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Coral fluorescence characteristics: excitation - emission spectra, fluorescence efficiencies, and contribution to apparent reflectance

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ABSTRACT

The fluorescence characteristics of Caribbean corals have been investigated through in situ and laboratory spectral measurements. Four pigments (as defined by spectral characteristics) are the source of most of the observed emissions. In vivo excitation/emission spectra were measured to determine Stokes shift and bandwidth (FWHM) of each of the types. Fluorescence efficiencies for natural fluorescence of the chlorophyll in the zooxanthellae were estimated from 18 in situ measurements, yielding values from 0.15 - 0.91%. Fluorescence efficiencies of the host pigments in 3 strongly fluorescent coral specimens were estimated from shipboard measurements and ranged from 4 - 6%. The quantitative data are being used in a computational model to investigate the contribution of fluorescence to apparent reflectance signatures.

Keywords: fluorescence, reflectance, corals, spectroscopy, fluorescence efficiency

1. INTRODUCTION

Reef-building corals are invertebrate animals that contain photosynthetic algae (zooxanthellae). The optical characteristics of corals are determined by the pigments that they contain[1], both photosynthetic and non-photosynthetic. In addition to the red-fluorescent chlorophyll in the zooxanthellae, many corals contain autofluorescent pigments in the host tissues[2,3] that can make a significant, and sometimes dominant, contribution to the appearance of the organism either under ambient daylight illumination[4] or when stimulated by an active sensing system[5,6,7]. It is possible in at least some cases that fluorescence from pigments in host tissues may contribute to photosynthesis[8]. Despite the striking nature of the phenomenon, there has been only sporadic attention to the topic and limited qualitative[4,5,6,9] or quantitative[10,11] data on the spectral characteristics of the fluorescence effects in corals.

Coral fluorescence has been investigated through in situ and shipboard measurement of excitation and emission properties. The predominant spectral types have been characterized. Estimates have been made of the fluorescence efficiency (ratio of photons fluoresced to photons incident) for several cases, including the natural fluorescence of chlorophyll under daylight illumination. Measurements have been made to document the contribution of fluorescence to apparent reflectance for some corals, and a mathematical approach to predicting that contribution has been implemented.

2. METHODS

2.1 In situ fluorescence and reflectance

Spectral reflectance and fluorescence emission data were collected with a prototype diver-operated instrument designated the Benthic SpectroFluorometer (BSF). The 1024-pixel CCD array in the instrument measures optical radiation over the spectral range of 300 to 800 nm with a spectral resolution of 5 nm. A one meter long, 600μ diameter optical fiber for light collection extends from the instrument housing to a hand-held probe. A separate fiber bundle carries excitation light from a halogen bulb to the measuring area. A four-position filter wheel provides the capability to select excitation wavelength. Data are stored on a hard disk in the instrument for post-processing, which consists of removal of electrical and dark current offsets and application of a correction factor to produce radiometrically correct values. A Savitzky-Golay smoothing function[12,13] may be applied to reduce residual pixel-to-pixel variation.

More than 300 in situ measurements of fluorescence and reflectance spectra have been made at sites at Lee Stocking Island, Bahamas; Key Largo , Florida Keys National Marine Sanctuary; and Dry Tortugas, Florida.

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2.2 Shipboard measurements of fluorescence excitation and emission

Shipboard measurements of in vivo fluorescence excitation and emission spectra were made in July 1996 at a site in the Dry Tortugas, Florida. 25 live coral specimens, representing 10 species, were collected from a depth of approximately 18 meters. Spectra were measured with a FluoroMax 2 spectrofluorometer (Spex Industries) fitted with a 2 meter fiber optic probe. The corals were maintained in flowing seawater between collection and measurement. For all specimens, emission spectra were measured with excitation wavelengths of 365, 450, and 488 nm (2 nm slit, 0.2 sec integration). Excitation spectra were measured with emission wavelength settings of 490, 530 and 690 nm (2 nm slit, 0.5 sec integration). For some specimens an additional excitation scan was made with an emission wavelength setting of 590 nm. Synchronous scans with a wavelength offset of 5 nm were made to determine the presence or absence of particular pigment types.

2.3 Fluorescence efficiency

Fluorescence efficiencies were determined for the host pigments in three coral specimens. The selected specimens each contained only one of either pigment 486 or 515. These measurements were made in a darkened laboratory on board ship, using a filtered light source for excitation. In addition, fluorescence efficiencies were determined for the in situ chlorophyll emission in 18 specimens representing 7 coral species, at a depth of 15 - 18 meters. The filtering effect of the water column effectively removed incident radiation at the wavelengths of chlorophyll emission.

Fluorescence efficiency was determined as the ratio of photons fluoresced to photons incident. The incident irradiance (μw-cm^{-2}-nm^{-1}) was measured by directing the fiber optic probe of the BSF at a reference surface (Spectralon®, 20% reflectance) at an angle of 45°. The data were then converted to units of photon flux (photons-sec^{-1}-cm^{-2}-nm^{-1}) by dividing by $\hbar c/\lambda$, the energy per photon at each wavelength λ ($\hbar$ = Planck's constant = $6.63\text{x}10^{-34}$ J-sec, c = velocity of light = $3\text{x}10^{8}$ m/sec). At each wavelength the intensity value was multiplied by the normalized value of the excitation spectrum at that wavelength. This was done to adjust for the fact that not all incident photons are equally likely to stimulate fluorescence. The data were then integrated over all excitation wavelengths to calculate the total number of incident photons, N_I, available to stimulate fluorescence.

The surface emittance was measured in the same manner as the incident irradiance. The emission spectrum was post-processed in a similar way to calculate the number of fluoresced photons, N_F. In this case, however, the integration was restricted to the portion of the spectrum in which the fluorescence occurs. Fluorescence efficiency was then calculated as the dividend N_F/N_I, the ratio of fluoresced photons to incident photons.

2.4 Fluorescence contribution to reflectance

In situ reflectance and fluorescence measurements were made to confirm the contribution of fluorescence to the appearance of corals under daylight illumination. In some cases the influence of fluorescence was obvious, as when a coral appeared orange or red at a depth of 15-20 meters, where photons of those wavelengths do not penetrate. In other cases the intensity of green coloration suggested a fluorescence contribution.

A mathematical treatment was formulated for the contribution of fluorescence to reflectance. The total apparent surface spectral emittance ($E_T(\lambda)$, μW-cm^{-2}-nm^{-1}) is:

$$E_T(\lambda) = E_D(\lambda)R(\lambda) + \sum F_i(\lambda), \qquad [1]$$

where $E_D(\lambda)$ is the incident irradiance, $R(\lambda)$ is the true (non-fluorescent) reflectance factor, and $F_i(\lambda)$ is the emittance at λ due to the fluorescence of pigment i. The emittance contributed by fluorescence for each pigment can be written as:

$$F_i(\lambda) = \frac{\varepsilon_i \varphi_i}{\lambda} \int_{\lambda_{ex}} E_D(\lambda_{ex}) \lambda_{ex} X_i(\lambda_{ex}) d\lambda_{ex}, \qquad [2]$$

where ε_i is the fluorescence efficiency, φ_i is the fraction of the total fluoresced photons emitted in the wavelength band around λ, and $X_i(\lambda_{ex})$ is the normalized (peak value = 1) value of the excitation spectrum for pigment i at excitation wavelength λ_{ex}. The integral, carried out over the full range of excitation wavelengths, represents the total number of potentially useful

incident photons, as defined above. Multiplying this quantity by ε_i yields the total photons emitted. Multiplication by φ_i yields the number of those photons that are fluoresced at wavelength λ, and division by λ converts the result from units of photon flux to units of energy flux.

3. RESULTS AND DISCUSSION

3.1 Excitation and emission characteristics

The in situ and shipboard data sets indicate that there are four pigments (as defined by spectral characteristics) that are the source of most of the observed emissions. For purposes of simplicity I have designated these as 486, 515, 575, and 685, naming them for the approximate location of the emission peak, although the actual location of the peak may vary by up to approximately 10 nm. One of these pigments (685) is chlorophyll contained in the zooxanthellae, while the other three are located in host tissues[11]. Excitation and emission spectra for representative samples of each type are shown in Figure 1, and the spectral characteristics are summarized in Table 1. Only chlorophyll is found in all symbiotic corals: the other pigments may occur singly or in mixtures within a given specimen (Figure 2).

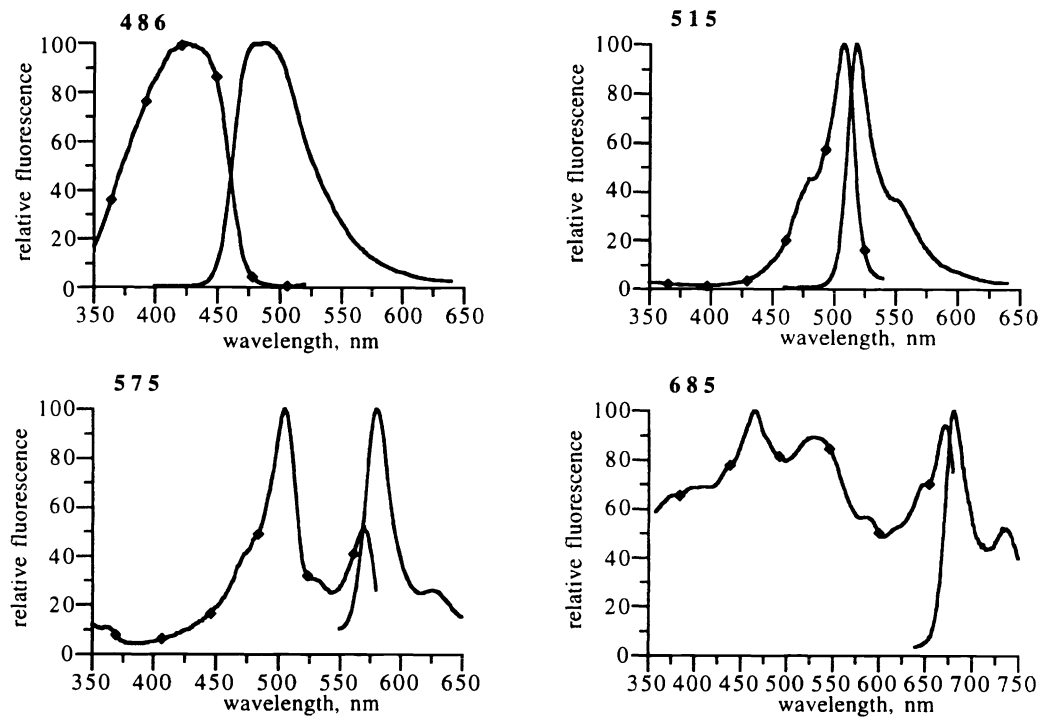

Figure 1. Excitation (—◆—) and emission (——) spectra for the four pigment types commonly found in corals. Species: 486 - *Agaricia* sp.; 515 - *Mycetophyllia lamarckiana*; 575 - *Montastrea cavernosa*; 685 - *Montastrea annularis*.

	bandwidth, nm	
peak, nm	FWHM	Stokes shift, nm
486	65	60
515	25	10-15
575	30	10/75
685	[chlorophyll]	

Table 1. Fluorescence characteristics of the four most common pigment types.

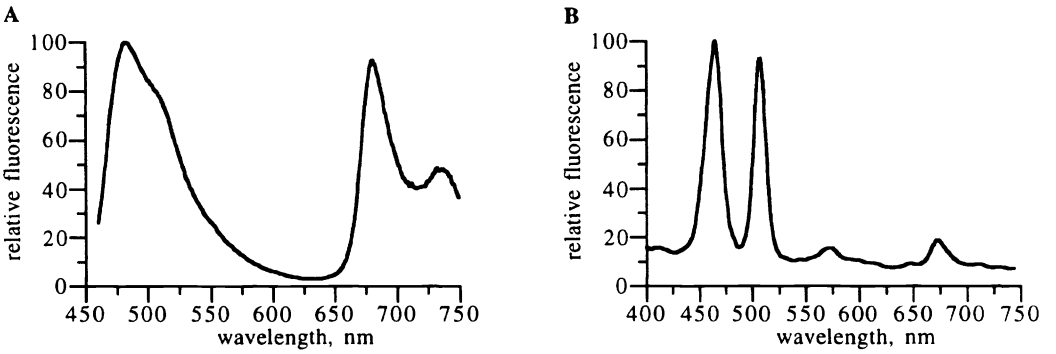

Figure 2. Fluorescence emission scan (excitation at 450 nm) (**A**) and synchronous scan (**B**) for a specimen of *Montastrea annularis*. The synchronous scan data show that the broad peak on the left is actually formed from the emissions of two separate pigments (486 and 515). The 685 pigment (chlorophyll) is clear in both graphs, and there is also some 575 pigment present, although it is not evident in **A**.

The four fluorescent pigments described above are the ones most commonly found. A fifth spectral type (yellow-fluorescent, emission peak at about 557 nm, bandwidth 55 nm FWHM, Stokes shift 50 nm) has been found in one species of *Agaricia* at several locations, and it is likely that continued investigation will reveal additional pigments. While most observations have been made of Caribbean species, limited measurements from Indo-Pacific species indicate the presence of the same general pigment types.

With the exception of the 685 pigment (chlorophyll), the pigments have not yet been identified biochemically. It has been suggested[2,14] that one or both of the shorter-wavelength pigments (486, 515) may be biliproteins. There is evidence[11,15] suggesting that the 575 pigment is phycoerythrin, a photosynthetic accessory pigment normally found in red algae and cyanobacteria. There is also evidence to suggest[15] that the 515 and 575 pigments are biochemically related. Note the two distinct peaks in the excitation spectrum, and the similarity of the shorter-wavelength of those two peaks to the excitation spectrum for the 515 emission.

3.2 Fluorescence efficiencies

Figures 3 and 4 illustrate representative data for incident and emitted radiation used in the computation of fluorescence efficiencies for coral pigments and for chlorophyll in zooxanthellae, respectively.

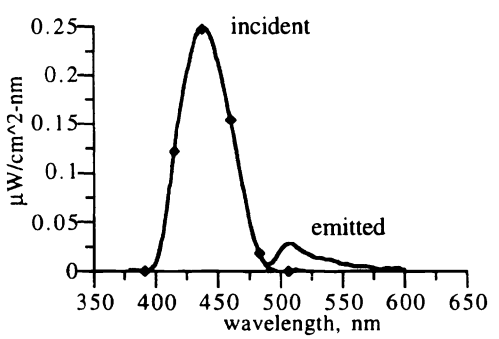

Figure 3. Incident (—◆—) and emitted (——) light for a brightly fluorescent specimen of *Scolymia* sp. containing only the 515 pigment. Shipboard measurement.

Computed fluorescence efficiencies for the coral host pigments were in the range of 4 - 6% for the three samples. One of the specimens contained only the 486 pigment, while the other two contained only the 515 pigment. All three specimens were chosen for their particularly strong fluorescence response under longwave ultraviolet illumination, and these values are liable to represent the high range of values for all corals.

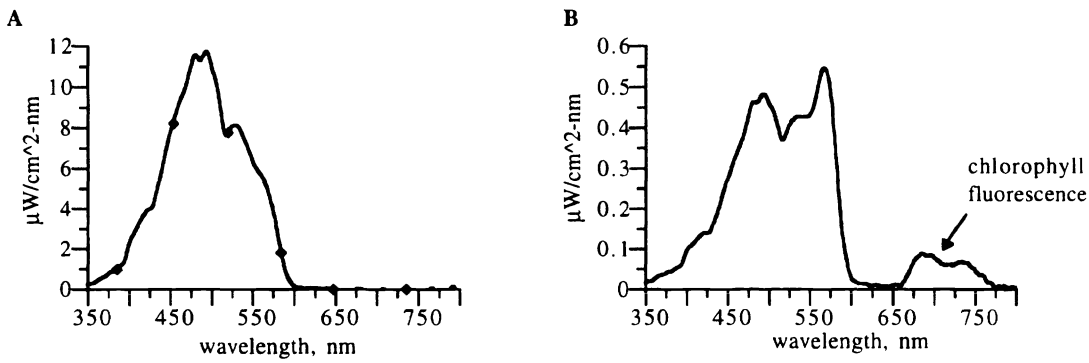

Figure 4. Spectra of downwelling (**A**) and reflected (**B**) light used for determination of natural fluorescence efficiency of chlorophyll in symbiotic algae. Specimen of *Montastrea annularis*, depth 18 meters.

Computed efficiencies for natural (daylight stimulated) chlorophyll fluorescence in 18 corals specimens ranged from 0.15 - 0.91% (mean 0.43%, standard deviation 0.21%). The 18 specimens were located at two sites that had significantly different ambient illumination levels due to differences in water clarity, and the measurements were not made at the same time of day. The sample size is as yet too small to determine if the variation between the values at the two sites is significant, and what might be causing the difference.

The definition of fluorescence efficiency (photons fluoresced/photons incident) differs from the traditional definition of fluorescence quantum yield, $\Phi_F = N_F/N_A$ [16], where N_F is the number of photons fluoresced and the divisor N_A is the number of photons absorbed rather than the number of photons incident. The measurement method used here views the undisturbed coral surface and is thus viewing a complex mixture of pigments, with no way of knowing what percentage of the incident photons were absorbed by the pigments producing the fluorescence. Since the absorbance by the fluorescing pigment must be <1, the quantum yield values for all cases would be larger than the fluorescence efficiency values reported here. The sense of fluorescence efficiency defined here is suitable for model computations related to optical closure or to the performance of active remote sensing systems.

3.3 Fluorescence contribution to apparent reflectance

Figure 5 shows the contribution of fluorescence to the in situ spectrum of light reflected from a coral at a depth of 18 meters under daylight illumination. The coral appeared orange to the eye. The peaks at 515, 575 and 685 nm are due to the fluorescent pigments in the host and the zooxanthellae. (The apparent peak at about 475 nm may be due to 486 pigment, or to the distribution of downwelling light.)

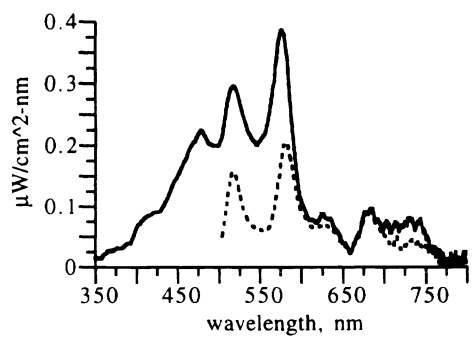

Figure 5. Reflected light spectrum (——) under daylight illumination and induced fluorescence (- - -) (excitation wavelength = 488 nm) for a specimen of *Montastrea cavernosa*, depth 18 meters. The scaling of the induced fluorescence is for comparison purposes only and is not the absolute magnitude of the fluorescence contribution.

The computational approach to fluorescence-enhanced reflectance was used to produce the model curves for natural chlorophyll fluorescence in Figure 6. Using a prototype spectrum for the chlorophyll fluorescence and the measured downwelling light spectrum as excitation source, a series of emission curves were generated for efficiencies from 0.4 - 1.0%.

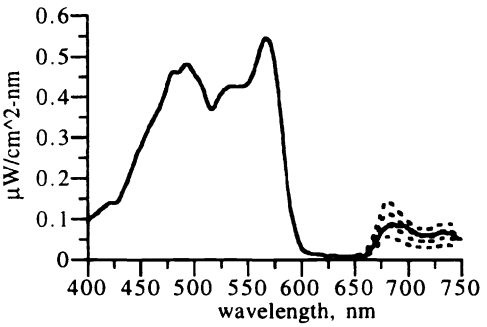

Figure 6. Measured (——) and modeled (- - -) spectra of light reflected from a coral surface (*Montastrea annularis*, depth 18 meters). The four model curves represent chlorophyll fluorescence efficiencies of 0.4 to 1.0%, in steps of 0.2%.

3.4 Future directions

While advances have been made in quantifying the spectral properties related to fluorescence effects in corals, there is much that remains to be done. The biochemical nature and role of the pigments in the host tissues are not known. The intensity and/or peak wavelength of fluorescence emission may vary within a species, and at times within a single specimen, but the causes and possible interpretive value of these variations are not understood. Additional measurements of fluorescence efficiency are needed to supplement the initial set reported here, with an effort to determine the cause of variations.

4. ACKNOWLEDGMENTS

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5. REFERENCES

1. G. Y. Kennedy, "Pigments of marine invertebrates," *Adv. Mar. Biol.*, 16, 309-381 (1979).

2. S. Kawaguti, "On the physiology of reef corals VI. Study on the pigments," *Palao Trop. Biol. Stn. Stud.*, 2, 617-674 (1944).

3. S. Kawaguti, "Electron microscopy on the fluorescent green of reef corals with a note on mucous cells," *Biol. J. Okayama University*, 2, 11-21 (1966).

4. C. Limbaugh and W. J. North, "Fluorescent, benthic, Pacific Coast coelenterates," *Nature* 178, 497-498 (1956).

5. R. Catala, "Fluorescence efffects from corals irradiated with ultra-violet rays," *Nature*, 183, 949 (1959).

6. R. Catala, "Nouveaux organismes marins présentant des effets de fluorescence par l'action des rayons ultra-violets," *C. R. Acad. des Sciences*, 250, 1128 (1960).

7. J. T. Hardy, F. E. Hoge, J. K. Yungel and R. E. Dodge, "Remote detection of coral 'bleaching' using pulsed-laser fluorescence spectroscopy," *Mar. Ecol. Prog. Ser.*, 88, 247-256 (1992).

8. D. Schlichter, H. W. Fricke, and W. Weber, "Light harvesting by wavelength transformation in a symbiotic coral of the Red Sea twilight zone," *Mar. Biol.*, 91, 403-407 (1986).

9. A. Logan, K. Halcrow, T. Tomascik, "UV excitation-fluorescence in polyp tissue of certain scleractinian corals from Barbados and Bermuda," *Bull. Mar. Sci.*, 46, 807-813 (1990).

10. C. H. Mazel, "Spectral transformation of downwelling radiation by autofluorescent organisms in the sea," *SPIE Vol. 1302 -- Ocean Optics X*, 320-327 (1990).

11. C. H. Mazel, "Spectral measurements of fluorescence emission in Caribbean cnidarians," *Mar. Ecol. Prog. Ser.*, 120, 185-191 (1995).

12. A. Savitzky, and M. J. E. Golay, "Smoothing and differentiation of data by simplified least squares procedure," *Anal. Chem.*, 36, 1627-1639 (1964).

13. J. Steinier, Y. Temonia and J. Deltour, "Comments on smoothing and differentiation of data by simplified least squares procedure," *Anal. Chem.*, 44, 1906-1909 (1972).

14. V. M. Buchsbaum, "Behavioral and physiological responses to light by the sea anemone *Anthopleura elegantissima* as related to its algal endosymbionts," Ph. D. Thesis, Stanford University (1968).

15. C. H. Mazel, "Fluorescence in Caribbean coral reef cnidarians," Ph. D. Thesis, Boston University (1993).

16. I. D. Campbell and R. A. Dwek, "Biological Spectroscopy," Benjamin/Cummings Publishing, Menlo Park (1984).