

Contribution of fluorescence to the spectral signature and perceived color of corals

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Abstract

Solar-induced fluorescence from pigments in the host tissues of reef corals can make a significant contribution to their spectral signature and can affect their apparent color as perceived by a human observer. The relative magnitudes of the reflectance and fluorescence contributions to the spectrum can vary as a function of illumination conditions. We have combined measured coral reflectance and fluorescence spectra with modeled downwelling spectral irradiances to investigate the contribution of fluorescence to coral spectra and color. Variations in depth, fluorescence efficiency, and solar zenith angle were modeled. Fluorescence enhancement is greatest when the coral absorbs light at wavelengths that are transmitted well by seawater and emits light efficiently at wavelengths that are moderately attenuated. The methodology provides a means of predicting the combined reflectance and fluorescence spectral signatures and perceived colors of fluorescent corals under arbitrary illumination conditions.

The striking patterns and hues of coloration in many corals and anemones have attracted attention for many years (Fox and Pantin 1944; Kawaguti 1944; Stephenson 1947), but an understanding of the possible function of such coloration remains elusive (Wicksten 1989). Observers long ago noted that in some cases the intense coloration is enhanced by or largely attributable to the fluorescence of pigments contained in the animal tissues (Phillips 1927; Kawaguti 1944; Shibata 1969). The effect is most striking when specimens appear orange or red (Limbaugh and North 1956; Marden 1956) at depths at which those colors are absent from the downwelling light because of the spectral filtering of seawater (Smith and Baker 1981). Investigations of coral fluorescence have focused on qualitative observations of the color of the fluorescence and quantitative measurements of its spectral characteristics (Catala 1959; Shibata 1969; Logan et al. 1990; Mazel 1995), and not on its contribution to spectral signature or perceived color under natural lighting conditions. Lythgoe (1979) noted that fluorescence played very little role in coloration in the terrestrial environment but that in the spectrally limited underwater environment it had a greater potential to produce vivid color effects.

Some of the fluorescent pigments in corals have recently been identified (Matz et al. 1999) as forms of the green fluorescent protein (GFP) originally found in the hydromedusa *Aequorea victoria* (Chalfie et al. 1994). The identification of the molecular nature of a pigment does not in itself reveal

its function. In *Aequorea*, GFP performs an energy transfer function in bioluminescence (Morin and Hastings 1971), converting the blue emission of the bioluminescence reaction to green. Corals, however, are not bioluminescent, and the function of the fluorescence has variously been ascribed to (1) providing photoprotection in high-light conditions (Kawaguti 1969; Salih et al. 2000), (2) enhancing photosynthesis in low-light conditions (Schlichter and Fricke 1990), or (3) both, depending on the positioning of the fluorescent pigment relative to the zooxanthellae (Salih et al. 1998; Dove et al. 2001). These functions have not been conclusively demonstrated for corals in general. Gleason (1993) found that the green fluorescent pigment in *Porites astreoides*, while more prevalent at shallower depths, did not provide protection against ultraviolet radiation. Mazel et al. (2003) concluded that the green fluorescent pigment in Caribbean corals is not performing either of the two functions mentioned above. Here, we explore the potential for fluorescence to influence the color of corals.

Corals can be colorful even without fluorescent pigments in the host tissues. The predominant color of corals is a beige or brown shade due to the absorption properties of light-capturing pigments in the symbiotic dinoflagellates (zooxanthellae) that are present in the gastrodermal tissues of most reef species. Other colors can be associated with nonfluorescent pigments in host tissues (Kawaguti 1944; Shibata 1969; Dove et al. 1995, 2001; Lukyanov et al. 2000).

Even when they are present, fluorescent pigments do not necessarily result in a perceived color effect under daylight illumination, at least in human observers. Many corals that exhibit striking fluorescence when illuminated with ultraviolet or blue light in darkness appear as ordinary brown specimens under ambient daylight illumination, indistinguishable from neighboring nonfluorescent specimens (pers. obs.). This can be explained by several factors, including the spectral distribution of the ambient illumination, the excitation and emission characteristics of the fluorescent pigments, and

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the fluorescence efficiencies of the pigments. We used in situ and laboratory measurements of coral reflectance and fluorescence and the modeling of downwelling spectral irradiance to explore the contribution of fluorescence to the spectral signatures of corals as a function of variation in depth, solar zenith angle, and fluorescence efficiency. Because human perception of coral color has been the basis of several studies of the ecological distribution of coral varieties (Gleason 1993, 1998; Takabayashi and Hoegh-Guldberg 1995), we compute a quantitative measure of the color enhancement effect as it would affect a human observer. The results could also be used by researchers interested in the interaction of coral color with the visual systems of reef fish or invertebrates.

Methods

Sampling and measurement locations—Fieldwork was conducted at sites around the Caribbean Marine Research Center facility on Lee Stocking Island, Exumas, Bahamas (23°46.5'N, 76°05.5'W). In situ measurements were made at North Norman's, Rainbow, North Perry, South Perry, and Horseshoe Reefs at depths ranging from 2 to 18 m. Coral samples for laboratory measurement were collected at the same sites. Sampled corals were maintained in flowing seawater tanks from the time of collection until laboratory spectral measurements were made. The tanks were shaded with Vexar netting to produce total illumination levels similar to those at the collection sites.

In situ spectral measurements—An updated version of the diver-operated spectrometer described by Mazel (1997a) was used to measure the light leaving the coral surface under ambient illumination conditions and the reflectance factor of the coral surface relative to a Spectralon (Labsphere) reference surface. The instrument contains a spectrometer (Model S2000, Ocean Optics) and data logging computer (Tattletale 8, Onset Computer) in a watertight housing connected to a probe head by a fiber optic light collector and an electrical cable. For measurements of surfaces under ambient illumination, the tip of the light collector was directed at the surface at an angle of ~45° and held so that the surface was not shaded. Data were stored in nonvolatile memory and downloaded after the dive. A correction factor was applied to the spectral signature data to compensate for the spectral sensitivity of the light-collecting optics and electronics. The correction curve was computed from a measurement of a source of known spectral irradiance (Optronics Industries).

For reflectance measurements, a combination of blue, white, and red light-emitting diodes (LEDs) in the measurement probe head provided broadband illumination from 390 to 800 nm. The light from the LEDs passed through a holographic diffuser (Physical Optics) and provided even illumination over the measurement area. A reading of the incident light provided by the measurement probe was made by placing a Spectralon reference surface in the measurement plane. Readings of the light reflected from coral specimens were divided by the incident light reading to compute reflectance. New reference measurements were made at least every 15 min to guard against slow instrument drifts.

Fluorescence excitation and emission spectra—Representative excitation and emission spectra for the fluorescent pigments most commonly found in Caribbean corals were determined as described previously (Mazel 1997b; Fux and Mazel 1999; Fuchs 2001). We call these pigments p486, p515, and p575, where the numbers correspond to the approximate wavelength of the emission peak. The excitation and emission spectra for the fluorescent pigments with peaks at 538 and 583 nm found in Indo-Pacific corals (zFP538 and drFR583, Matz et al. 1999) appeared nearly identical in shape to our measured spectra for p515 and p486, respectively. We approximated those spectra for analysis by shifting our measured spectra by the appropriate amounts on the wavelength axis. These pigments are designated p538 and p583 in this manuscript for consistency. The prototypical excitation and emission spectra for the pigments are denoted by F_{xn} and F_{en} , respectively, in the equations that follow, where the "n" in the subscript indicates that the spectrum is normalized to a peak value of unity.

Laboratory measurement of fluorescence efficiency—Fluorescence quantum yield is defined as the ratio of photons fluoresced to photons absorbed (Mobley 1994). This is a difficult quantity to measure in vivo for the coral fluorescent pigments, however, because they occur in a complex micro-environment that can include assorted cellular material, non-fluorescent pigments, zooxanthellae, and more, all overlain by a layer of mucus. The variety of absorbing substances, their physical distribution in the tissues, and how absorbed photons are partitioned among them are not known. We devised a more practical measure of fluorescence efficiency that could be determined relatively easily, with a methodology that permitted the inverse calculation of emitted photons under arbitrary illumination conditions (Fuchs 2001). This practical fluorescence efficiency (referred to simply as "efficiency" from now on) is computed as the ratio of photons fluoresced to photons available to be absorbed. "Photons available to be absorbed" is computed as the product of the incident irradiance and the normalized fluorescence excitation spectrum. This scaling step is required because not all incident photons are equally likely to stimulate fluorescence, and the excitation spectrum is equivalent to the relative probability of absorption. This approach makes no assumptions about the details of the allocation of photons within the sample but simply looks at the problem as the amount of light out relative to light in. Because the number of photons available to be absorbed will always be greater than the number of photons actually absorbed, a determination of the conventional fluorescence quantum yield for the pigment would produce a larger value.

As described in Fuchs (2001), the practical fluorescence efficiency, Φ_p , can be expressed as Eq. 1.

$$\Phi_p = \int F_{e0}(\lambda) d\lambda / \int E_{d0}(\lambda) F_{xn}(\lambda) d\lambda \quad (1)$$

$F_{e0}(\lambda)$ is the measured fluorescence emission as a function of wavelength λ and $E_{d0}(\lambda)$ is the incident downwelling irradiance (both in photons $s^{-1} cm^{-2} nm^{-1}$). $F_{xn}(\lambda)$ is the nor-

malized excitation spectrum for the pigment in question. A “0” in the subscript indicates a value associated with the experimental determination of efficiency, and not with modeled irradiance and emission.

The data required to compute the efficiency were acquired from a sequence of laboratory measurements made with a model S2000 spectrometer (Ocean Optics). Blue LEDs (peak wavelength 460 nm, bandwidth 30 nm full width at half maximum) or long-wave ultraviolet light (350–400 nm range) were used to excite fluorescence, depending on the pigment being measured. The excitation was selected so that it would not overlap the emission, eliminating the need to separate fluoresced from reflected light in this measurement. The incident irradiance was measured by directing the light sources at a Spectralon reference surface at normal incidence and measuring the intensity with the spectrometer’s fiber optic light collector directed at the surface at a 45° angle. Fluorescence was measured by replacing the reference surface with the coral sample and measuring the fluoresced light.

Efficiency measurements for p486, p515, and p575 were made using this method from intensely fluorescing specimens of *Agaricia* sp., *Colpophyllia natans*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, *Montastraea faveolata*, and *Scolymia* sp. Practical fluorescence efficiencies were not measured for p538 and p583, but values similar to those for the other pigments were assumed for this analysis.

Modeling of downwelling light—Downwelling irradiance spectra for varying depths and solar zenith angles were computed using Hydrolight (Sequoia Scientific) (Mobley 1994). The Hydrolight calculations utilized measurements of inherent optical properties (absorption and attenuation) made with an ac-9 (WET Labs) at the field site around the same time as sample and in situ data collection. The Hydrolight calculation assumed a flat sea surface (no wind). Downwelling irradiance spectra were computed for depths of 2, 5, 10, and 20 m, with a fixed solar zenith angle of 30°, and at solar zenith angles of 0, 30, 60, 75, and 80°, at a fixed depth of 20 m. In situ measurements of downwelling irradiance were made to verify the modeled spectra.

Computation of the spectrum of light leaving the coral surface—Model runs for the influence of fluorescence on spectral signature were made for a range of conditions that could influence the relative contributions of the fluorescence and reflectance components of light leaving a coral surface by varying (1) the practical fluorescence efficiency (0, 2.5, 5, and 10%) at fixed depths (2 and 20 m) and fixed solar zenith angle (30°), (2) the depth (2, 5, 10, and 20 m) with fixed solar zenith angle (30°) and fixed efficiency (7%), and (3) the solar zenith angle (0, 30, 60, 75, and 80°), at fixed depth (20 m) and fixed efficiency (7%).

The fluorescence emission from chlorophyll, with a peak in the vicinity of 685 nm, is nearly ubiquitous on the reef, but it was not included in the modeling effort because (1) the emission is weak, with measured efficiency values on the order of 1%; (2) the human eye is relatively insensitive to the deep red wavelengths of the chlorophyll fluorescence; (3) the emitted light is rapidly attenuated by seawater; and

(4) experimental model runs confirmed very little influence of the chlorophyll emission on perceived color.

The term *exitance* is defined as the flux leaving a surface per unit area, without regard to the source of that flux (reflectance, fluorescence, or transmittance). We use the term here to denote the flux leaving a coral surface under ambient illumination (S). The exitance in this case is the sum of the reflected and fluoresced components (Grum 1980; Leland et al. 1997; Fuchs 2001), where the reflected portion at any wavelength is the product of the reflectance and the incident irradiance.

$$S(\lambda) = E_d(\lambda) \times R(\lambda) + F_e(\lambda) \quad (2)$$

S is the total exitance and R is the elastic reflectance. We assume that the surface is Lambertian. For non-Lambertian surfaces, a more thorough but straightforward mathematical treatment would be required (Leland et al. 1997). We also assume that the addition of the fluorescent pigment does not produce a significant change in the reflectance. The validity of this assumption is addressed in the Discussion.

We need to find the total photon flux, N_F , in the fluorescence emission, F_e , produced by any incident irradiance, E_d (Eq. 1).

Ed = incident

Fe = emission

$$N_F = \Phi_p \times \int E_d(\lambda) F_{xn}(\lambda) d\lambda = \int F_e(\lambda) d\lambda \quad (3)$$

Fx = excitation normalized

The spectral distribution of these photons will have the shape of the fluorescence emission spectrum. We can compute a scale factor C that relates the number of photons computed in Eq. 3 to the total number of photons in the prototypical normalized emission spectrum (Eq. 4).

$$C = N_F / \int F_{en}(\lambda) d\lambda \quad (4)$$

We can rewrite Eq. 2 as follows.

$$S(\lambda) = E_d(\lambda) \times R(\lambda) + C \times F_{en}(\lambda) \quad (5)$$

With this result, we can compute the exitance for any coral for which we have a reflectance spectrum, the representative or measured fluorescence excitation and emission spectra, and a measurement or estimate of efficiency. The fractional contributions of reflected and fluoresced light to the exitance at each wavelength are $(E_d(\lambda) \times R(\lambda))/S(\lambda)$ and $(C \times F_{en}(\lambda))/S(\lambda)$, respectively. The fractional contributions of reflected and fluoresced light to the total exitance are the integrals of these two expressions over the full visible spectrum.

Computation of chromaticity coordinates—The color effect that will be produced in a “standard” human observer by any spectral distribution can be computed using techniques defined by the Commission Internationale de l’Eclairage (CIE) (Boynton 1990). Three tristimulus values, X , Y , and Z , are determined by computing the integrals of the products of the spectrum of interest and specified observer weighting functions, $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, and $\bar{z}(\lambda)$. For the case we are considering here, these quantities are defined by Eqs. 6.

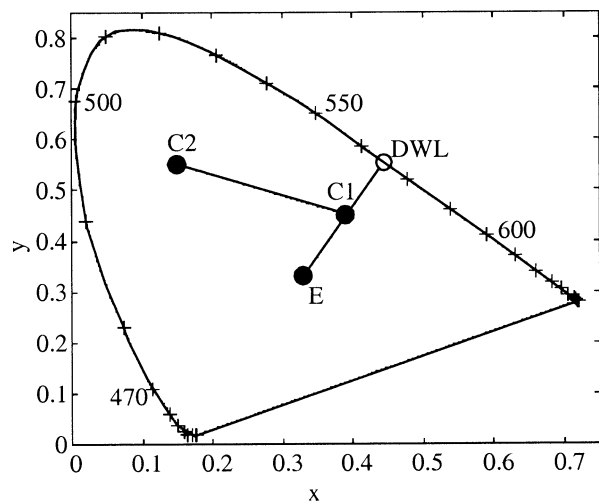


Fig. 1. Example of a CIE chromaticity diagram. A spectral distribution that mapped to the position C1 would be perceived as a mixture of the pure spectral color (dominant wavelength, indicated by DWL) on the periphery of the diagram and a “white” light source, indicated by the point labeled E. If the spectral distribution that mapped to C1 was mixed with a second distribution that mapped to C2, the combination would map to a point on the straight line connecting the two points. The position along the line would depend on the relative intensities of the two distributions.

$$\begin{aligned} X &= \int S(\lambda) \bar{x}(\lambda) d\lambda \\ Y &= \int S(\lambda) \bar{y}(\lambda) d\lambda \\ Z &= \int S(\lambda) \bar{z}(\lambda) d\lambda \end{aligned} \quad (6)$$

Chromaticity coordinates x , y , and z are then computed as in Eqs. 7.

$$\begin{aligned} x &= X/(X + Y + Z) \\ y &= Y/(X + Y + Z) \\ z &= Z/(X + Y + Z) \end{aligned} \quad (7)$$

These coordinates are plotted on a CIE chromaticity diagram that can be interpreted to describe the perceived color effect. For this study, we use the weighting functions developed for objects that fill a 2° field of view of the observer because corals tend to be small objects within a larger visual field. (The results computed for an observer with a 10° field of view are not substantially different.)

The positions to which spectral distributions map on the chromaticity diagram provide information about how those distributions will be perceived and how they can combine to create new color effects. The most important characteristics relative to this discussion are illustrated in Fig. 1. The point marked **E** is at coordinates $x = y = 0.33$ and corresponds to the CIE standard illuminant E, a normalized reference with correlated color temperature of 5500 K. This position can be considered “white,” the mixture of all colors. The periphery of the diagram corresponds to pure spectral colors, or monochromatic light, at wavelengths from 400 to 700 nm.

The *dominant wavelength*, indicated by the point marked DWL in the diagram, is the wavelength at which a line from point E through a spectral distribution’s chromaticity coordinates intersects the periphery of the diagram. The dominant wavelength is the pure spectral color that could be mixed with white to produce the same color effect as that spectral distribution. In the example in Fig. 1a, spectral distribution that maps to the point indicated by C1 has the coordinates $x = 0.39$, $y = 0.45$. The line from E through C1 intersects the periphery at a dominant wavelength of approximately 565 nm, which falls in the yellow-orange part of the spectrum. The *saturation* of a color is defined as the ratio of the distance from its position on the chromaticity diagram to the standard illuminant, divided by the distance from the dominant wavelength to the standard illuminant. The point C1 falls about halfway between white and “pure” and thus would be on the order of 50% saturated. That is, not a pure spectral orange, but a somewhat washed-out version of the color. The chromaticity diagram alone does not fully describe the color because the absolute intensity of the light is also important. The spectral distribution indicated by C1 could be perceived as orange if the intensity is high, and brown if it is low.

The chromaticity diagram provides a basis for predicting how mixtures of colors will be perceived. If a spectral distribution that mapped to the point C2 were added to the one that mapped to C1, the combination would map to a position on the straight line that connects the two points. The position along this line would be determined by the relative intensities of the two sources. The contribution of fluorescence to perceived color can be thought of as the addition of a fluorescence spectral distribution to a reflectance spectral distribution, with the chromaticity coordinates of the composite spectrum falling on the line connecting the coordinates of the fluorescence and reflectance contributions considered separately.

Results

The fluorescence characteristics and the chromaticity properties of the coral fluorescent pigments are summarized in Table 1. Note that the dominant wavelength, as defined in the chromaticity diagram, is typically at a longer wavelength than the emission peak. Four of the emission spectra produce strongly saturated colors, whereas p486 is relatively unsaturated in chromaticity terms. All of the spectra can vary somewhat from one specimen to another in shape and in the location of the peaks. The emission peak of the green pigment (p515), in particular, has been observed to occur at wavelengths over the range of 500 to 520 nm. The excitation spectrum shifts in a corresponding manner, maintaining a constant Stokes shift of 10 to 11 nm. The excitation spectrum for the orange emission (p575) can be quite different from one specimen to another. There are often two excitation peaks at ca. 508 and 567 nm (Mazel 1997b; Mazel et al. 2003). In some cases, the two peaks are nearly equal in magnitude, whereas in others, one or the other of the two peaks is substantially stronger. For any of the pigments, the emission spectrum is independent of excitation wavelength.

Table 1. Summary of spectral and chromaticity characteristics of five coral fluorescent pigments. The wavelength of the emission peak is not a constant for each pigment type, and the value given is a typical one. The approximate width of the emission spectrum is denoted by the column headed FWHM (full width at half maximum). Note that the dominant wavelength, as defined in the chromaticity diagram, is typically at a longer wavelength than the emission peak. Four of the emission spectra produce strongly saturated colors, whereas p486 is relatively unsaturated in chromaticity terms.

Pigment	Emission peak (nm)	FWHM (nm)	Stokes shift (nm)	Dominant wavelength (nm)	Saturation (%)
p486	486	58	43	496	33
p515	515	25	11	540	92
p538	538	25	11	557	98
p575	575	22	67/10*	584	100
p583	583	58	43	590	100

* The excitation spectrum for p575 often has two peaks, the relative amplitudes of which may vary.

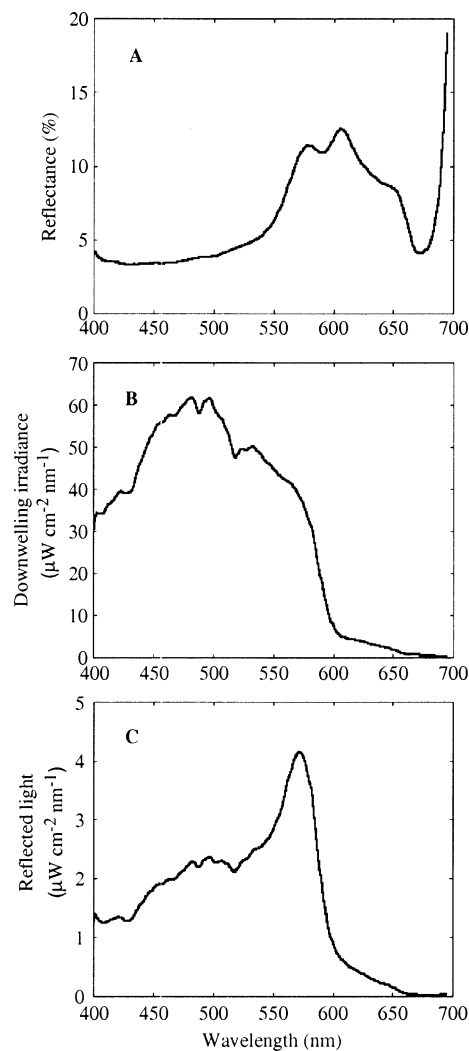


Fig. 2. Illustration of the method for computing the elastic reflectance portion of the light leaving the surface of a coral. (A) Elastic reflectance spectrum of a nonfluorescent coral; (B) downwelling irradiance at a depth of 10 m, computed by Hydrolight; and (C) light reflected from the coral surface, the wavelength-by-wavelength product of the data plotted in A and B.

Efficiency values for a number of brightly fluorescing corals ranged from 3 to 5% for p486, 10 to 12% for p515, and 8 to 10% for p575. These values should represent the high end of the range for the broader population of corals, since brightly fluorescent corals were specifically selected for measurement.

The computation of the reflectance component of the exittance is illustrated in Fig. 2. Figure 2A is a representative reflectance spectrum measured from a nonfluorescent specimen of the coral *Montastraea faveolata*. This spectrum is the one used in all our model runs. Not all corals have identical reflectance spectra, but the spectrum here is similar to those we have measured from many other specimens and to published examples (Hochberg and Atkinson 2000). Figure 2B shows the output of a Hydrolight computation of downwelling irradiance for a depth of 10 m and a solar zenith angle of 30°. The Hydrolight spectrum compares well with downwelling light spectra that we measured in situ at the same depth. The spectrum in Fig. 2C is the product of the reflectance spectrum of Fig. 2A and the downwelling irradiance spectrum of Fig. 2B at each wavelength. Although the distinctive peak at approximately 565 nm is suggestive of a sharp fluorescence peak, it occurs as a by-product of the characteristics of the reflectance and downwelling irradiance spectra. The downwelling irradiance decreases sharply from 570 to 600 nm as a result of water attenuation. This drop in available light accounts for the sharp drop in this wavelength range in the computed spectrum in Fig. 2C. The fall-off on the left side of the peak is attributable to the marked decrease in reflectance below 570 nm illustrated in Fig. 2A.

Figure 3 illustrates the influence that fluorescence can have on the spectrum of light that leaves a coral surface. The plot shows the normalized spectra of light leaving the surfaces of two neighboring colonies of the coral *Montastraea faveolata* that appeared brown and green at a depth of 10 m. The two measurements were made under ambient illumination in situ within 5 min of each other on a clear day. The relative increase in the 450–550-nm range in the green specimen is due to the presence of green fluorescent pigment in the host tissues, with a distinct peak at approximately 508 nm. The signature at wavelengths longer than 660 nm for both specimens arises from solar-induced fluorescence of

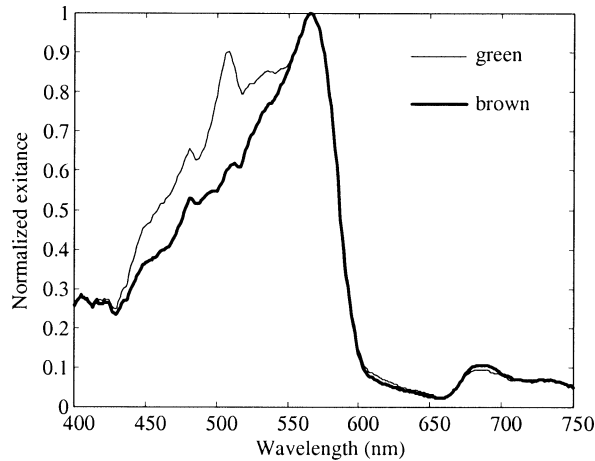


Fig. 3. Normalized exitance spectra of light leaving the surfaces of two neighboring colonies of the coral *Montastraea faveolata* that appeared green and brown at a depth of 10 m. The two measurements were made within 5 min of each other. The relative increase in the 450–550-nm range in the green specimen is due to the presence of fluorescent pigments in the host tissues, with a distinct peak at approximately 508 nm. The signature at wavelengths longer than 660 nm arises in both cases from the fluorescence of chlorophyll in the zooxanthellae. The distinct peak at 565 nm does not arise from fluorescence, but from the product of the incident irradiance and the reflectance spectrum.

chlorophyll in the zooxanthellae. The peak at 565 nm does not arise from fluorescence, but from the product of the incident irradiance and the reflectance spectrum, as described for Fig. 2C.

Figure 4 is a CIE chromaticity diagram showing the positions corresponding to the spectral distributions of the pure fluorescence emissions of the five coral fluorescent pigments and of a nonfluorescent coral at depths of 2, 5, 10, and 20 m. The combination of fluorescence and reflectance would produce spectral distributions that occupy points on the imaginary line connecting the nonfluorescent point and the point associated with the fluorescence emission. The position along the line would depend on the relative strengths of the two contributions.

Modeled results compared well with in situ measurements of exitance from fluorescing corals, as illustrated in Fig. 5. The measurement is from a specimen of *Scolymia* sp. that appeared orange under ambient illumination at a depth of 19 m. The model conditions were 20 m depth, 30° solar zenith angle, and p575 at 7% efficiency.

Figure 6 illustrates the effect of an increase in efficiency on the spectral exitance. The graphs show the effect in the 2- and 10-m cases for p515 at a fixed solar zenith angle of 30°. The model was run for practical fluorescence efficiencies of 0, 2.5, 5, and 10%, but only the 0 and 10% cases are shown for clarity. The intermediate efficiencies fall between these two extremes. The fluorescence contribution amounts to adding a properly scaled version of the emission spectrum to the spectrum of a nonfluorescent coral illuminated by the modeled irradiance. The results are similar for the other fluorescent pigments, with the fluorescence enhancement occu-

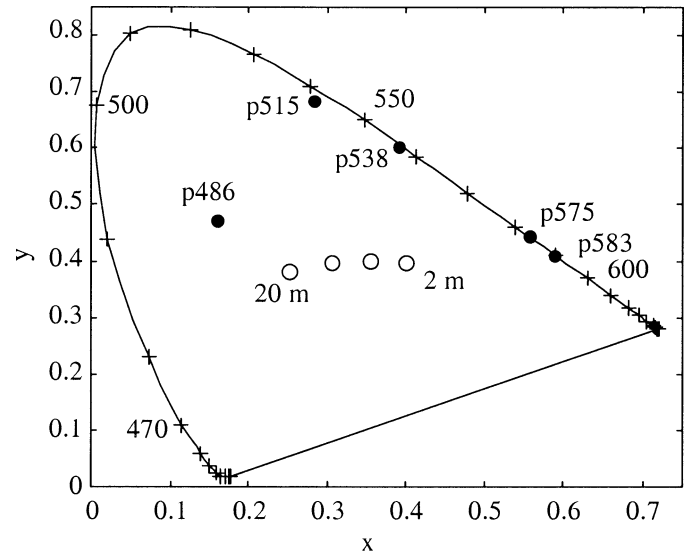


Fig. 4. CIE chromaticity diagram showing the positions corresponding to the spectral distributions of the pure fluorescence emissions of the five coral fluorescent pigments (filled circles) and of a nonfluorescent coral at depths of 2, 5, 10, and 20 m (open circles). The combination of fluorescence and reflectance would produce spectral distributions that occupy points on the line connecting the nonfluorescent point and the point associated with the fluorescence emission. The position along the line would depend on the relative strengths of the two contributions.

pying the wavelength range corresponding to the fluorescence emission spectrum of that pigment.

The effect on dominant wavelength and percent saturation for increasing efficiency of each of the fluorescent pigments is summarized in Table 2, for depths of 2 and 20 m. At each depth, the 0% efficiency column corresponds to a nonfluorescent coral, for which the perceived color is a function of the downwelling light and the reflectance spectrum. The

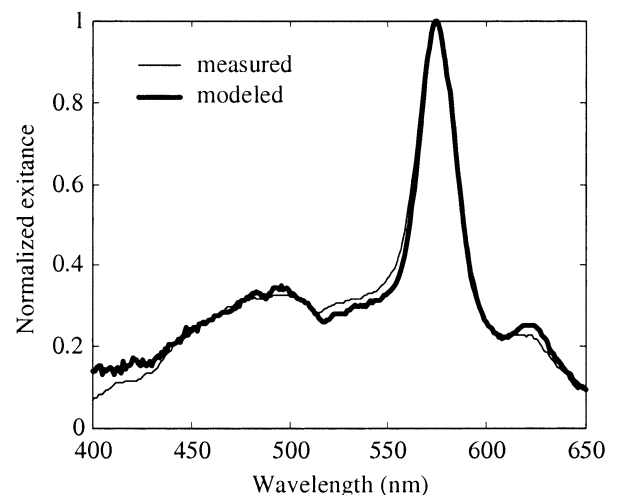


Fig. 5. Comparison of measured and modeled exitances for a fluorescing coral. Measurement: *Scolymia cubensis*, depth 19 m. Model: p575 at 7% practical fluorescence efficiency, 20 m depth, 30° solar zenith angle.

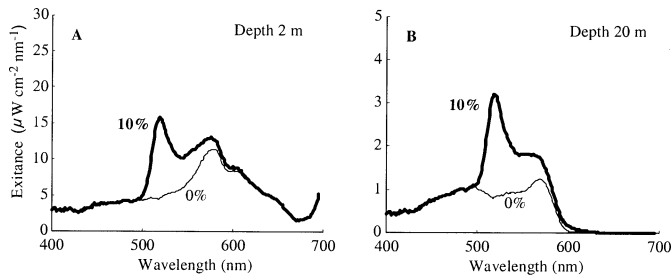


Fig. 6. The plots illustrate the effect of an increase in the practical fluorescence efficiency of the p515 pigment on the spectral exitance from a coral surface at depths of (A) 2 m and (B) 10 m. The 0% efficiency line corresponds to a nonfluorescent coral. The addition of the fluorescence affects only the portion of the spectrum corresponding to the emission of that pigment. Lower values of practical fluorescence efficiency would fall between the 0 and 10% lines. The results would be similar for the other coral fluorescent pigments.

dominant wavelength for the nonfluorescent case falls in the orange part of the spectrum (575 nm) at 2 m and in the blue-green region (494 nm) at 20 m. Corals are strongly absorbing, so the relatively low level of the exitance (in absolute terms) at 2 m would result in a brown appearance. At 20 m, the longer wavelengths have been removed from the downwelling irradiance by attenuation, shifting the color toward the blue.

At 2 m, increasing efficiency does not significantly shift the dominant wavelength for any of the pigments. For p486, the dominant wavelength remains in the yellow-orange region of the spectrum, shifting only 18 nm toward the blue, with a small decrease in saturation. For the other pigments, there is even less of a shift in dominant wavelength, but there is an increase in saturation. At 20 m, increasing efficiency of p486 does not shift the dominant wavelength at all because the dominant wavelength for the pure p486 fluorescence (Table 1; Fig. 4) is essentially the same as that for the nonfluorescent coral. There is an increase in saturation, but it is not large because the saturation of the pure pigment itself is not large. For the other pigments, there are significant shifts in dominant wavelength. For p515, the dominant wavelength of 518 nm at 10% efficiency is in the green part of the spectrum, whereas the other three pigments shift the dominant wavelength to the yellow/orange part. For p575 and p583, there is also a significant increase in saturation.

The relative contribution of fluorescence to the total exitance for these cases of increasing efficiency is summarized in Table 3. Note that p486 contributes relatively more photons to the total exitance than p515 for each set of conditions, whereas we saw from the above analysis that p515 has a greater effect on perceived color than p486. In the most extreme cases (p575 and p583, 10% efficiency, 20 m depth) nearly half of the total exitance arises from fluorescence.

Figures 7A and 7B show the downwelling irradiances computed by Hydrolight for depths of 2, 5, 10, and 20 m and a fixed 30° solar zenith angle. In Fig. 7A, the outputs are plotted in absolute units, emphasizing the decrease in intensity with depth, whereas in Fig. 7B, the outputs have been normalized to emphasize the narrowing in spectral dis-

Table 2. Dominant wavelength and percent saturation as a function of practical fluorescence efficiency for the exitance spectra for corals with each of the fluorescent pigments at a fixed 30° solar zenith angle and depths of 2 and 20 m.

Pigment	Practical fluorescence efficiency (%)							
	0				2.5			
	Dominant wavelength (nm)				Saturation (%)			
Depth 2 m								
p486	575	571	566	557	40.4	37.1	34.2	29.5
p515	575	571	567	562	40.4	43.2	45.6	49.7
p538	575	573	570	567	40.4	43.6	47.8	53.5
p575	575	577	578	580	40.4	47.4	53.1	61.1
p583	575	578	580	583	40.4	45.3	51.5	58.9
Depth 20 m								
p486	494	494	495	495	23.9	27.4	29.1	33.3
p515	494	500	507	518	23.9	23.0	23.7	29.9
p538	494	500	514	533	23.9	19.9	19.2	29.3
p575	494	522	556	570	23.9	12.6	23.8	40.9
p583	494	506	555	573	23.9	12.0	19.6	35.9

tribution with depth due to the filtering effect of the water column. The decrease in energy at the longer wavelengths favors a color enhancement effect by fluorescence emission in that portion in the spectrum. The normalized spectral exitances from the coral surface at depths of 2 and 20 m for these irradiances and 7% efficiency of the host pigments are shown in Fig. 8. Only these two depths are presented in the interest of clarity, and the spectra are normalized to emphasize the change in spectral shape. The curves for the 5- and 10-m cases would occupy positions intermediate between these two.

The effect of increasing depth on dominant wavelength and percent saturation is summarized in Table 4. The variation in chromaticity with depth for a nonfluorescent coral (first row of Table 2 and open circles in Fig. 4) is due to the changing downwelling spectral irradiance distribution. For p486 the chromaticity values are not very different than

Table 3. Percentage of total photons leaving the coral surface contributed by fluorescence for practical fluorescence efficiencies of 2.5, 5, and 10% at depths of 2 and 20 m for the coral host fluorescent pigments.

Pigment	Practical fluorescence efficiency (%)		
	2.5	5	10
Depth 2 m			
p486	7.1	13.3	23.4
p515	5.8	11.0	19.9
p538	6.0	11.3	20.3
p575	10.5	19.0	32.0
p583	9.9	18.1	30.6
Depth 20 m			
p486	12.9	22.8	37.2
p515	12.0	21.4	35.3
p538	11.1	20.0	33.4
p575	17.3	29.5	45.6
p583	16.0	27.5	43.2

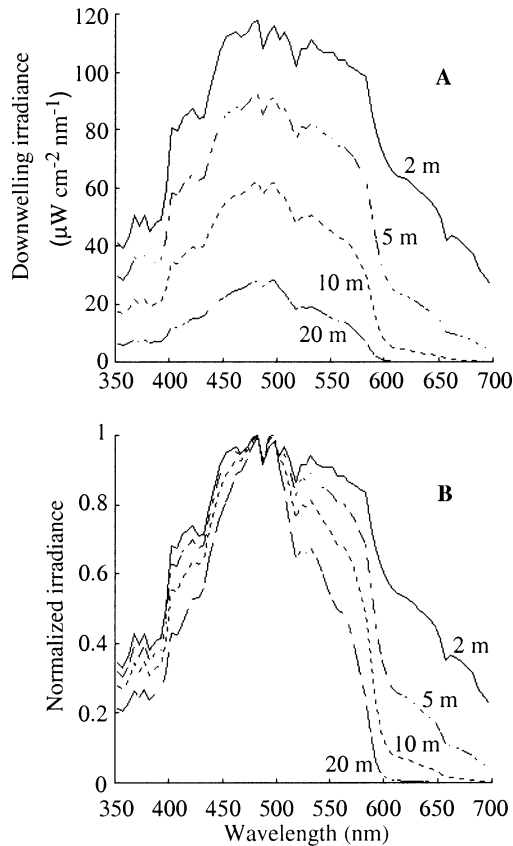


Fig. 7. (A) Plots of Hydrolight output for downwelling irradiance at depths of 2, 5, 10, and 20 m, in absolute units. (B) The same data normalized to emphasize the change in spectral distribution. As depth increases, the downwelling irradiance decreases in intensity and becomes narrower spectrally because of the removal of the longer wavelengths by water column attenuation.

those for a nonfluorescent coral, with little difference in saturation. For p515 and p538, the dominant wavelength is constrained to a narrower range in the yellow to green, with an increase in saturation. For p575 and p583, the dominant wavelength is constrained to a very small variation (15 nm) as depth increases. There is a decrease in saturation from the 2- to the 20-m case for all of the pigments except p486. The downwelling light decreases more rapidly in the long-wavelength portion of the spectrum with depth. As a result, at 20 m there is relatively (not absolutely) more light in the range of 400–550 nm than at 2 m. This tends to “pull” the color toward the blue side of the chromaticity diagram. A specimen can only have chromaticity coordinates in the orange part of the diagram with fluorescence, and even with relatively low saturation it will stand out against its long-wavelength-deficient surroundings.

As solar zenith angle increases from 0° (overhead) to 80° (near the horizon), the light intensity decreases. There is a small relative increase in downwelling irradiance at wavelengths shorter than 450 nm, and a small relative decrease at wavelengths longer than 500 nm. This is shown in Fig. 9 for a depth of 20 m. The results for other depths are similar. The dominant wavelength does not shift substantially for any of the pigments as solar zenith angle changes (Table 5). For

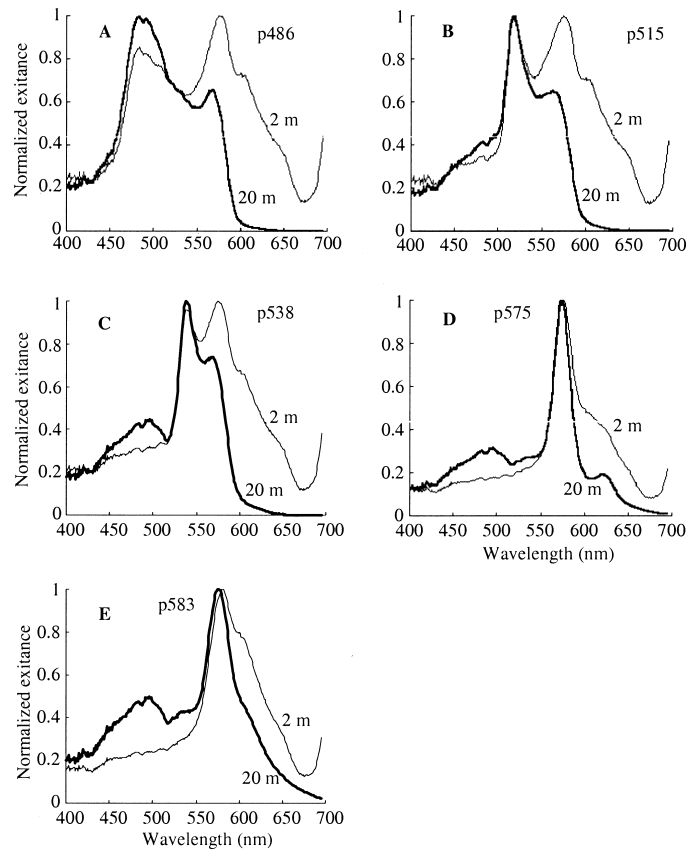


Fig. 8. Plots of the normalized spectral exitances at depths of 2 and 20 m for the fluorescent pigments for model conditions of 30° solar zenith angle and 7% practical fluorescence efficiency.

p486 and p515, there is an increase in saturation due to reinforcement of the shorter wavelengths, whereas for the other pigments, there is a decrease in saturation due to the relative increase in shorter wavelength light.

Discussion

Fluorescence can be a dominant factor in the color of some corals under natural illumination, and the methods pre-

Table 4. Dominant wavelength and percent saturation as a function of depth for the exitance spectra for a nonfluorescent coral and for corals with each of the fluorescent pigments at a fixed 30° solar zenith angle and 7% practical fluorescence efficiency.

Pigment	Depth (m)							
	2	5	10	20	2	5	10	20
	Dominant wavelength (nm)				Saturation (%)			
None	575	562	526	494	40.4	28.3	14.4	23.9
p486	562	541	507	495	31.9	21.8	18.4	31.0
p515	565	553	534	512	47.5	38.7	29.7	25.5
p538	569	560	546	524	50.4	42.3	32.6	23.0
p575	579	575	570	564	56.7	50.2	42.2	31.5
p583	581	577	572	566	55.0	47.8	38.9	27.0

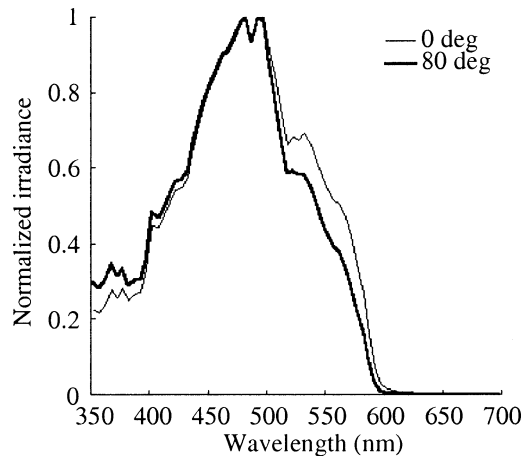


Fig. 9. Plots of Hydrolight output for downwelling irradiance at solar zenith angles of 0 and 80° and a depth of 20 m, normalized to emphasize the change in spectral distribution. As solar zenith angle increases, there is a small relative increase in downwelling irradiance at wavelengths shorter than 450 nm and a small relative decrease at wavelengths longer than 500 nm. The spectra for intermediate solar zenith angles fall between the two cases illustrated here.

sented here provide a means for analyzing the fluorescence enhancement quantitatively. Some of the fluorescent pigments have more potential than others to produce a color effect in humans, but the mere presence of a pigment does not guarantee that there will be a noticeable effect. It is convenient to distinguish “overt fluorescence,” for the cases in which the fluorescence is evident in natural illumination, and “covert fluorescence,” for which it is necessary to illuminate the coral with an appropriate light source in darkness. In the case of covert fluorescence, the emission still occurs under daylight illumination but is overwhelmed by the reflected light. Our study has demonstrated that some of the fluorescent pigments are more likely than others to produce an overt color effect, which is due to the characteristics of the fluorescence excitation and emission spectra, in combination with the fluorescence efficiency and the spectral composition of the ambient irradiance. Fluorescent substances that (1) absorb wavelengths that are transmitted well by seawater, (2) have high practical fluorescence efficiency, (3) emit at wavelengths that are only moderately attenuated, and (4) emit at wavelengths to which human eyes are most sensitive

are able to produce the most striking effects in human observers. This combination is especially true of p575 and p583, which emit in the orange portion of the spectrum. They absorb in the green portion of the spectrum and emit in a wavelength range that is attenuated enough that there is little competing downwelling light, yet not so strongly that the emitted light is reduced too rapidly. The green pigment (p515) most widespread in corals emits in a spectral range for which the downwelling irradiance is not strongly attenuated, which would argue against a strong color effect, but the emission often occurs with high efficiency and falls in a wavelength range to which the human eye is very sensitive.

The blue-green pigment (p486) produces less of a visual effect. It absorbs wavelengths that are transmitted well by seawater, but its efficiency tends to be low. It emits at wavelengths that are also transmitted well by seawater, so the fluorescence is in competition with strong reflected light. Finally, the pure emission itself produces a relatively unsaturated color, especially as compared to the highly saturated colors of the other pigments. These factors combine to minimize the visual effect of this pigment and make the green and orange emissions the fluorescence enhancement colors most commonly seen by divers. These results correspond to underwater observations: p486 has rarely been seen to have a noticeable effect on perceived color, even when present in relatively large quantities (as represented in the measured strength of the emission). Divers have often noted an intense blue color in some specimens of *Porites* sp. and other corals. We have examined these and found that the color is due to reflectance and not fluorescence (unpubl. data). Their reflectance in the blue part of the spectrum is much higher than that of the coral from which the spectrum used in the model (Fig. 2A) was measured. This, in combination with the preponderance of blue in the downwelling irradiance, produces this visual effect in the human observer.

Chlorophyll in the zooxanthellae is an efficient absorber of light at the wavelengths transmitted by seawater, but its fluorescence emission at 685 nm and longer wavelengths is strongly absorbed by seawater. It is also characterized by low practical fluorescence efficiency and emission at wavelengths to which human vision is not very sensitive (Boyn-ton 1990). Consequently, chlorophyll fluorescence under ambient illumination is measurable by underwater instruments but is not readily perceived by humans.

The simplifications we made here in modeling the contri-

Table 5. Dominant wavelength and percent saturation as a function of solar zenith angle for the exitance spectra for a nonfluorescent coral and for corals with each of the fluorescent pigments at fixed depth of 20 m and 7% practical fluorescence efficiency.

Pigment	Solar zenith angle (degree)									
	0	30	60	75	80	0	30	60	75	80
	Dominant wavelength (nm)					Saturation (%)				
None	495	494	492	489	488	22.2	23.9	28.0	32.3	33.6
p486	495	495	494	492	491	30.4	31.0	33.8	37.4	38.9
p515	513	512	508	504	503	25.2	25.5	26.8	27.3	27.1
p538	526	524	519	511	508	23.5	23.0	21.9	19.7	19.3
p575	565	564	563	562	562	32.5	31.5	29.5	24.5	22.4
p583	567	566	564	562	562	28.1	27.0	24.3	18.5	16.2

bution of fluorescence to total exitance were (1) using only one baseline reflectance spectrum, (2) assuming that only one fluorescent pigment was present in a coral specimen, and (3) assuming that there is no significant contribution to reflectance associated with the fluorescent pigments. We will discuss each of these briefly here.

The reflectance spectrum shown in Fig. 2A was used for all computations. The reflectance of all corals is not identical, but the spectrum chosen is reasonably representative of those measured by us and other researchers from many different corals. If it were desirable to model the fluorescence contribution in a particular species or individual, the appropriate reflectance spectrum could simply be substituted for $R(\lambda)$ in the equations.

The modeling was applied to only one fluorescent pigment at a time, whereas corals often contain more than one of these pigments. When multiple pigments are present, the fluorescence emissions can be modeled separately and their contributions to the spectral exitance summed. This approach works in many cases and has been used to decompose a composite fluorescence emission spectrum into the contributions of the individual fluorescing constituents and then predict the response to a new excitation wavelength (Fux and Mazel 1999). If there is direct energy transfer (such as by fluorescence resonance energy transfer) from one pigment to another, there would be some error in the analysis. This kind of energy coupling has been reported for extracts of p486 and p515 (Salih et al. 2000), and we have measured varying levels of coupling between these two pigments in vivo (Fuchs 1999). If coupling does occur, and the amount of coupling is known, it could be incorporated into the computation.

Finally, we assumed that the introduction of a fluorescent pigment only adds a fluorescence component to the exitance and does not affect the baseline reflectance significantly. Reflectance spectra of fluorescing corals measured using a broadband light source indicate that, in most cases, this is a reasonable assumption. Such spectra generally look very similar to the reflectance spectrum of a nonfluorescing coral, with the introduction of a new peak at the wavelength of the fluorescence emission (Fuchs 2001). When analytical techniques were used to decompose these spectra into their fluorescence and elastic reflectance components, a new reflectance minimum was sometimes revealed in a wavelength range corresponding to the fluorescence excitation spectrum (Fuchs 2001). This was evident for fluorescing corals containing p515, but not for specimens with p486. Even when the new minimum was present, the altered reflectance spectrum would not have produced results significantly different from those presented here. In no case was a broadband reflectance effect observed. Salih et al. (2000) described a broadband reflectance increase associated with fluorescent pigments, but this effect was restricted to polyp mouths with high concentrations of the pigments that produced a nearly opaque silver/white appearance under natural illumination (Salih pers. comm.).

The presence of color, of fluorescent origin or not, does not in itself imply that there is a function for the color. Wicksten (1989) provides a thorough overview of the nature and possible role of color in sessile marine invertebrates, without

coming to any definitive conclusions. Color is an obvious characteristic differentiating morphs of a species, and has been the defining variable in several studies of ecological distributions (Gleason 1993, 1998; Takabayashi and Hoegh-Guldberg 1995). Although these investigators did find differences in the distributions of the morphs, there was no clear cause and effect relationship between the color and the ecological zonation.

There is certainly a reason for the presence of the pigments that result in coloration, but that reason might have to do with other properties of the pigments, with the color merely being an adaptively neutral by-product. In one case we are aware of, in which fluorescent coloration serves a direct biological function, Arnold et al. (2002) demonstrated that the fluorescence in feathers in crowns and cheeks of parrots is a positive factor in sexual preference. In the case of a crab that has claws with dark tips, Melnick et al. (1996) found that the change in color was associated with a change in material that imparted desirable mechanical characteristics to the claw. The physical properties of the material were the important characteristic, with no evidence that the associated color effect had ecological significance. Growns and Ritz (1994) investigated color variations in southern Tasmanian echinoderms and found some correlation between color morphs and habitat. All of the color variants seemed to belong to the same species, and the correlations could have been the result of environmental forcing factors or ecological isolation of subpopulations.

The CIE method for color analysis is a useful way to track the effect of changing spectral distributions on human-perceived color, but it does not provide an absolute indication of how a color will appear. The chromaticity diagram does not take into consideration the absolute intensity of the illumination. The same spectral distribution can produce a marked color effect at one intensity and a very muted response at a lower intensity. As was noted earlier, orange and brown occupy the same region on a chromaticity diagram, and the perception of one versus the other is a function of relative intensities. There are many other factors involved in the perception of color, such as the spectral distribution in the broader environment that encompasses the sample of interest.

The discussion of color from the point of view of human observers begs the question of how natural inhabitants of the reef might perceive a spectral distribution. Measurements of the spectral response of the visual receptors of reef fish indicate a general adaptation to the wavelengths of light present in the marine environment (McFarland 1991). There is no evidence that piscine visual receptors are preferentially tuned to wavelengths of fluorescence emissions in corals, and no special interaction between fluorescent corals and fish or other mobile organisms in the reef environment has been suggested. McFarland and Munz (1975) discuss the special case of the visible spectrum during twilight, the period that marks the change from diurnal to nocturnal activity. The main change noted in the available irradiance is a reduction of yellow and orange wavelengths relative to the blue. This effect complements the natural reduction of those wavelengths by the filtering effect of water, and would provide

the same conditions as have already been noted in regard to fluorescence enhancement.

The early study of human vision demonstrated that knowledge of an input spectral distribution and of the spectral sensitivities of the cone cells is not sufficient to predict how a color will be perceived (Boynton 1990), so we should not expect a purely mechanistic analysis to work for fish. Color perception is in the realm of psychology, and it was necessary to test many observers to develop the general methodology and observer weighting functions that are used to predict perceived color in humans. We do not have this luxury with fish or other marine organisms, but progress has been made in developing discriminability models to estimate the "distance" between colors when the visual receptor spectral sensitivities are known (Vorobyev et al. 1998, 2001; Marshall 2000). These approaches could be applied to the modeled spectra here to estimate the visual effect that the fluorescence enhancement might have for various reef fish.

The methods described here are general and could be used to compute the contribution of fluorescence to spectral signatures under any arbitrary combination of conditions. They could be used to design an illumination scheme that either preferentially emphasizes or minimizes the fluorescence component of total exitance for measurement or display purposes. There is a great deal of current interest in characterizing the reflectance spectra of corals and other marine organisms to develop remote sensing data interpretation algorithms (Hochberg and Atkinson 2000). Reflectance spectra measured from fluorescent specimens will include a fluorescence component, the relative magnitude of which will depend on the spectral distribution of the light source, whether the sun or an artificial source, used to illuminate the reference and sample surfaces. Different illumination sources could produce quite different measured reflectances. The potential for this to occur could be estimated using the methods presented here.

Several separate lines of research should eventually contribute to a clearer understanding of the function of color, and fluorescent color in particular, in corals. These include: additional ecological studies examining the relationship between habitat and color morph distribution, controlled field and laboratory experiments to determine the factors controlling the expression of coral color, analysis and experimentation to determine the relationship between exitance spectra and fish vision, and additional analysis of the fluorescing pigments at the molecular scale to better understand the relationship between structure and function. Once we learn more about the function of color and the controls on fluorescent and nonfluorescent color expression, we may find that color can be a valuable bioindicator of environmental conditions.

References

- ARNOLD, K. E., I. P. F. OWENS, AND N. J. MARSHALL. 2002. Fluorescent sexual signaling in parrots. *Science* **295**: 92.
- BOYNTON, R. M. 1990. Human color perception, p. 211–253. *In* K. N. Liebovic [ed.], *Science of vision*. Springer-Verlag.
- CATALA, R. 1959. Fluorescence effects from corals irradiated with ultra-violet rays. *Nature* **183**: 949.
- CHALFIE, M., Y. TU, G. EUSKIRCHEN, W. W. WARD, AND D. C. PRASHER. 1994. Green fluorescent protein as a marker for gene expression. *Science* **263**: 802–805.
- DOVE, S. G., M. TAKABAYASHI, AND O. HOEGH-GULDBERG. 1995. Isolation and partial characterization of the pink and blue pigments of pocilloporid and acroporid corals. *Biol. Bull.* **189**: 288–297.
- , O. HOEGH-GULDBERG, AND S. RANGANATHAN. 2001. Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral Reefs* **19**: 197–204.
- FOX, D. L., AND C. F. A. PANTIN. 1944. Pigments in the coelenterata. *Biol. Rev.* **19**: 121–134.
- FUCHS, E. 1999. Fluorescence in reef corals. Ph.D. thesis, Massachusetts Institute of Technology.
- . 2001. Separating the fluorescence and reflectance components of coral spectra. *Appl. Opt.* **40**: 3614–3621.
- FUX, E., AND C. H. MAZEL. 1999. Unmixing coral fluorescence emission spectra and predicting new spectra under different excitation conditions. *Appl. Opt.* **38**: 486–494.
- GLEASON, D. F. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astreoides*. *Limnol. Oceanogr.* **38**: 1452–1463.
- . 1998. Sedimentation and distributions of green and brown morphs of the Caribbean coral *Porites astreoides* Lamarck. *J. Exp. Mar. Biol. Ecol.* **230**: 73–89.
- GROWNS, J. E., AND D. A. RITZ. 1994. Colour variation in southern Tasmania populations of *Heliocidaris erythrogramma* (Echinodermata: Echinoidea). *Aust. J. Mar. Freshw. Res.* **45**: 233–242.
- GRUM, F. 1980. Colorimetry of fluorescent materials, p. 235–288. *In* F. Grum and C. J. Bartleson [eds.], *Color measurement*. Academic Press.
- HOCHBERG, E. J., AND M. J. ATKINSON. 2000. Spectral discrimination of coral reef benthic communities. *Coral Reefs* **19**: 164–171.
- KAWAGUTI, S. 1944. On the physiology of reef corals VI. Study on the pigments. *Palao Trop. Biol. Stn. Stud.* **2**: 617–674.
- . 1969. Effect of the green fluorescent pigment on the productivity of the reef corals. *Micronesica* (abstr.) **5**: 313.
- LELAND, J., N. JOHNSON, AND A. ARECCHI. 1997. Principles of bi-spectral fluorescence colorimetry. *SPIE* **3140**: 76–87.
- LIMBAUGH, C., AND W. J. NORTH. 1956. Fluorescent, benthic, Pacific Coast coelenterates. *Nature* **178**: 497–498.
- LOGAN, A., K. HALCROW, AND T. TOMASCIK. 1990. UV excitation-fluorescence in polyp tissue of certain scleractinian corals from Barbados and Bermuda. *Bull. Mar. Sci.* **46**: 807–813.
- LUKYANOV, K. A., AND OTHERS. 2000. Natural animal coloration can be determined by a nonfluorescent green fluorescent protein. *J. Biol. Chem.* **275**: 25,879–25,882.
- LYTHGOE, J. N. 1979. *The ecology of vision*. Clarendon Press.
- MARDEN, L. 1956. Camera under the sea. *Natl. Geogr.* **109**: 162–200.
- MARSHALL, N. J. 2000. Communication and camouflage with the same 'bright' colours in reef fishes. *Phil. Trans. R. Soc. Lond. B* **355**: 1243–1248.
- MATZ, M. V., A. F. FRADKOV, Y. A. LABAS, A. P. SAVITSKY, A. G. ZARAIISKY, M. L. MARKELOV, AND S. A. LUKYANOV. 1999. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* **17**: 969–973.
- MAZEL, C. H. 1995. Spectral measurements of fluorescence emission in Caribbean cnidarians. *Mar. Ecol. Prog. Ser.* **120**: 185–191.
- . 1997a. Diver-operated instrument for in situ measurement of spectral fluorescence and reflectance of benthic marine organisms and substrates. *Opt. Eng.* **36**: 2612–2617.
- . 1997b. Coral fluorescence characteristics: Excitation-emis-

- sion spectra, fluorescence efficiencies, and contribution to apparent reflectance. *SPIE* **2963**: 240–245.
- , M. P. LESSER, M. Y. GORBUNOV, T. M. BARRY, J. H. FARRELL, K. D. WYMAN, AND P. G. FALKOWSKI. 2003. Green fluorescent proteins in Caribbean corals. *Limnol. Oceanogr.* **48**: 402–411.
- McFARLAND, W. N. 1991. The visual world of coral reef fishes, p. 16–38. *In* P. F. Sale [ed.], *The biology of fishes on coral reefs*. Academic Press.
- , AND F. W. MUNZ. 1975. The visible spectrum during twilight and its implications to vision, p. 249–270. *In* G. C. Evans, R. Bainbridge, and O. Rackham [eds.], *Light as an ecological factor: II*. Blackwell Scientific.
- MELNICK, C. A., Z. CHEN, AND J. J. MECHOLSKY, JR. 1996. Hardness and toughness of exoskeleton material in the stone crab, *Menippe mercenaria*. *J. Mater. Res.* **11**: 2903–2907.
- MOBLEY, C. D. 1994. *Light and water*. Academic Press.
- MORIN, J. G., AND J. W. HASTINGS. 1971. Energy transfer in a bioluminescent system. *J. Cell. Physiol.* **77**: 313–318.
- PHILLIPS, C. E. S. 1927. Fluorescence of sea anemones. *Nature* **119**: 747.
- SALIH, A., O. HOEGH-GULDBERG, AND G. COX. 1998. Photoprotection of symbiotic dinoflagellates by fluorescent pigments in reef corals, p. 217–230. *In* J. G. Greenwood and N. J. Hall [eds.], *Proceedings of the Australian Coral Reef Society 75th Anniversary Conference*.
- , A. LARKUM, G. COX, M. KUHLE, AND O. HOEGH-GULDBERG. 2000. Fluorescent pigments in corals are photoprotective. *Nature* **408**: 850–853.
- SCHLICHTER, D., AND H. W. FRICKE. 1990. Coral host improves photosynthesis of endosymbiotic algae. *Naturwissenschaften* **7**: 447–450.
- SHIBATA, K. 1969. Pigments and a UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant and Cell Physiol.* **10**: 325–335.
- SMITH, R. C., AND K. S. BAKER. 1981. Optical properties of the clearest natural waters (200–800 nm). *Appl. Opt.* **20**: 177–184.
- STEPHENSON, T. A. 1947. The colours of marine animals. *Endeavour* **6**: 152–159.
- TAKABAYASHI, M., AND O. HOEGH-GULDBERG. 1995. Ecological and physiological differences between two colour morphs of the coral *Pocillopora damicornis*. *Mar. Biol.* **123**: 705–714.
- VOROBYEV, M., D. OSORIO, A. T. D. BENNETT, N. J. MARSHALL, AND I. C. CUTHILL. 1998. Tetrachromacy, oil droplets and bird plumage colours. *J. Comp. Physiol. A* **183**: 621–633.
- , N. J. MARSHALL, D. OSORIO, N. H. DE IBARRA, AND R. MENZEL. 2001. Colourful objects through animal eyes. *Color Res. Appl.* **26**: S214–S217.
- WICKSTEN, M. K. 1989. Why are there bright colors in sessile marine invertebrates? *Bull. Mar. Sci.* **45**: 519–530.

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