Spectral measurements of fluorescence emission in Caribbean cnidarians

Charles H. Maze

Dept of Ocean Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

ABSTRACT: Fluorescence effects in cnidarian host tissues have been investigated only sporadically and have largely been described qualitatively. In past studies specimens were selected for investigation based on their apparent fluorescence under either daylight or ultraviolet (UV-A or UV-B) illumination, thereby missing relationships between the 2 effects. In this study samples of a variety of Caribbean cnidarian species were selected based on their in situ responses to daylight and/or UV-A illumination. Fluorescence emission spectra (excitation wavelength 365 or 405 nm) were measured for sources in the host tissues. A sorting of specimens by similarity in spectral shape resulted in groupings containing members of different species. Emission spectra measured from separate specimens of a single species exhibited clear differences in some cases. There was no correlation between the spectral shape and the relative fluorescence intensity observed under illumination by ultraviolet light (UV-A, emission maximum $\lambda = 360$ nm). Emission maxima fell in the range of 480 to 590 nm. The only source of fluorescence at wavelengths greater than 650 nm was chlorophyll in zooxanthellae. Some specimens contained material with an emission spectrum identical to that of phycoerythrin ($\lambda = 575$ nm). The results are of interest for studies of photoecology and for applications of coral reef optical remote sensing.

KEY WORDS: Cnidarians - Fluorescence - Photoecology - Remote sensing

INTRODUCTION

The red fluorescence ($\approx 685$ nm) of chlorophyll in single-celled algae, including those existing as endosymbionts in many coral reef cnidarians, is well documented (e.g. Dustan 1985). The fluorescence of substances in host tissues has received much less attention despite having been reported early in the century (Phillips 1927). There have been several descriptive reports of fluorescence emissions under ultraviolet light excitation (Limbaugh & North 1956, Catala 1959, 1960, 1964, Jokiel & York 1982, Logan et al. 1990) and several reports of measured absorption spectra (Kawaguti 1944, Buchsbaum 1968, Shibata 1969). Hardy et al. (1992) measured the in vivo laser-induced fluorescence in several species of corals. They were primarily concerned with chlorophyll fluorescence under 532 nm excitation, but they also measured the fluorescence from 1 specimen using 337 nm excitation. Read et al. (1968) investigated several corals and sponges that fluoresced under daylight illumination but only measured emission spectra of alcohol extracts from 2 species of sponge. Schlichter et al. (1986) measured the emission spectrum of a chloroform extract of a fluorescent substance in cytoplasm of the Red Sea coral Leptoseris fragilis. As part of his investigation of the reflection and absorption spectra of corals, Shibata (1969) measured excitation spectra in vivo for fluorescence at wavelengths longer than 600 nm, but did not measure emission spectra. The work reported here is the first to make a systematic effort to record quantitative emission spectra from fluorescent substances in the host tissues of a variety of freshly sampled cnidarian specimens.

Investigators of cnidarian fluorescence have differed in the way they selected specimens for examination. Limbaugh & North (1956) and Read et al. (1968) collected organisms that appeared to exhibit fluorescence under daylight illumination. Catala (1959) and Logan et al. (1990) collected a variety of specimens to determine how they responded to ultraviolet light (UV-A and UV-B, respectively) in the laboratory, but did not
base the initial selection on any particular characteristic of appearance. For this investigation I used both appearance under daylight illumination and appearance under ultraviolet light (UV-A) illumination in situ as selection criteria. Observations of ultraviolet-induced fluorescence during night dives (Mazel 1993) revealed wide inter- and intraspecific variations in fluorescence color, pattern, and intensity, including numerous specimens with no apparent fluorescence. Some of the specimens that appeared to be strongly fluorescent in daylight exhibited only weak fluorescence under ultraviolet illumination, while others that fluoresced strongly under ultraviolet excitation did not appear to be fluorescent in daylight (even though underwater daylight includes some level of UV-A radiation). The study reported here quantitatively assesses the spectral emissions underlying these qualitative variations in appearance.

Optical remote sensing techniques may one day rapidly provide valuable data on coral reefs over large areas. In the laboratory, Hardy et al. (1992) evaluated the potential of using pulsed-laser-induced fluorescence as a means of remotely assessing bleaching in corals. If an optical sensing technique such as this were to be applied in the field, it would be important to know whether corals contain any non-zooxanthellate source of fluorescence at the wavelengths of chlorophyll emission and that the received signal originated from zooxanthellae and not from fleshy or encrusting algae. Measurements of fluorescence spectra from coral tissue provide the data needed to address these issues.

**MATERIALS AND METHODS**

**Samples.** Specimens were collected from patch reefs in a 2 to 10 m depth range in Biscayne National Park, Florida, USA. Samples were selected based on their fluorescent appearance under either daylight excitation or ultraviolet light (Model B-14N lamp, Spectronics Inc., with F4TSBLB bulb, UV-A emission 340 to 380 nm) excitation during night dives (Mazel 1993). Small pieces of coral were chipped from coral heads and placed in plastic bags. On the surface the specimens were transferred to a 20 l bucket of seawater. Spectral measurements were made as soon as the specimens were returned to the laboratory. Specimens were examined under ultraviolet light prior to measurement to ensure that the qualitative appearance had not changed noticeably. The interval between collection and measurement never exceeded 4 h.

Additional measurements were made from samples obtained from commercial suppliers and maintained in aquaria.

**Fluorescence measurements.** Fluorescence emission spectra were measured with a microspectrofluorometer built around a Zeiss Standard epifluorescence microscope (Mazel 1990, 1993). The excitation source was a xenon arc lamp. For most measurements the Zeiss 487702 filter set was used. This set includes a 365 nm glass bandpass filter, a 420 nm dichroic mirror, and a 418 nm cutoff barrier filter. For samples in which the fluorescence was not strongly excited by ultraviolet light, a 405 nm interference filter was substituted for the 365 nm filter. With both configurations, reliable

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus/species</th>
<th>(\lambda_{\text{max}}), nm</th>
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<td>Condylactis gigantea</td>
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Table 1. Fluorescence emission spectral peaks for samples of Caribbean cnidarians. Where a species name appears more than once, the measurements were made from separate specimens. *Specimens were acquired from commercial vendors and maintained in aquaria. All other samples collected from Biscayne National Park, Florida, USA, \(\lambda_{\text{max}}\), wavelength of the peak fluorescence emission, \(\lambda_2\) and \(\lambda_3\): secondary or tertiary peaks or shoulders. Primary and any secondary or tertiary emissions appeared to originate from a common source, except for the emission in the vicinity of 575 nm, which was associated with distinguishable features within the tissues. The data summarized here do not include the deep red (= 685 nm) zooxanthellar chlorophyll fluorescence that was present in all of the samples.
measurements of emitted light intensity could be collected over the wavelength range from 450 to 700 nm. Light exiting the microscope camera port passed through a scanning monochromator (100 nm min⁻¹) (International Light Model GM100-2) to a photomultiplier tube (International Light Model PM271D) with S20 spectral response. The photomultiplier output was processed by an International Light Model IL700 Research Radiometer and printed on a strip chart recorder (Linear Model 1200). The chart recordings were read at 3.3 nm increments and the values entered into a computer. A correction factor was applied to the raw data to remove the effects of the relative spectral sensitivity of the monochromator/photomultiplier combination and the absorption characteristics of the filters in the emission path. The resulting values were then normalized to set the peak emission to a value of 100 in order to facilitate comparisons between emission spectra from the various samples.

**RESULTS**

The most common instances of fluorescence appeared green to blue-green to the eye, with wavelengths of peak emission ranging from approximately 480 to 520 nm (Table 1). The spectra of many of the samples contained secondary peaks or shoulders. A number of specimens, including representatives of different genera and families, had virtually identical emission spectra. These could be roughly divided into groups (Fig. 1) based on the shapes of the normalized emission spectra. The specimens in Group I are characterized by an emission peak in the range of 483 to 487 nm, an apparent shoulder at 510 to 515 nm, and a spectral width of 55 to 60 nm (measured between the points on either side of the peak at which the intensity is half of the maximum). The specimens in Group II are characterized by an emission peak in the range of 497 to 505 nm, an apparent shoulder at 530 to 535 nm, and a spectral width of approximately 30 nm. The specimens in Group III are characterized by an emission peak in the range of 513 to 518 nm, an apparent shoulder at about 543 to 550 nm, and a spectral width of approximately 30 nm.

These groupings do not include all of the measured spectra. A form of Agaricia sp. appeared yellow under ultraviolet light and had an emission maximum at 557 nm. Some specimens of various species (see Table 1) contained an orange-fluorescent substance in the host tissues with an emission peak at 573 to 580 nm. Numerous colonies of Ricordea florida were observed in the field in which this substance was the predominant source of emission. In all of the specimens that contained this material, green-fluorescent substances were also found (Fig. 2). The green and orange emissions originated from separate sources in the tissues, as determined by microscopic examination (Mazel 1993).

In a few specimens of Ricordea florida that were predominantly green-fluorescent, the tissue immediately surrounding the mouth exhibited an orange-red fluorescence. The measured emission peak fell at 587 to 590 nm. This was the longest wavelength emission peak observed for substances in cnidarian host tissues.
Ricordea florida

Fig. 2. *Ricordea florida*. Fluorescence emission spectrum for a specimen of a corallimorpharian. The emission peaks at 510 and 574 nm originated from separate structures in the ectodermal tissues. The material producing the 574 nm peak has spectral properties similar to those of phycoerythrin, but does not appear to be associated with intact cyanobacteria.

Many specimens exhibited only a single fluorescence color, while others, such as the *Ricordea florida* referred to above, exhibited more than 1 response. Some specimens of *Montastrea annularis* had regions in the tissues that were distinctly blue-fluorescent and others that were distinctly green-fluorescent. These regions tended to merge smoothly from one to the other. The measured emission spectra (Fig 3) showed the shift in dominant emission peak from one wavelength range to the other, although neither disappeared entirely.

In some instances separate specimens from a given species or genus contained fluorescent materials with very different emission peaks. Members of the genus *Agaricia* were observed in situ with fluorescent colors including blue-green, green, and yellow. The emission spectra for these varieties (Fig. 4) corresponded to the field observations. Even when separate specimens of a given species appeared to the eye to have the same fluorescence color, there could be small but measurable variations in the emission spectra. Green-fluorescent specimens of *Ricordea florida* were found which had similar spectral shapes, but with emission peaks ranging from 510 to 520 nm.

Investigation under the microscope and measurement of emission wavelengths revealed that in some cases the fluorescent color observed in situ was an optical illusion. Some specimens of *Favia fragum, Montastrea annularis, Meandrina meandrites, Manicina areolata*, and *Scolymia* sp. contained regions that appeared to fluoresce weakly with an orange color under UV-A illumination. Under the microscope only green fluorescence from substances in the host tissues and deep red fluorescence from chlorophyll in the zooxanthellae were visible. This was verified by quantitative measurement (Fig. 5). Without the benefit of magnification these distinct sources merged to produce the impression of orange.

All of the specimens contained zooxanthellae that exhibited a red fluorescence from the chlorophyll with an emission peak at 675 to 685 nm. At no time did I observe a similar emission from substances elsewhere in the host tissues. Conversely, the blue-,
DISCUSSION

Many coral reef cnidarians contain substances in the host tissues that fluoresce when stimulated by ultraviolet and/or visible light. Prior investigators (Limbaugh & North 1956, Catala 1959, Buchsbaum 1968, Read et al. 1968, Jokiel & York 1982, Logan et al. 1990) made only qualitative observations of the color of the emitted light in vivo. Schlichter et al. (1986) reported the quantitative emission spectrum of a chloroform extract of coral tissue. Shibata (1969) measured fluorescence excitation spectra with a filter over the detector that passed all wavelengths longer than 600 nm and made no mention of the specific characteristics of the emission. Hardy et al. (1992) measured the in vivo fluorescence emission of a single coral specimen under ultraviolet (337 nm) excitation as part of a study primarily concerned with measurements of chlorophyll fluorescence. The data reported here comprise the first compilation of emission spectra recorded from fresh samples of cnidarian tissue. Quantitative measurements are of value as a means of more accurately characterizing the phenomenon and as an aid in identifying the fluorescing substances and their distribution. The spectral data is also of interest for application of remote sensing to coral reefs.

Catala (1959) suggested that members of a genus shared ultraviolet-induced fluorescence characteristics. Both qualitative in situ observations (Mazel 1993) and the spectral data reported here indicate that this generalization does not apply to the Caribbean cnidarians. While all specimens of some species appeared to have a similar fluorescent appearance, in many cases separate samples from a given species or genus possessed distinct fluorescence characteristics (e.g. the green- and orange-fluorescent varieties of Ricordea florida and the varied emissions from Agaricia spp.)

Spectral measurements provide a more precise means than qualitative color descriptions for describing fluorescence emission. Logan et al. (1990) labeled all of the instances of in vivo fluorescence they observed as ‘green’. The measurements reported here demonstrate that there can be small but measurable differences in emission spectra for specimens that appear to the eye to exhibit the same fluorescence color response. As in the case of the apparent orange fluorescence in Favia fragum and other species, it is possible for the eye to be deceived entirely. The addition of green and red fluorescence emissions from intermingled microscopic sources produced the impression of an intermediate color, orange, when viewed without the benefit of the microscope (Mazel 1993). It is only through microscopic investigation and quantitative measurement that the sources underlying the macroscopic effects can be reliably determined.

The spectral data provide a basis for comparison with fluorescence measurements in related investigations. The fluorescence emission spectrum for Solenastrea bournoni under 337 nm excitation (Hardy et al. 1992) exhibited a peak extending from about 350 to 550 nm, much broader than the peaks reported here. Their published data show a secondary peak at 465 nm that they attribute to uncorrected instrument response characteristics. The emission spectra measured from
chloroform extracts of the Red Sea deep-water coral *Leptoseris fragilis* (Schlichter et al. 1986) exhibited a peak at shorter wavelengths (420 to 455 nm) than were found in any of the Caribbean specimens. The emission spectra from cnidarian tissues do not match those reported by Boto & Isdale (1985) for either the coral skeleton background or the yellow-green fluorescent skeletal bands attributed to fulvic acid concentrations from terrestrial run-off. Nor do they match the emission spectra for fluorescent metabolites found in seawater in the vicinity of coral reefs (Gentien 1981). Shibata (1969) measured excitation spectra for fluorescence emission at wavelengths longer than 600 nm. If the specimens he examined contained fluorescent sources with the same general characteristics as those reported here, he could have measured emission either from chlorophyll in zooxanthellae or from the long-wavelength tail of the sources with emission peaks in the 575 to 590 nm range. The excitation spectra did not exhibit strong peaks in the ultraviolet, and there has been no explicit connection made between fluorescence emission at any wavelength and the ultraviolet-absorbing pigments investigated by Shibata or others (Jokiel & York 1982, Dunlap & Chalker 1986).

In situ observations revealed that some cnidarian specimens appeared to be fluorescent under both daylight and UV-A illumination, others appeared fluorescent under only 1 of these conditions, and still others did not appear to fluoresce in either case. Specimens representing each of these types were sampled for this investigation. Under the microscope, and with selection of the appropriate excitation filter, fluorescent substances could be found in the host tissues of each type. While the fluorescence might vary greatly in intensity, representatives of the different in situ 'types' shared identical spectral emission shapes. These results indicate that daylight- and UV-A-induced fluorescence phenomena are closely related. A sampling program that selects specimens either at random or with a criterion that excludes any of the types of responses may be inadequate for providing insight into the general phenomenon of fluorescence in cnidarians. Factors that can contribute to variation in response under different illumination conditions include the excitation spectrum (presence or absence of a peak in the ultraviolet; Mazel 1993), the fluorescence quantum yield, the total amount of the fluorescing substance present, and the absorption efficiency.

The measured spectra do not in themselves provide an indication of a possible functional role of the fluorescence. The comparisons of ultraviolet- and daylight-induced responses do, however, provide a basis for assessing the suggestion by Kawaguti (1969) that ultraviolet-excited green fluorescence might act to enhance photosynthesis by converting short wavelengths to longer wavelengths more readily absorbed by chlorophyll. The in situ observations revealed that it is the green emissions that are the most likely to exhibit the inverse relationship in the intensity of fluorescence under the 2 forms of illumination. Thus the corals that appear intensely green-fluorescent in daylight are not primarily converting ultraviolet to green wavelengths, but rather are converting blue to green. This would not seem to provide an advantage for photosynthesis.

A strong case for this function has been made (Schlichter et al. 1986, Fricke et al. 1987) for the fluorescent substances in the Red Sea deep-water coral *Leptoseris fragilis*. Those results cannot be generalized since the fluorescent substances in that species were not contained in the same ectodermal tissues noted by others (Kawaguti 1966, Logan et al. 1990, Mazel 1993) and there were additional structural adaptations (Schlichter et al. 1986). The emission spectra measured from chloroform extracts indicated fluorescence at shorter wavelengths (420 to 455 nm) than were found in any of the measurements reported here.

It is possible that even small variations in fluorescence response could serve as an indicator of state or process in an organism, thus providing a valuable tool for the coral reef biologist. Hardy et al. (1992) have studied the laser-induced fluorescence emission from chlorophyll in zooxanthellae, but there has been no comparable investigation of factors that might affect the fluorescence originating from substances in the coral tissues. Small changes in the local environment (e.g. pH) of fluorescent molecules can produce shifts in the position of emission peaks or in fluorescence intensity (Campbell & Dwek 1984). It is not known what factors play a role in the variations reported here. There is a need for additional qualitative observations and quantitative measurements coupled with controlled experiments to better understand the underlying causes of fluorescence variations.

Numerous specimens contained a substance in the host tissues with peak emission wavelengths in the range of 573 to 580 nm. The fluorescence emission and excitation spectra of this substance were essentially identical to those for phycoerythrin (Mazel 1993), a photosynthetic accessory pigment in cyanobacteria and red algae (Glazer et al. 1982). The emission did not appear to come from intact cyanobacteria in the cnidarian tissues. In *Ricordea florida* both the green- and orange-fluorescent materials were found in columnar epidermal cells (Mazel 1993) that fit the description of those previously identified with the green-fluorescent pigment by Kawaguti (1966) and Logan et al. (1990). Emission spectra measured from orange-fluorescent regions contained no indication of fluorescence at the wavelengths characteristic of
phycoerythrin or chlorophyll, which we would expect to observe if a complete photosynthetic mechanism were present.

The spectral data are of interest for studies related to coral reef optical remote sensing. The detection and measurement of chlorophyll in zooxanthellae by its fluorescence has been investigated as an indicator of productivity (Dustan 1985) and of bleaching (Hardy et al. 1992). The general utility of such approaches could be limited if there were additional sources of energy in the wavelength band of interest (e.g. Spitzer & Dirks 1985). The measurements reported here detected no sources other than zooxanthellae for emission in the wavelength range for chlorophyll detection (675 to 685 nm). The longest emission peak wavelengths (587 to 590 nm) found in any of the cnidarian tissues originated from substances in polyp tissue surrounding the mouth of several specimens of *Ricordea florida*. The 575 nm emission from the tissues of some corals is a potentially complicating factor for remote detection and measurement of phycocerythrin on the reef.

If techniques such as airborne oceanographic LIDAR (light detection and ranging) (Hoge & Swift 1981, Hardy et al. 1992) are to be applied to coral reefs, it may be necessary to distinguish the portion of the upwelling chlorophyll emission signal that originates from zooxanthellae from the portion that originates from macroalgae and encrusting algae. The widespread presence in corals of substances that fluoresce at wavelengths substantially shorter than those of chlorophyll emission may provide a tool for making such a distinction.

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