelatinous plankton such as the ctenophore *Euplokamis dunlapae*, and the siphonophore *Nanomia cara*, while visible and apparent to divers, were rarely detected in the pumped samples. This may be in part because the pumped samples averaged less than 490 liters, and these organisms were not present in sufficient densities to be detected in such volumes.

For the seven most common bioluminescent species, the characteristics of the spectra varied enough that it appeared possible to distinguish organisms based on these qualities (Table 1, Fig. 1). The organism with the most distinctly separated spectrum, the polychaete *Tomopteris* sp., is unfortunately also the least abundant of the bioluminescent species investigated.

Analysis of vertical profiles of bioluminescence intensity will be presented elsewhere, but the spectra corresponding to intensity peaks (Fig. 2) were analyzed for potential correlation with dominant species from plankton samples.

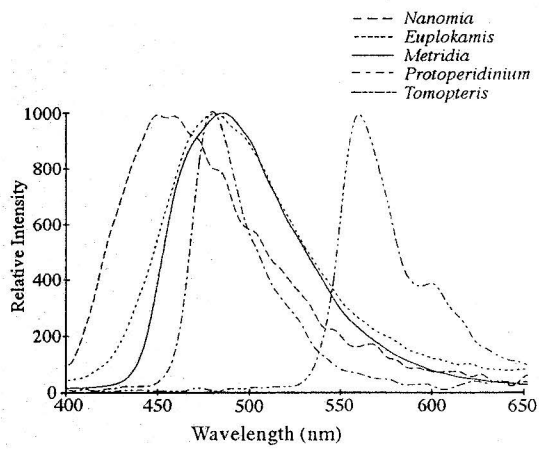

Fig. 1. Representative spectra of common organisms found in the Gulf of Maine. Most species are distinguishable either by maximum wavelength or by the width of the spectrum. The polychaete *Tomopteris* is atypical in that it emits yellow light, but the others luminesce at more typical blue to blue-green wavelengths.

Discussion

Because of the relatively low diversity and high abundance of bioluminescent species, the Gulf of Maine was considered an ideal location to test the feasibility of reconstructing plankton distributions from spectral information. If such a system could be made to work effectively, it would be extremely useful for determining the vertical distributions of species, which are very difficult to assay without repeated discrete-depth net tows. More recently, acoustic methods have also shown promise in this area (Holliday et al., 1989; Greene et al., 1992).

Unfortunately, several factors conspire to make accurate determination difficult under these circumstances. The main obstacle to effective characterization appears to be the effect of integrating spectra from two different organisms. The maximum wavelength of two organisms may be separated by 40 or more nanometers (Fig. 3A), but when their spectra are summed, the resulting curve will be unimodal (Fig. 3B). This summation masks the component spectra being summed, whether their individual intensities are equal or not (Fig 3B, solid line).

As noted above, the spectrometer was originally designed to measure the bulk luminescence spectrum of the total plankton assemblage, in order to improve calculations of attenuation of a radiant source through the water, and to allow accurate calibration of photometers. Its resolution, which is adequate for these purposes, is not as well suited for discrimination of subtle differences in wavelength.

Nonetheless, it does not seem untoward to check for some correlation between the spectra measured and the organisms found in the pumped samples. Spectra from deeper than 110 m had a strong blue component that indicates the presence of the siphonophore *Nanomia* (Fig. 2F-H). These physonects were not abundant enough to be well represented in the plankton samples, although they were among the most abundant planktonic carnivores seen on blue-water and submersible dives. The samples did not extend deep enough to reach beyond the lower limit

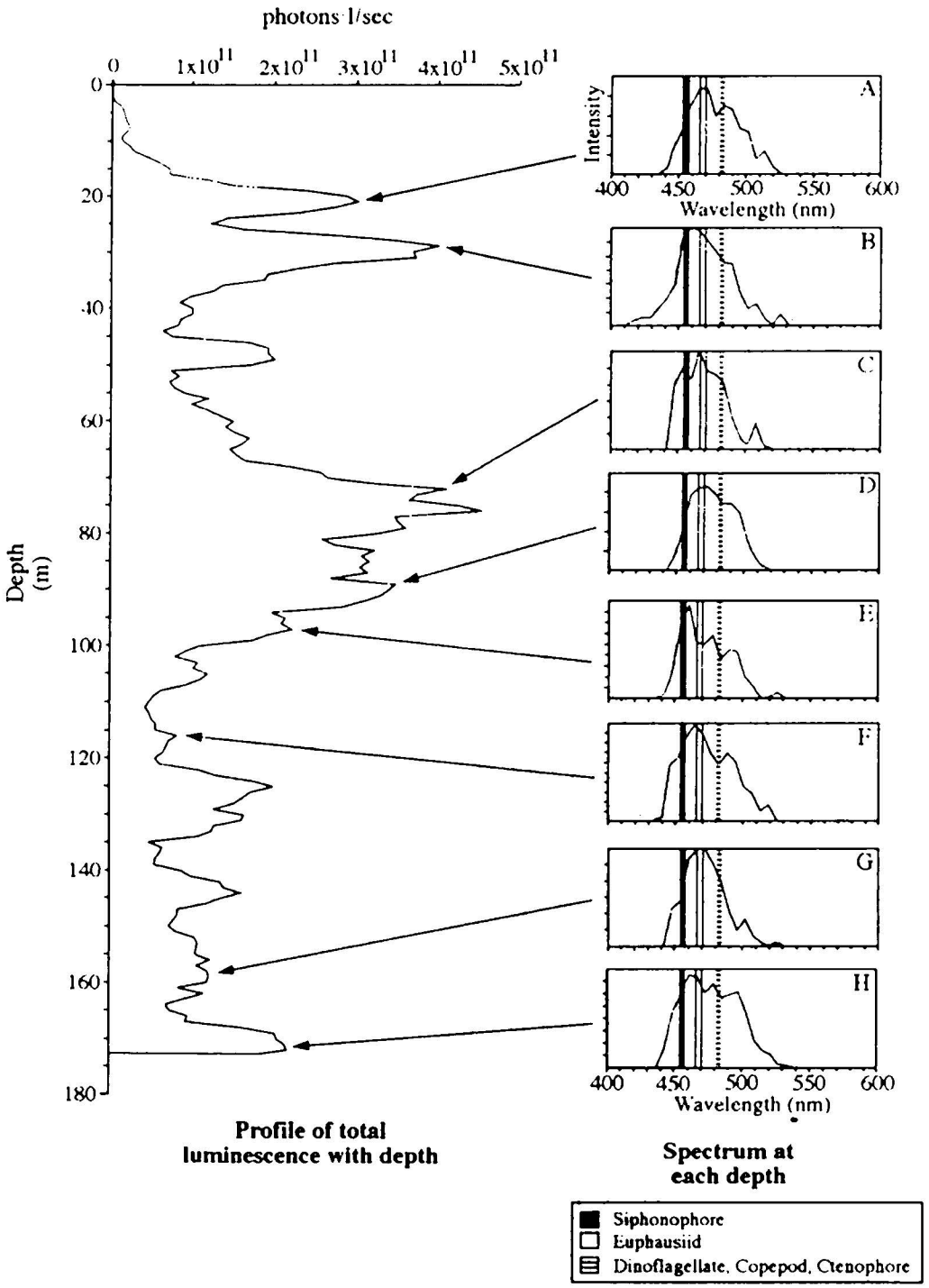

Fig. 2. Vertical profile of bioluminescence intensity and associated spectra. The bioluminescence intensity shows two main subsurface peaks with complex structure overlaid. Representative spectra from eight depths are given on the right (A-H). The vertical lines (black, white, striped) on the spectra graphs indicate where the maximum wavelengths of commonly found organisms would be found. 'Shoulders' of short wavelength luminescence, possibly from the siphonophore *Nanomia* are apparent in certain samples (C, F-H). Some curves (A, D, F, H) have high intensities at longer wavelengths characteristic of dinoflagellates and the copepod *Metridia*, while many (A, C, D, F, G) have spectra which could have resulted from euphausiid luminescence. The sharp peaks in the spectra are a result of noise and do not indicate a sharp peak of a single wavelength.

of dinoflagellates (*Protoperidinium* 104 ind.m^{-3} at 225 m), but the subsurface peak in luminescence intensity, which corresponds to the fluorescence maximum (unpublished HIDEX data), is probably due to dinoflagellates or copepods based on the spectrum recorded (Fig. 2D).

To improve the effectiveness of a spectral approach to plankton community characterization, we suggest the following changes would be required.

Higher resolution.

Sensitivity constraints of the instrument made it necessary to combine our samples into 5 nm bins, which has the effect of broadening spectral peaks. According to the sampling theorem (Shannon, 1949), to discriminate two narrow peaks, an instrument must be able to resolve a difference which is half the distance between the peaks. This physical restriction means that given the instrument's resolution, even in ideal circumstances we can only accurately resolve differences in spectra greater than 10 nm. Furthermore, the pressures of natural selection have fine-tuned luminescence emissions of organisms so that they have converged at a relatively small range of wavelengths, and many organisms differ from each other only slightly (Table 1, Fig. 1). To discriminate between these subtle differences, and to detect differences in the width of the emission curve, one would ideally have access to better resolution than 5 nm.

Higher sensitivity

The problem of resolution is tied to the question of sensitivity in two ways. If higher sensitivity was available, one could use a narrower dispersion slit and not have to bin adjacent detector units. Perhaps more importantly, higher sensitivity would make it possible to integrate the signal for a shorter time. As the integration time decreases, the number of individual organisms which are being integrated decreases, and the broadening of the spectra through summation (Fig. 3B) is lessened. Increasing sensitivity would be the single most effective way to improve the power of a spectral approach to identification.

Kinetics information.

In addition to containing spectral information, the bioluminescence emission from a particular species usually has a kinetic signature which may be measured. Some organisms emit millisecond flashes, while others can glow for several seconds. Nealson et al. (1986) found that spectra or kinetics alone provided some discrimination power, but the ability to accurately identify organisms improved dramatically when both characters were used together. Although it is beyond the immediate scope of this experiment, one could potentially combine this type of information with spectral information to increase the ability to determine which organisms are present.

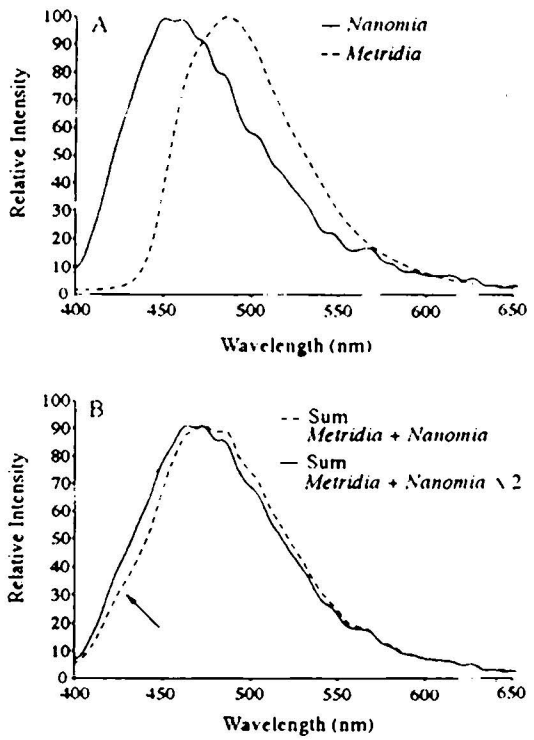

Fig. 3. The effect of summation on spectral curves. A. It is difficult separate out the components which make up an integrated spectral signal, because what appears to be a biologically significant separation in maximum wavelength may not be very large relative to the width of the individual curves. B. In this example, the short-wavelength spectrum of the siphonophore *Nanomia* has been added to the spectrum from the copepod *Metridia* either in equal parts (- - -) or at twice the intensity of *Metridia* (—). The only evidence that there were two spectra going into the integrated signal is a small distortion (arrow) caused by the *Nanomia* spectrum.

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REFERENCES

CASE, J.F., E.A. WIDDER, S. BERNSTEIN, K. FERER, D. YOUNG, M.I. LATZ & M. GEIGER, 1993. Assessment of marine bioluminescence. Nav. Res. Rev., 45: 31-41.

COWLES, T.J., R.A. DESIDERIO & S. NEUER, 1993. In situ characterization of phytoplankton from vertical profiles of fluorescence emission spectra. Mar. Biol., 115: 217-222.

FOSSÅ, J.H., 1992. Mass occurrence of Periphylla periphylla (Scyphozoa, Coronatae) in a Norwegian fjord. Sarsia, 77: 237-251.

GREENE, C.H., E.A. WIDDER, M.J. YOUNGBLUTH, A. TAMSE & G.E. JOHNSON, 1992. The migration behavior, fine structure, and bioluminescent activity of krill sound-scattering layers. Limnol. Oceanogr., 37: 650-658.

HOLLIDAY, D.V., R.E. PIEPER & G.S. KLEPPEL, 1989. Determination of zooplankton size and distribution with multifrequency acoustic technology. J. Cons. Int. Explor. Mer., 46: 52-61.

NEALSON, K.H., A.C. ARNESON & M.E. HUBER, 1986. Identification of marine organisms using kinetic and spectral properties of their bioluminescence. Mar. Biol., 91: 77-83.

SHANNON, C.E., 1949. Communication in the presence of noise. Proc. Inst. Radio Eng., 37: 10-21.

WIDDER, E.A., J.F. CASE, S.A. BERNSTEIN, S. MACINTYRE, M.R. LOWENSTINE, M.R. BOWLBY & D.P. COOK, 1993. A new large volume bioluminescence bathyphotometer with defined turbulence excitation. Deep-Sea Res. I, 40: 607 627.

WIDDER, E.A., M.I. LATZ & J.F. CASE, 1983. Marine bioluminescence spectra measured with an optical multichannel detection system. Biol. Bull., 165: 791-810.

YOUNGBLUTH, M.J., 1984. Manned submersibles and sophisticated instrumentation: tools for oceanographic research. SUBTECH '83 Proceedings: Society for Underwater Technology. London. 335-344.