Separating the fluorescence and reflectance components of coral spectra

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The optical signal leaving the surface of fluorescing corals is a combination of elastic and inelastic scatter. A new experimental method was developed to separate the fluorescence and the reflectance components that involves measurements with and without a long-pass cutoff filter that eliminates the fluorescence contribution to the signal. The required measurements were performed underwater to demonstrate the applicability of the method for *in situ* applications. Computations with prototype rather than individually measured fluorescence emission spectra do not significantly compromise the accuracy of the results. A model was developed for calculating the interaction of the reflectance and the fluorescence components with new incident illumination conditions. The model calculations were supported by field experiment. We show that the contribution of fluorescence to some coral's spectra in various illumination conditions justifies consideration in optical models. The results are applicable to modeling the spectra of fluorescing corals under any irradiance spectrum and interpreting remote-sensing data in the relevant wavelength range. Further research is necessary to examine the significance of fluorescence near coral reefs at various scales.

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1. Introduction

Fluorescence can be a significant factor in the appearance of reef corals. An early study of coral color in the South Pacific revealed that a fluorescent pigment was responsible for much of the green coloration observed.[1] In the 1950s Scuba (self-contained underwater breathing apparatus) divers observed that some corals displayed orange and red colors that should not have been possible in ambient light conditions and correctly assumed that they were due to fluorescence.[2,3] In recent years attention to fluorescence in the scientific community has increased owing to the potential evolutionary and biological information that might be revealed and the possible use of fluorescence in applications such as remote sensing and reef monitoring.[4–6]

The optical signal leaving the surface of fluorescing corals is a combination of elastic scatter (light reflected from a surface at the same wavelength in which it arrived) and inelastic scatter (light that has been absorbed at one wavelength and reemitted at a longer wavelength). The relative contributions of these two components depend on the physical properties of the fluorescing pigments, such as absorbance, fluorescence efficiency, shape and range of the emission spectrum of the fluorescing substance, and local illumination conditions.

Here I distinguish the two components quantitatively for the purpose of modeling the fluorescence enhancement effect as a function of incident illumination. Such a model would be applicable to remote-sensing data interpretation and to ecological studies of the significance of color in the marine environment.[7] Here I separate the reflectance and the fluorescence components of fluorescing corals, evaluate the significance of the two components in some corals, and develop an analytical tool to demonstrate how to incorporate the two components in optical analyses.

Five pigments, as defined by spectral excitation and emission characteristics, have been found to fluoresce in Caribbean reef corals.[8] One is chlorophyll contained in the symbiotic algae (zooxanthellae) in coral endodermal tissues. The others are contained in coral host tissues. Here I concentrate on the two pigments that have fluorescence emission peaks in the vicinity of 486 and 515 nm. These pigments are chosen because: (1) they are known to contribute to the daylight appearance of some corals and (2) they can occur in some specimens as the sole fluorescent

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pigment other than the chlorophyll in the zooxanthellae.[8,9]

Several methods have been developed to separate the fluorescence and the reflectance components of fluorescing materials. The dual-monochromator method utilizes a source and a detector synchronized to measure true (elastic) reflectance.[10] Another method utilizes a stepped monochromatic source for illumination and a CCD array for simultaneous direct measurement of the reflected and the fluoresced components after they have been spatially separated by a diffraction grating.[11] These methods utilize instruments that are not suitable for underwater applications. Another method was developed by Allen in the analysis of fluorescent whiteners for the textile industry.[12] Allen used a sequence of fluorescence-weakening and fluorescence-eliminating filters to collect the data needed to separate the two light components. The fluorescing pigments used in Allen's study have fairly broad emission and excitation peaks and large stocks shifts. For the corals, however, the pigment in the vicinity of 515 nm, which is covered in this paper, is characterized by sharp peaks and small Stokes shifts. In addition the elastic reflectance spectra found by Allen's method contain a singularity that is inherent in this method and prohibit the calculation for the entire visible spectrum, thus inspiring the development of a new method that can be applied *in situ* to intact corals. Our method does not require laboratory experiments for one to determine the specific optical properties of each substance in the coral's tissue or extraction of coral materials. We take advantage of our ability to measure the fluorescence characteristics of the experimental subject directly at the coral surface or to use premeasured fluorescence prototype spectra. The magnitudes of both the reflected and the fluoresced components at any wavelength depend on the intensity and spectral distribution of the irradiance, but the dependence differs for the two components. The relative contribution of each varies with changes in the incident irradiance spectrum. Once the elastic and the inelastic scatter components are separated, further analysis is performed to predict new spectra under different illumination. We perform separate calculations of the interaction of each component with the new illumination conditions and compare the predicted with the measured spectra.

2. Theoretical Background

Consider the spectral changes that occur when a fluorescing substance is added to a nonfluorescing material. The thick solid curve *AEF* in Fig. 1(a) shows a schematic spectral radiance curve of a theoretical nonfluorescing material. When a hypothetical fluorescent substance is added to this material, it's spectral radiance curve changes to the thin solid curve *ABCDF*, showing an emission peak at D and an absorption trough at B. The elastic reflectance curve is the *ABCEF* curve. The *CDF* area represents the fluorescent emission. The *ABCE* area represents the absorbed light, which provides a source of fluoresced photons. We use the location of this absorption trough below in the analysis. In the wavelength range of C to E the simultaneous absorption of exciting energy and emission of fluorescing energy occurs. This region of the spectrum is known as the crossover region. If a sharp cutoff filter is used to mask wavelengths below E completely, no energy that could excite fluorescence would reach the sample. Thus all the fluorescence emission would be eliminated. The elastic reflectance curve at wavelengths longer than E can then be determined. Such a filter is called a fluorescence-eliminating filter. On the other hand, the elastic reflectance curve at wavelengths shorter than C can be measured directly without a filter, because there is no fluorescence emission in that region. Accordingly, in the crossover region determination of elastic reflectance curves is not easy. Figure 1(b) shows the schematic excitation and the emission spectra of this hypothetical fluorescence substance, which illustrates that, to maximize the difference between the signal with and without fluorescence, the cutoff wavelength of the fluorescence-eliminating filter should be the end of the excitation curve (solid thin curve) of the fluorescing substance. This is denoted as wavelength λ_0.

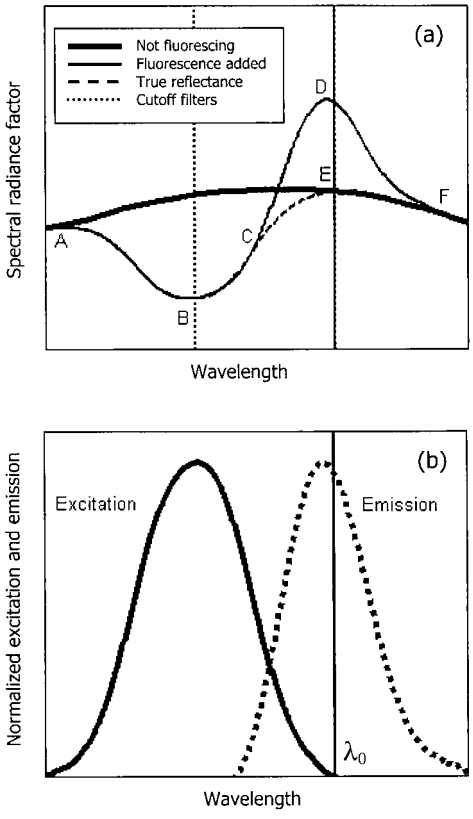

Fig. 1. (a) Schematic of fluorescence emission and absorption effects on the spectral radiance factor; (b) dashed thin curve, weakening-filter cutoff location; solid thin curve, killing fluorescence filter relative to excitation and emission spectra.

The spectrum of light that leaves a fluorescent surface per unit area (exitance) consists of both reflected

and fluoresced components. For a Lamberian surface it can be expressed as

$$E(\lambda) = E_i(\lambda) \times R_{EL}(\lambda) + F(\lambda), \quad (1)$$

where $E(\lambda)$ is the total exitance, $E_i(\lambda)$ is the incident irradiance, $R_{EL}(\lambda)$ is the elastic reflectance, and $F(\lambda)$ is the fluorescence. E, E_i, and F are in units of photons $s^{-1}\,cm^{-2}\,nm^{-1}$. The ratio of the total exitance to the incident irradiance is the irradiance reflectance. Under the assumption of Lambertian surfaces the spectral radiance factor (SRF) is the measurable equivalent of the irradiance reflectance[13] so that $SRF(\lambda) = E(\lambda)/E_i(\lambda)$. A slightly different way of writing Eq. (1) is

$$E(\lambda) = E_i(\lambda) R_{EL}(\lambda) + \gamma F_N(\lambda) \quad (2)$$

or, divided by $E_i(\lambda)$,

$$SRF(\lambda) = R_{EL}(\lambda) + \gamma \frac{F_N(\lambda)}{E_i(\lambda)}, \quad (3)$$

where $F_N(\lambda)$ is the fluorescence emission spectrum normalized to one at its maximum. (Different ways to obtain F_N are discussed below.) The γ is a parameter that converts $F_N(\lambda)$ from relative units to sample measurement units (photons $s^{-1}\,cm^{-2}\,nm^{-1}$). The γ is expected to be constant for any given incident illumination conditions but can vary when different incident illumination is used, which makes γ an apparent optical property of the sample. To isolate γ, rewrite Eq. (3) as

$$\gamma = \frac{[SRF(\lambda) - R_{EL}(\lambda)]E_i(\lambda)}{F_N(\lambda)}. \quad (4)$$

$R_{EL}(\lambda)$ can be measured with a fluorescence-eliminating filter in the $\lambda > \lambda_0$ range. [R_{EL} is equivalent to the EF portion of the spectrum in Fig. 1(a).] Once R_{EL} is known, we can calculated γ in the $\lambda > \lambda_0$ range by using Eq. (4). We expect a constant value for γ in the $\lambda_0 < \lambda < \lambda_n$ range, where λ_n is the wavelength at which the signals with and without fluorescence converge and γ can no longer be determined reliably. Although γ can theoretically be calculated at any single wavelength within this range, we use the entire $\lambda_0 < \lambda < \lambda_n$ range to get a better estimate for γ. The constant γ applies to the entire fluorescence spectrum, however, and once found can be used to calculate a new R_{EL} for the entire spectrum:

$$R_{EL}(\lambda) = SRF(\lambda) - \gamma \frac{F_N(\lambda)}{E_i(\lambda)}. \quad (5)$$

This is the new technique that is offered. The last term in Eq. (5) is the fluorescence enhancement to reflectance: $R_F = \gamma(F_N/E_i)$. This method is applicable for mixtures of fluorescing and nonfluorescing materials in which either only one fluorescing pigment exists or there is more than one fluorescing material in the mixture, but their excitation and emission spectra are separated and do not overlap.

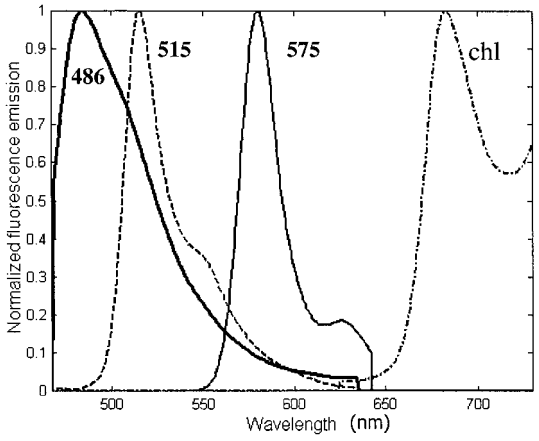

Fig. 2. Fluorescence emission spectra (normalized to one) of the major pigments found in Caribbean corals to date.

In the case of coral fluorescence the emission of different pigments can overlap significantly (Fig. 2). Hence the analysis here is restricted to samples with only one pigment other than the chlorophyll, which does not overlap with other pigments.

To calculate the light that returns from a fluorescing sample under new incident illumination, the two components of the exitance spectrum should be considered in different ways. The reflected light portion is a simple multiplication of the reflectance spectrum by the new incident illumination. For the fluorescence portion, however, the calculations are more involved. The photons that were absorbed by the pigment are the source for the fluorescence-emitted photons. If we knew the absorption coefficient of the fluorescing substance in the coral tissue, we could calculate the exact number of photons that were absorbed. Since we have no way to determine the number of absorbed photons, we use a different quantity in the calculations: the maximum available photons for absorption. Photons reach the sample surface from the incident illumination. The excitation spectrum defines the probability distribution by which these photons are absorbed. Hence the maximum number of photons per unit area that can be absorbed $N_A(\max)$, is

$$N_A(\max) = \int E_i(\lambda) Ex(\lambda) d\lambda, \quad (6)$$

where Ex is the sample excitation spectrum normalized to a peak of one. Once the fluorescence and elastic reflectance components of the exitance are separated, we can calculate the actual number of photons that fluoresced N_F:

$$N_F = \int \gamma F_N(\lambda) d\lambda. \quad (7)$$

The ratio is Φ_P,

$$\Phi_P = N_F/N_A(\max), \quad (8)$$

the practical fluorescence efficiency, which is a lower bound on the fluorescence efficiency in the traditional sense (the number of photons that fluoresced divided by the number of photons that were absorbed[14]). By using the practical fluorescence efficiency definition, we do not need to determine the actual number of photons that were absorbed by the different substances in the coral tissue nor do we need to determine the material's absorption properties.

Inasmuch as the practical fluorescence efficiency is a property of the sample and does not depend on incident light, once found, Φ_P can be used to compute the fluorescence component in new illumination conditions $E_{i,\mathrm{new}}$. Using Eq. (6) to calculate the available number of photons under the new incident irradiance, we can rearrange Eq. (8) to yield the fluorescence component under the new incident irradiance:

$$N_{\mathrm{F,new}} = \Phi_P \int E_{i,\mathrm{new}}(\lambda)\, Ex(\lambda)\mathrm{d}\lambda. \qquad (9)$$

We can now calculate a new exitance spectrum by adding the reflected component and the calculated new fluorescence component.

3. Methods

Coral specimens were collected in the spring of 1998 on Lee Stocking Island, Bahamas. For laboratory measurements, coral specimens were brought to the surface from depths of 15–18 m and maintained in flowing seawater in the shade. Four samples with strong 515-nm emission (*Scolymia* sp., *Mycetophyllia* sp., *Montastraea annularis*, *Colpophyllia natans*) and one sample with strong 486-nm emission (*Diploria labyrinthiformis*) were collected. Measurements were taken within a few hours of collection. A sequence of measurements per specimen lasted 1–2 min.

In vivo measurements were made with a Fluoromax-2 spectrofluorometer (SPEX Industries) fitted with a fiber-optic adapter. The randomized bifurcated fiber was directed at the surface of a specimen at a 45-deg angle in a darkened enclosure. For fluorescence measurements, both excitation and emission monochromators were used with 2-nm bandpass slits. For exitance measurements, for which a broadband excitation source was needed, an incandescent bulb was used to illuminate the sample. Two successive readings were taken: one from the sample and one from a 20% reflectance Spectralon surface placed near the sample. The ratio between these two readings determines the radiance factor. Long-pass cutoff filters were placed in the light path as fluorescence-eliminating filters. Excitation spectra were corrected by scanning in the ratio mode. Emission spectra were corrected by using manufacturer-supplied data for a relative spectral sensitivity of the detector. All scans were corrected for dark current offset and smoothed with a 17-point Savitzky–Golay smoothing algorithm.[15]

In situ measurements were made at the same location in January 1999 with the Benthic Spectro-Fluorometer (BSF), a prototype diver-operated instrument for reflectance and fluorescence measurements.[16] The BSF has a five-step filter wheel that allows the operator to choose different excitation conditions by placing different filters between the instrument's broadband light source (50-W halogen bulb) and a liquid light guide that leads the excitation light to the sample. A 600-μm optical fiber leads light from the sample to a CCD array spectrometer. An adapter was built to hold the end of the excitation liquid light guide perpendicular to the sample while holding the fiber at 45° at a fixed distance from the sample surface. The adapter prevented ambient light from getting to the detector. Exitance is measured over the full visible spectrum with a resolution of 10 nm. Fluorescence spectra were excited through a bandpass filter centered at 430 nm with a full width at half-maximum of 10 nm. Data were corrected for dark current and electrical offsets and for the spectral sensitivity of the detector optics. Scans were smoothed with the Savitzky–Golay smoothing algorithm. For reflectance measurements the BSF light source was used to illuminate the sample. Long-pass cutoff filters replaced the filters in the filter wheel to act as fluorescence-eliminating filters. The BSF filter wheel was fitted with appropriate long-pass filters that were used as fluorescence-eliminating filters. Previous familiarity with the shape of the excitation spectra for the 486 and 515 pigments[8,17] guided us to choose the appropriate filters. For samples containing the 486 pigment, we used a 495-nm cutoff filter as a fluorescence-eliminating filter. For the 515 pigment, a 515-nm cutoff filter was used. Two spectra were measured from each specimen: S1 (the sample illuminated by white light) contains all the fluorescence that was excited by the source, and RE (the sample illuminated through a fluorescence eliminating filter) is the reflectance spectrum where all the fluorescence had been eliminated.

We can get the fluorescence emission spectra that are necessary for the calculations above in two ways. One way is to measure directly from the sample. An alternative is to use premeasured prototype spectra: Prior measurements from Caribbean corals[8] identified five constituents of coral fluorescence signals, defined by characteristic excitation and emission spectra. To be consistent with previous publications[8,17] we use typical emission peaks of each pigment as a naming shorthand and denote them as the 486, 515, 557, 575, and 685 (chlorophyll) pigments. The emission peak of each of the individual pigments can vary both in intensity and in the precise location of the wavelength peak (variations over a range of 6 nm for the 486 pigment and 20 nm for the 515 pigment were observed), whereas the shape of the spectrum remains constant.[8,9] The excitation spectra for these pigments follow the same shifts in wavelength as the emission, maintaining a constant Stokes shift. We focus here on the fluorescence from pigments that can be traced to coral tissue[6,8] in the 400–650-nm

wavelength range. The 557 pigment is rare in abundance and has so far been found in only one species of coral and will not be treated further here. The 575 pigment always occurred together with either 486 or 515 or both and will not be analyzed here as well. These conditions restrict our current analysis to the 486 and 515 pigments.

Prototype spectra were selected by identifying specimens that contained only one pigment in the relevant wavelength range.[9,17] Eight 486 pigment spectra were normalized to have a peak value of one and shifted so that all peaks met at 486 nm. A prototype was computed as the means of these spectra. The same process was applied to 13 samples for the 515 pigment. An average Stokes shift of 40 nm was found for the 486 pigment and 11 nm for the 515 pigment.[9] The shape of the emission spectra remains relatively constant. The reflectance spectra of corals with no fluorescence pigments (other than chlorophyll) indicate that in the wavelength range of interest (450–550 nm) there are no sharp peaks.[8] Hence the fluorescence peak when fluorescence is present is distinctive (Fig. 3) and can be used to place the fluorescence prototype correctly. We demonstrate that use of prototype emission spectra does not compromise the results by repeating the calculations in two ways. First, the fluorescence emission spectrum is measured directly from each sample and used for the analysis. Second, we use the relevant pigment's prototype emission curve in the calculations.

To test the validity of the separation method for predicting new spectra in different illumination conditions, we conducted a field experiment. The exitance spectrum from a specimen of *Scolymia* sp. was measured *in vivo*, and the practical fluorescence efficiency was calculated by the method described above. Next the sample was hand carried by a diver to two different depths (8 and 17 m) at which new spectra were measured in local illumination conditions. The measured spectra were compared with the predicted spectra in two ways: (1) by treating the *in vivo* measured SRF simply as a reflectance curve and predicting new exitance by multiplying SRF by the local incident irradiance at the two new locations and (2) by calculating the practical fluorescence efficiency and predicting new exitance by the method described above [Eqs. (6)–(9)].

4. Results

The fluorescence and reflectance components of all the collected samples were separated by use of the new method. An example is shown in Fig. 3 for *Colpophyllia natans* that is dominated by the 515 pigment and in Fig. 4 for a *Diploria labyrinthiformis* sample that is dominated by the 486 pigment. All other samples showed similar results. An absorption peak (or reflectance minimum) is revealed in the elastic reflectance curves for all the samples with 515 pigment. The absorption peak is less evident for the 486 samples. We included a normalized excitation spectrum (dotted curves in Figs. 3 and 4) to demonstrate the agreement between the absorption feature

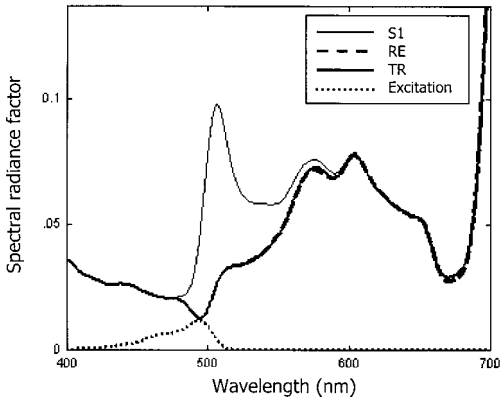

Fig. 3. Elastic reflectance spectrum of *Colpophyllia natans* calculated by the new method, TR. The dominating fluorescence pigment is 515. S1, sample illuminated by white light; RE, fluorescence eliminating filter; Excitation, excitation spectrum.

that was revealed and the wavelength location of the excitation peak. As expected, γ was constant in the $\lambda_0 > \lambda > \lambda_n$ range (Fig. 5). Practical fluorescence efficiency values for the coral samples were calculated by Eq. (6)–(8). The resulting values were 10.7%, 12%, 11.6%, 10.3% for the 515 pigment and 3.6% for the 486 pigment.

The difference between values of γ that were calculated with directly measured fluorescence spectra and γ that were calculated with prototype spectra was less than 5%. The significance (or lack) of this difference is demonstrated by the elastic reflectance curves in Fig. 6.

The fluorescence enhancement to reflectance ($R_F = \gamma F_N/E_0$) depends on the spectral energy distribution of the incoming light. Figure 7(a) shows both the reflectance and the fluorescence components for a *Colpophyllia natans* sample. To illustrate the contribution of fluorescence to the exitance signal, in Fig. 7(b) we show the relative contribution of each component to the exitance signal as a function of wavelength. For these particular illumination conditions, around 500 nm, almost 70% of the re-

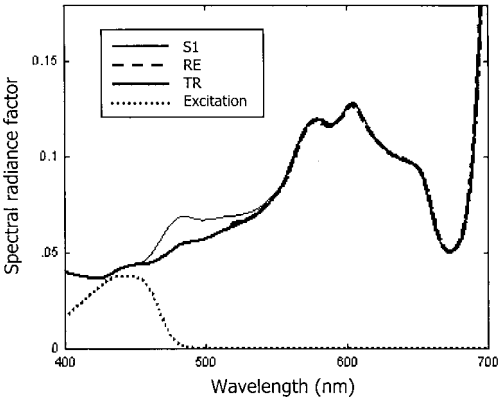

Fig. 4. Elastic reflectance spectrum of *Diploria labyrinthiformis* (the dominant fluorescing pigment is 486), calculated with the modified fluorescence method. Lines notation is as in Fig. 3.

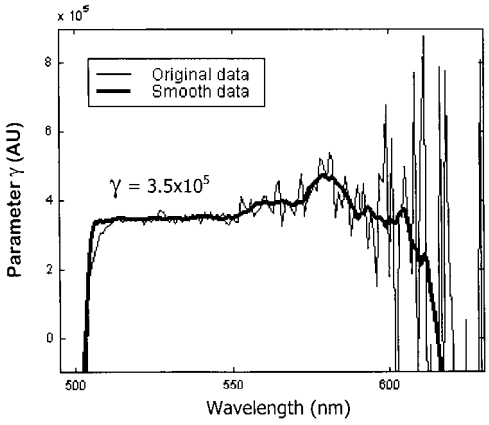

Fig. 5. Example of finding parameter γ for a *Colpophyllia natans*. The mean value in the flat region (515–550 nm) is used to calculate elastic reflectance.

turned photons can be attributed to fluorescence. The same analysis was performed on data that were measured *in situ* with the BSF. Results are in full agreement with results from the *in vivo* measurements.

In Fig. 8 we plot the normalized spectra of the downwelling irradiance that was measured at 8 and 17 m for a sample of *Scolymia* sp., showing the difference in their shape caused by seawater attenuation. Predicted spectra were calculated in two ways as described above. In Fig. 9(a) the exitance curves for the calculated and the measured spectra at a depth of 8 m are shown, whereas the curves in Fig. 9(b) are calculated for 17 m. The separation method (thin solid curves) clearly yields closer predictions to the measured data (thick solid curves).

5. Discussion

Allen applied the filter method to laundry whiteners that exhibit wide excitation and emission fluorescence spectra with relatively large Stokes shifts.

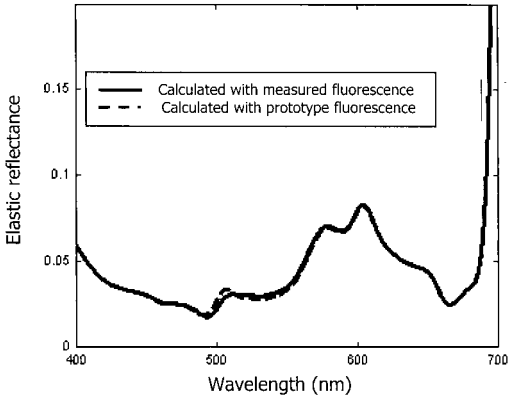

Fig. 6. Elastic reflectance curves of *Mycetophyllia* sp. calculated with the modified, fluorescence-based method. The solid curve was calculated with fluorescence measured from the sample, compared with the dashed curve, where fluorescence was taken from a library of premeasured prototypes.

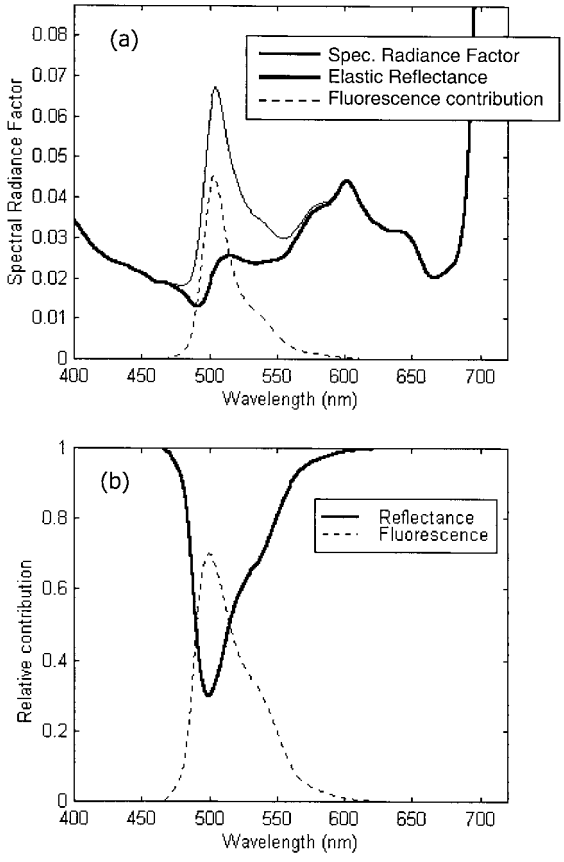

Fig. 7. (a) Separated reflectance and fluorescence components of *Colpophyllia natans*. FLR, dotted curve, is the normalized fluorescence, FLR = $\gamma \times F_N(\lambda)/E_0(\lambda)$. (b) Relative contribution of each component to the exitance signal.

The 515 fluorescence pigment in corals is characterized by sharp excitation and emission peaks and small Stokes shifts. Furthermore, the singularity that is embedded in this method prevents calculation of the elastic reflectance for the entire visible spectrum. Our new method is better suited for coral applications. In Eq. (5) R_{EL} covers the entire

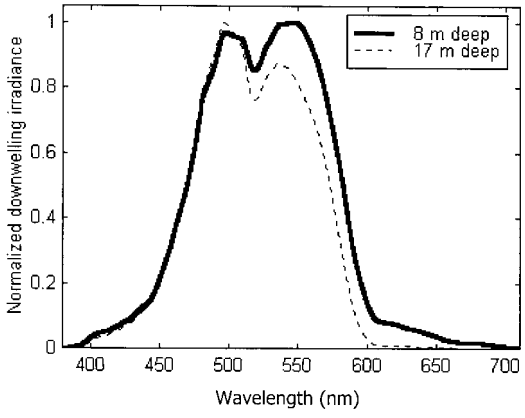

Fig. 8. Normalized downwelling irradiance at depths of solid curve, 8 m and, dashed curve, 17 m of coastal waters measured near Lee Stocking Island.

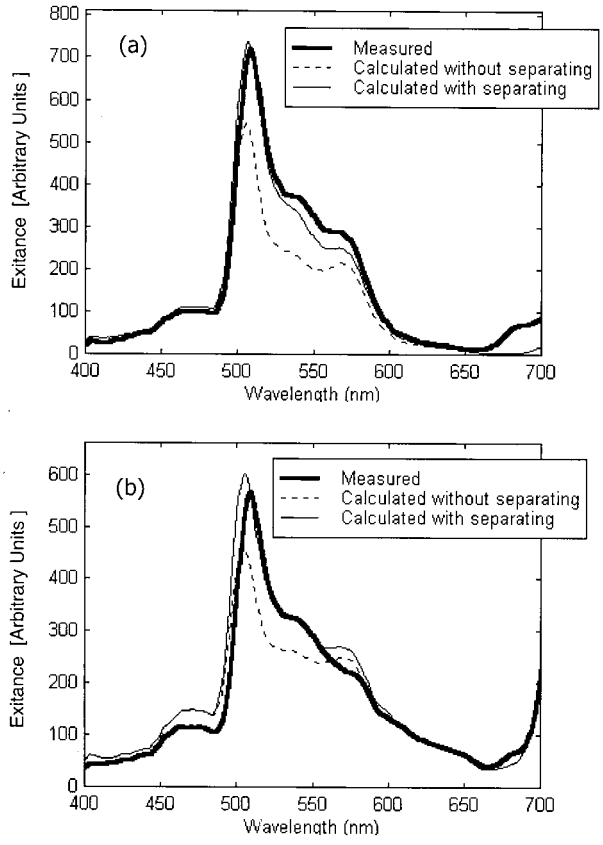

Fig. 9. Comparison of predicted exitance spectra for incident irradiance at depths of (a) 8 m and (b) 17 m with measured spectra for *Scolymia* sp. Sample: thick solid curve, *in situ* measured data; thin dashed curve, calculated without separation; thin solid curve, calculated after separating the reflectance and fluorescence components.

spectrum. It does not contain the inherent singularity that was inevitable in Allen's method and it requires fewer measurements. We see good agreement between the reflectance minimum that is revealed in the elastic reflectance spectrum and the position of the excitation spectrum. The new method was applied *in situ* and produced results similar to the conclusions drawn from *in vivo* tests.

Results of the analysis when using fluorescence emission that was measured directly from the sample and calculations made with prototype fluorescence spectra show that the two options are comparable. This has a direct implication on the number of measurements that are required. The data that need to be collected for elastic reflectance analysis are a reading of the spectral radiance factor, S1 (this is really two measurements as described above), a reading with a fluorescence-eliminating filter, RE, and an emission spectrum of the specimen's fluorescence (either measured or taken from a prototype library). The new method is applicable only to samples with single pigments (in addition to chlorophyll). For samples with multiple pigments, other methods, such as the use of dual monochromators, will be necessary. Instruments for *in situ* applications of these methods are not available to date.

Once the fluorescence and the reflectance portions of the signal are separated, an additional analysis is possible to calculate the interaction between corals and different lighting conditions in the marine environment. A new quantity, fluorescence practical efficiency Φ_P, was defined and used to calculate fluorescence emission under new incident illumination. Although different from the traditional definition of fluorescence efficiency, the practical fluorescence efficiency is a valuable quantity for remote-sensing application and oceanographic models. Although Φ_P is a property of each sample, we are not required to determine the absorption coefficient of the sample to calculate it. Further research is necessary to determine the statistical variations of Φ_P between different species and its stability in response to ecological parameters. Additional research is also necessary to expand this analysis to include specimens with more than one fluorescing pigment.

We show that there is a significant difference between spectra that were calculated without the separation of the two components and spectra that were separated. Water attenuation considerably changes the shape of the downwelling light with depth (Fig. 8), which in turn affects the reflectance and the fluorescence portions differently. When calculating light interaction in a coral-reef environment, one might err significantly (depending on the wavelength range of interest) if the contribution of fluorescence is not accounted for. Note, however, that the values that were found for Φ_P in the analysis here are likely to be on the high side, inasmuch as we chose our samples based on the bright fluorescence that they exhibited. Further research is necessary to determine how representative these values are to the entire coral community. Additional parameters that would need to be considered before the significance of fluorescence in the reef environment can be determined are the statistical coverage of fluorescing corals over the reef and the scales of interest. The research presented here refers only to individual corals at close proximity. As a detector moves away from the reef the contribution of fluorescence becomes less evident. This analysis can be valuable for optical models (such as Hydrolight or Monte Carlo) that calculate light traveling through the water column and interacting with the bottom. To date these models treat the bottom as a reflective material. A different approach may be needed when a coral environment is considered in small scale.

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