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REPORT

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Investigation of luminescent banding in solid coral: the contribution of phosphorescence

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Abstract This study investigates the nature and components of annual luminescent banding in massive *Porites* coral skeletons, with a view to refining the technique for using this banding to reconstruct past environmental conditions. Three-dimensional excitation-emission-matrix spectroscopy and optical fibre beam delivery have been used to investigate the luminescence properties of the bright and dull bands of solid coral. Six characteristic excitation/emission peaks have been identified: 280/450–600, 340/450, 370/470, 390/485, 420/505 and 450/530 nm. The first peak corresponds to protein-type fluorescence. The others are characteristic of humic acid luminescence. The difference in luminescence intensity between bright and dull bands has been quantified and characterised spectroscopically. The luminescence of the bright bands is up to 25% more intense than their neighbouring dull bands with the greatest increase in relative intensity in the long wavelength emission region, between 500 and 600 nm. The contribution of long-lived phosphorescence to the total luminescence intensity has been determined by time-resolved measurements on the 100 ms timescale. Both bright and dull bands show long-lived phosphorescence with decay times up to 1.5 s. This phosphorescence accounts for about 10% of the total luminescence intensity of bright bands. The difference in phosphorescence intensity between bright and dull bands is substantially greater than the difference in total luminescence intensity: the phosphorescence of bright bands is up to twice as intense as that of dull bands. This suggests that phosphorescence plays an important role in defining luminescent banding in coral. Furthermore, the large observable difference in phosphorescence between bright and dull bands indicates that measurement of phosphorescence profiles across growth bands in corals may prove to be a more sensitive indicator of past environmental conditions than measurements of total luminescence.

Key words Coral · Humic substances · Fluorescence spectroscopy · Phosphorescence

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Introduction

Massive reef-building corals record in the structure and composition of their aragonite skeletons information about the environmental conditions in which they grow. This, combined with the presence of annual bands to provide a chronology, and the longevity of individual colonies (up to several centuries) make corals excellent archives for environmental reconstruction. The objective of these studies is to provide a basis for the use of luminescence measurements to extend the instrumental record of environmental and climatic variability and hence gain a better understanding of the mechanisms and control of such changes.

Luminescent banding in the aragonitic skeletons of massive reef-building corals was discovered by Isdale (1984) and has been proposed as a valuable tool for retrospective environmental analysis. Under UV light, the coral skeleton displays a series of defined bands, which can be categorised as "bright" (yellow/green) and "dull" (blue). In Isdale's (1984) study, X-radiography used to reveal seasonal variations in skeletal density provided temporal analysis of a core of *Porites* coral from the Great Barrier Reef. Periods of heavy (monsoonal) rainfall led to increased runoff from the surrounding landmass, and Isdale noted that these coincided with the laying down of the bright bands within the coral skeleton. Hence, Isdale postulated that

the luminescent banding pattern could be utilised to reconstruct the record of coastal runoff over the lifetime of a coral colony, which can be up to 400 years. Extraction and separation techniques determined the banding as due to the inclusion of humic substances (Boto and Isdale 1985; Klein et al. 1990; Susic et al. 1991). These humic substances appeared to be mainly derived from the breakdown of terrestrial organic species which were subsequently washed from the land during periods of heavy rainfall. The correlation between rainfall, runoff and bright band deposition holds for many samples, but is not a complete description. For example, corals from Phuket, Thailand (Theodorou 1995), exhibit bright bands laid down during the dry season, and corals that are far removed from any terrestrial input, such as those found off the coast of Oman, may also exhibit the banding pattern (Tudhope et al. 1996). This points to some potential pitfalls in attempting to reconstruct past conditions from simple measurements of coral luminescence. Consequently, in order to realise the full potential of luminescent banding as an environmental proxy, there is a need for further analysis to elucidate the nature and components of coral luminescence and to identify the differences between bright and dull bands.

For the most part, analysis of humic substances has involved preliminary extraction from the host matrix by strong base, e.g. sodium hydroxide or pyrophosphate, to remove the alkali-soluble fraction. This can result in a number of molecular alterations, including base hydrolysis of esters and incorporation of oxygen into the humic structure. Although the effects of alkaline extraction can be minimised, it is potentially better to observe the luminophores in situ in the solid coral skeleton. Previous work has shown this to be technically challenging (Matthews et al. 1996), but here we report successful application of in-situ luminescence analysis.

Humic substances contain numerous individual luminescing species that give rise to a complex signal. For a multi-component system, the most effective way to characterise the luminescence is as a function of both the excitation and emission wavelength. This gives rise to the excitation-emission matrix (EEM) (Coble et al. 1990; Matthews et al. 1996). The EEM gives a three-dimensional isometric or contour plot, with excitation wavelength, emission wavelength and luminescence intensity as the three variables. Analysis of extracted solutions from bright and dull bands showed that the structure of the EEMs, i.e. the peak positions and relative intensities are essentially identical. This suggests that there is not an intrinsic difference in the nature of the fluorescent material in the bright and dull bands and that other factors are responsible for the banding effect. Therefore, we have extended these studies by recording the luminescence variations directly from the surface of the solid coral skeleton, and have obtained the first reproducible EEM of the system. Applying this technique to samples from different geographical locations allows further insight into the banding pattern.

We also report here on the use of a fibre-optic probe to scan across the surface of the coral and record variations in luminescence. Remote analysis using a fibre-optic probe has been successful in recording excitation and emission spectra of bright and dull bands (Boto and Isdale 1985; Miano et al. 1988). This technique has previously been utilised to scan across the coral surface at a fixed excitation and emission wavelength (Milne and Swart 1994). We use varying excitation and emission wavelengths to maximise characterisation of the complex signal.

Photophysics of luminescent banding

Luminescence is the radiative emission associated with the de-excitation of an electronically excited state. There are two distinct types of luminescent emission: fluorescence and phosphorescence. The processes that give rise to these emissions can be summarised in a Jablonskii diagram, as shown in Fig. 1.

In the condensed phase, molecular fluorescence is the radiative emission due to the transition from the lowest vibrational level of the first excited singlet electronic state, S_1, to the ground electronic state, S_0, incurring no change in spin multiplicity. Phosphorescence is the radiative transition from the first excited triplet electronic state, T_1, to the ground electronic state, S_0, and involves a change in spin multiplicity. The T_1 state is the lowest excited electronic state and phosphorescence thus occurs at lower energy (longer wavelength) than fluorescence. Since the transition between T_1 and S_0 is spin-forbidden, the phosphorescence lifetime is much longer than the fluorescence lifetime; typically, fluorescence decays on the timescale 10^{-7} to 10^{-12} s, whereas phosphorescence decay occurs on the timescale 10^{-6} to 10 s. For a

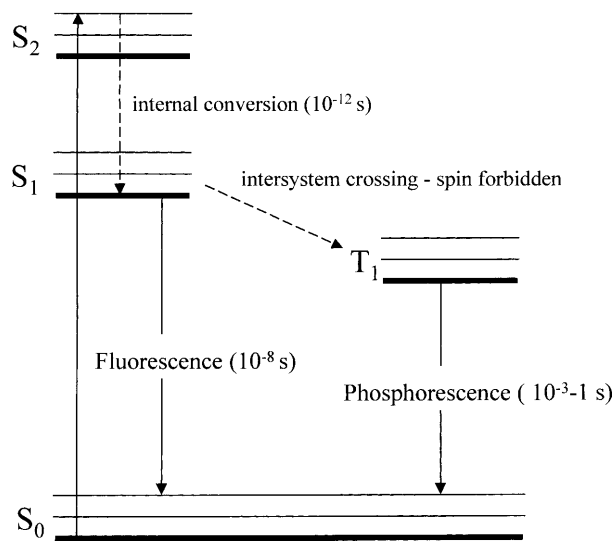

Fig. 1 A Jablonskii diagram showing the transitions giving rise to fluorescence and phosphorescence. The *solid arrows* show radiative (absorption and emission) processes. The *dashed arrows* show non-radiative processes

multi-component system, such as humic substances, the fluorescent and phosphorescent emissions overlap in terms of wavelength, and can only be distinguished by the timescales over which the emissions occur. We utilise this property to characterise the type and intensity of luminescence giving rise to the banding pattern in coral skeletons. The phosphorescence of fossil corals has been documented previously as a visible glow once ultraviolet illumination had been removed (Klein et al. 1990), but the study did not extend to modern corals. We have studied the wavelength-dependent phosphorescence response of modern corals and have found it to be an important indicator of the banding pattern. In solution, the phosphorescence is quenched by collisional deactivation of the triplet state by solvent molecules and oxygen. These processes occur on shorter timescales than the phosphorescence emission. Hence, coral extract solutions exhibit no phosphorescence.

Methods

Work was carried out on coral cores from Laing Island and Madang in Papua New Guinea (PNG) and coral cores from Wadi Ayn on the Arabian Sea coast of Oman. Laing Island Reef is situated on the Bismark Sea coast of Papua New Guinea (4°9′S; 144°53′E; Tudhope et al. 1995). The coastal rainfall averages about 3000 mm/year with a dry season from June to September. The Laing Island Reef lies close to the mouths of two large rivers, the Sepik River (50 km distant) and the Ramu River (25 km distant). Using staining techniques (Tudhope et al. 1995), it has been ascertained that the bright bands are deposited during the wet season and the dull bands during the dry season. Cores taken from Madang (5°13′S; 145°49′E) show a correlation between periods of increased rainfall and bands of bright luminescence. For each year of growth, there are two bright bands and two dull bands laid down in the skeleton (Tudhope 1995). Both cores taken from Papua New Guinea can be considered to be seasonally influenced by coastal runoff. Cores taken from the Wadi Ayn region of Oman (17°N; 55°E) are not subject to subannual inundation from coastal runoff and hence can be considered to be free from terrestrial input. The samples analysed were not bleached as this has been found to reduce fluorescence intensity (Matthews et al. 1996). The cores were cut into slices along the axis of maximum growth, yielding pieces around 10 mm thick and 20 × 50 mm in area.

Recording EEMs

The luminescence measurements were carried out using a Spex Fluoromax fluorimeter and the data were recorded and processed using associated Datamax software. The excitation source was a 150 W continuous ozone-free xenon lamp. Emission was monitored by a photomultiplier tube (PMT), sensitive between 200 and 680 nm. A solid sample holder, available commercially, held the coral pieces in the sample compartment. Data acquisition was performed using 1.5-nm slit widths for both the excitation and emission monochromators and a 1-s integration time. The wavelength increment in spectral scans was set at 1 nm. The sample was fixed at 45° relative to both the incident radiation and the detection system for the duration of the investigation. The excitation beam was focused on to the coral surface, giving an illuminated area of 1 × 5 mm. Since individual bright and dull luminescent bands are each a few millimetres in width (the coral extends 10–20 mm annually) the excitation beam could be located within a bright or dull band on the coral surface. Consequent excitation spectra were collected by scanning the excitation wavelength between 250 and 580 nm, with a fixed emission wavelength at 20 nm greater than the maximum excitation wavelength, to avoid first-order Rayleigh-Tyndall (RT) scattering. Second order RT peaks were deleted from each individual scan after collection. All excitation spectra were corrected for the wavelength dependence of the lamp intensity. Excitation spectra were collected at 5-nm emission wavelength increments, giving a total of 63 spectra per sample. The recorded emission was corrected for the wavelength- dependent response of the PMT. The spectra were then collated into a matrix to produce an EEM for each sample.

Remote scanning using a fibre-optic probe

These measurements were carried out using optical fibre bundles interfaced to the Spex Fluoromax fluorimeter. The coral sample was mounted outside the fluorimeter on a precision linear translation stage that was driven incrementally by a stepper motor. The excitation beam was delivered from the excitation monochromator to the sample in a fused silica optical fibre bundle (LOT – Oriel standard grade; transmittance from 250–1100 nm, bundle diameter 3.2 mm) terminating approximately 2 mm above the surface of the coral. Luminescent emission was collected using a glass optical fibre bundle (LOT – Oriel; 400-1300 nm transmittance, bundle diameter 3.2 mm) and delivered to the emission monochromator. The fibres were positioned to maximise the emission intensity from the coral surface, and then clamped in place for the duration of the experiment. The tips of the excitation and emission bundles were positioned at 90° to each other and at 45° to the vertical. The sample was passed beneath the fibre bundles at a fixed velocity of 6.0×10^{-5} ms^{-1}, allowing a scan of the variation in luminescence intensity as a function of lateral position. By positioning the sample to correspond to a bright band intensity maximum or dull band intensity minimum, the EEM of the band could be collected. Excitation spectra were recorded in the range 280–580 nm, with the maximum excitation wavelength for a particular spectrum set at 20 nm less than the emission wavelength. The minimum emission wavelength used was 420 nm, dictated by the transmittance properties of the glass optical fibre bundle. Both the excitation and emission slit widths were set at 4 nm. The integration time was 0.4 s and the wavelength increment was 1 nm.

Measuring phosphorescence decay

All measurements were carried out using a Spex Fluoromax fluorimeter with DM3000 software. The coral sample was fixed in the solid sample holder and the excitation source was focused on to a particular band as described in the procedure for the recording of EEMs. After approximately 2 s, the excitation source was cut off using a manually controlled shutter with a shutter speed in the region of 100 ms. The decaying emission signal was recorded as a function of time over a time interval of 10 s at 0.01-s increments. The total luminescence of the coral is made up of short-lived fluorescence and long-lived phosphorescence. By closing the shutter in the excitation beam, the short-lived fluorescence signal is cut off but the long-lived phosphorescence persists and the decay of its intensity with time can be measured. This phosphorescence decay can be fitted to an exponential function, which gives information on the lifetimes of the decaying species. By extrapolation, we obtained an estimate of the percentage contribution of phosphorescence to the total luminescence of the system. Since the shutter has a closing speed of 100 ms, only long-lived components with phosphorescence persisting for times greater than 100 ms could be detected. This method does not take into account short-lived phosphorescing species and is thus an underestimation of the total phosphorescence of the system.

Results and discussion

The EEM for a bright band of Laing coral is shown in Fig. 2. The x-axis shows the emission wavelength, the

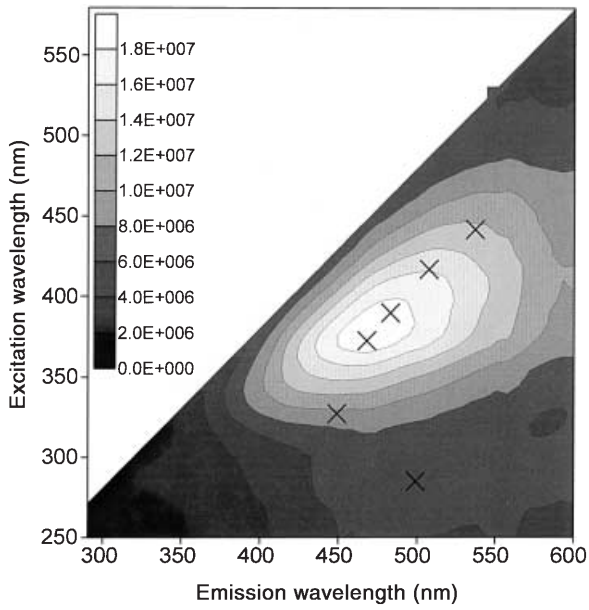

Fig. 2 An excitation-emission-matrix (EEM) of a bright band of solid coral. The sample originates from Laing Island, Papua New Guinea. The contours are plotted between zero and 1.8×10^7 cps, at intervals of 2×10^6 cps. The *crosses* mark the peak positions estimated by Gaussian curve fitting

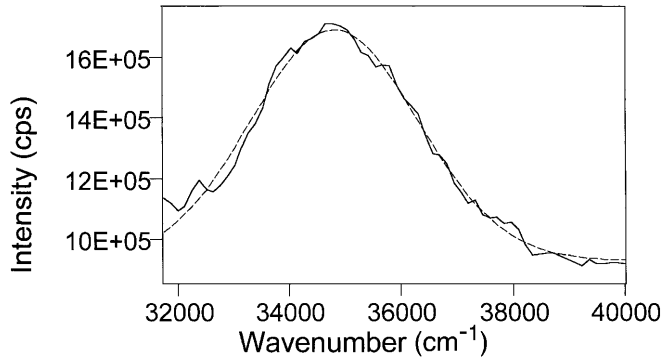

Fig. 3 Excitation spectrum of coral from Laing Island, Papua New Guinea. The spectrum was recorded between 250 and 315 nm excitation and at a fixed emission wavelength of 335 nm. The excitation has been fitted to a Gaussian curve (*dotted*) with an excitation wavelength maximum of 280 nm

y-axis shows the excitation wavelength, and the contours link points of equal intensity. Sections parallel to the *y*-axis represent individual excitation spectra. The diagonal line running from bottom left to top right shows the cut-off where first order Rayleigh-Tyndall scattering would start to interfere with luminescence detection. For a single luminescing species, the wavelength of maximum emission intensity, λ_{em}^{max}, is independent of excitation wavelength, and the wavelength of maximum excitation intensity, λ_{ex}^{max}, is independent of emission wavelength. Thus, the EEM would consist of a rectangular array of peaks running in lines parallel to the *x*-and *y*-axes. In the EEM of coral in Fig. 2, the dependence of λ_{em}^{max} on excitation wavelength and λ_{ex}^{max} on emission wavelength indicates the contribution of many individual emitting species to the total observable signal. The EEM is dominated by broad, asymmetric features, with a peak at $\lambda_{ex}^{max}/\lambda_{em}^{max}$ 370/470 nm, with ridges extended at various angles to the excitation and emission axes, showing that this feature is the composite of a number of underlying peaks. It is apparent in the EEM that quite well-defined peaks are present in the excitation dimension. In comparison, the emission is very broad, extending over a wide spectral range. This anisotropy is the consequence of electronic energy transfer, together with the contribution of phosphorescence to the total luminescence intensity. As a result of energy transfer, excitation of a particular chromophore can result in luminescence from many other luminophores of lower excitation energy. The occurrence of phosphorescence as well as fluorescence further broadens the range of wavelengths over which emission occurs.

The positions of the component excitation peaks were estimated by fitting the individual excitation spectra to the sum of several Gaussian peaks, using the GRAMS/386 peak fitting program (CurveFit) which is incorporated in the Datamax software package. This program employs an iterative non-linear least-squares curve fitting procedure based on the Marquadt algorithm. The spectra were fitted in the wavenumber (cm^{-1}) domain and the component excitation peaks were constrained to have a full width at half-maximum (FWHM) in the range 2000–4000 cm^{-1}, typical of a single absorbing chromophore. Although spectra are measured in the wavelength domain for practical reasons (the use of diffraction gratings in spectrometers), the true spectrum is a measure of intensity as a function of photon energy and is, therefore, correctly represented in the frequency or wavenumber (reciprocal wavelength) domain. A Gaussian function in the *wavenumber* domain, with a FWHM in the range 2000–4000 cm^{-1}, is a reasonable approximation to the spectral profile of a typical aromatic chromophore at room temperature. Thus, when analysing the contribution of different component spectra to the observed composite spectrum it is correct to work in the wavenumber domain.

At short emission wavelengths (320–350 nm), the excitation spectra can be fitted by a single peak with λ_{ex}^{max} of 280 nm, as illustrated in Fig. 3. At longer emission wavelengths, up to five component peaks are required to fit the excitation spectra. For example, the excitation spectrum at an emission wavelength of 450 nm can be fitted well by the sum of four component peaks, as shown in Fig. 4. The shortest wavelength (highest wavenumber) peak in this excitation spectrum corresponds to the 280 nm peak of Fig. 3. The broad peak at lower wavenumbers can be fitted by three Gaussian components with λ_{ex}^{max} of 340 nm (29,500 cm^{-1}), 370 nm (27,000 cm^{-1}) an 395 nm (25,500 cm^{-1}).

Using this procedure, it was found that the excitation spectra over the range of emission wavelengths from 330 to 570 nm could be fitted by combinations of six different component peaks. The λ_{ex}^{max} values and

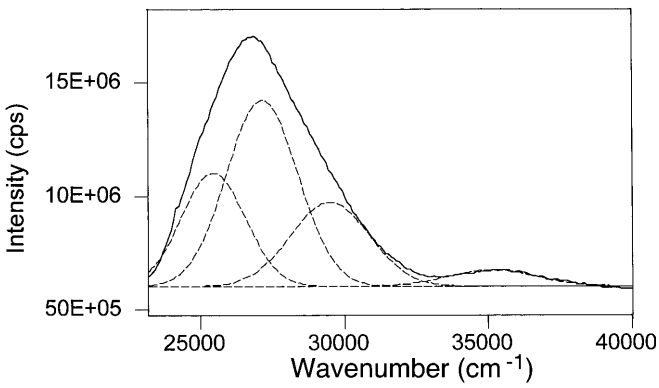

Fig. 4 Excitation spectrum of coral from Laing Island, Papua New Guinea. The spectrum was recorded between 250 and 430 nm excitation and at a fixed emission wavelength of 450 nm. The excitation has been fitted to the sum of four Gaussian peaks (*dotted*)

Table 1 Excitation/emission peak positions estimated by Gaussian curve fitting for bright band regions of samples from various locations

Location	Excitation/emission peaks, $\lambda_{ex}^{max}/\lambda_{em}^{max}$/nm					
Laing	285/450–600	340/450	370/470	395/485	420/505	450/530
Madang	280/450–600	335/430	370/470	400/490	425/510	455/535
Oman	280/470–600	340/470	365/475	395/500	420/515	450/540

corresponding λ_{em}^{max} values are listed in Table 1 and the peak positions are indicated on the EEM in Fig. 2. This suggests that a minimum of six different chromophores (or families of closely related chromophores) is predominant in the excitation of luminescence over this spectral range. Although only six excitation peaks are identified here, it is likely that there are substantially more species contributing to the luminescence, with closely overlapping spectra. In this multichromophoric system, much of the emission may come from species that are excited indirectly via energy transfer, rather than by direct absorption of the excitation light. Thus the chromophores that are dominant in the excitation process may differ from and be fewer than those that are dominant in emission.

The positions of the excitation/emission peaks may be compared with those identified previously by Matthews (1996), in Thai corals. The first peak identified has an excitation maximum at 280 nm and corresponds to the amino acid tryptophan. The characteristic tryptophan excitation/emission peak occurs at 280/350 nm. Our results show a broad emission from 450 nm to 600 nm. Due to energy transfer, an absorption at 280 nm may lead to an emission from many different luminophores that have lower excitation energies than tryptophan. The tryptophan peak has been identified in coral extracts, but preliminary studies by Matthews did not locate it in the solid coral EEM. The peak at 340/450 nm corresponds to a peak identified in the solid coral by Matthews at 350/440 nm. The peak identified at 370/470 nm has not been isolated before, and may have been obscured by the 395/485 nm peak identified in Matthews' work. Two further peaks are identified at 420/505 and 450/530 nm.

The immediately adjacent dull band showed similar peak positions, although the intensity of emission was reduced across the wavelength range. However, bright bands are not always more intensely luminescent than dull bands. A bright band from one sample may in fact be less intense than a dull band from another. This is not surprising since throughout the core, variations in architecture, extent of seasonal impacts and geometry of luminescence collection may all cause fluctuations in the measured intensity. It is important to consider bands that are spatially and temporally close when making a direct comparison of intensity. Comparative, quantitative measurements between bright and dull bands were carried out using a fibre-optic probe and are presented below.

Component peak positions for samples from all three geographical regions studied are shown in Table 1. All show similar peak positions, which implies a broad similarity in the constituent luminophores. It is notable that samples from different locations show a greater variation in emission peaks than in excitation peaks. This suggests that similar chromophores are dominant in the absorption processes in each sample, but there are variations in the luminophores contributing to emission. This behaviour again demonstrates the important role of energy transfer in the excitation process in these systems, and may also be symptomatic of differences in the phosphorescence properties of different samples.

The Oman samples are not subject to inundation from terrestrial sources, but nonetheless show similar luminescence characteristics to corals from PNG. Tudhope et al. (1996) suggested that the banding pattern in corals from Oman may relate to seasonality in marine productivity, with the bright band being deposited shortly after the peak of productivity caused by SW monsoon-induced coastal upwelling. If correct, the implication is that general luminescence measurement of corals may not be able to differentiate between marine and terrestrially derived humic luminophores. This ability is fundamental for accurate and reliable environmental reconstruction and emphasises the requirement for more sophisticated characterisation of the components of luminescence.

Remote measurements using a fibre-optic probe

The variations in luminescence intensity with position on the coral surface, exciting at 350 nm and detecting at 550 nm, are shown in Fig. 5. At this combination of wavelengths, there is a significant difference between the intensity of the bright band and the intensity of the neighbouring dull band. At 300/500 nm, the ratio of bright:dull intensities (BB1:DB2) is 1.05. At 350/500 nm, it was 1.10 and at 350/550 this ratio increased to 1.15.

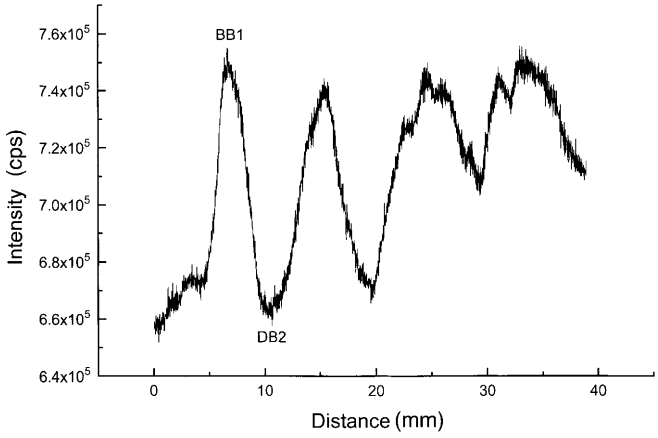

Fig. 5 The intensity variation across the surface of solid coral from Laing Island, Papua New Guinea (PNG), recorded perpendicular to the direction of the banding pattern at excitation/emission wavelengths of 350/550 nm

Hence at this position the bright band is 15% more intense than the neighbouring dull band.

There are four bright bands in this region of the coral skeleton, bordered on each side by dull bands. To the eye, the first bright band appears approximately 3 mm in width. The full width half maximum (FWHM) of the first peak in Fig. 5 is 2.7 mm. The second bright band appears to be wider, and the corresponding peak shows an increased FWHM of 3.6 mm. The last two bands are visibly wider and more diffuse. In Fig. 5, these are seen as broad peaks, with a reduced intensity difference between the neighbouring bright and dull bands. Previously, luminescence intensities obtained by scanning across the surface of a coral specimen have been cubed to reduce the influence of 'background' luminescence (Isdale 1984). However, in our measurements, the difference between bright and dull bands is directly evident without the need for manipulation of the data. The 'background' luminescence (or luminescence of the dull regions of the skeleton) is not constant and shows a gradual increase as the scan progresses from left to right. This illustrates why it is important to compare neighbouring bright and dull bands when considering their relative intensities.

The EEM recorded at the maximum intensity of a bright band (Fig. 6) shows similar features to the EEM recorded within the sample chamber (Fig. 2). There is a central peak at 370/470 nm, and several ridges or 'shoulders', suggesting that the luminescence comprises several individual emitting species. In comparison with the EEM recorded within the sample chamber (Fig. 2), the overall intensity of the recorded peak luminescence is reduced by a factor of ten, from 1.8×10^7 to 1.8×10^6. This would be expected since the efficiency of the excitation and detection systems is reduced by the use of the fibre-optic assembly. The EEM recorded at the minimum intensity corresponding to dull band two (DB2) shows a reduction in absolute intensity across the wavelength range (Fig. 7).

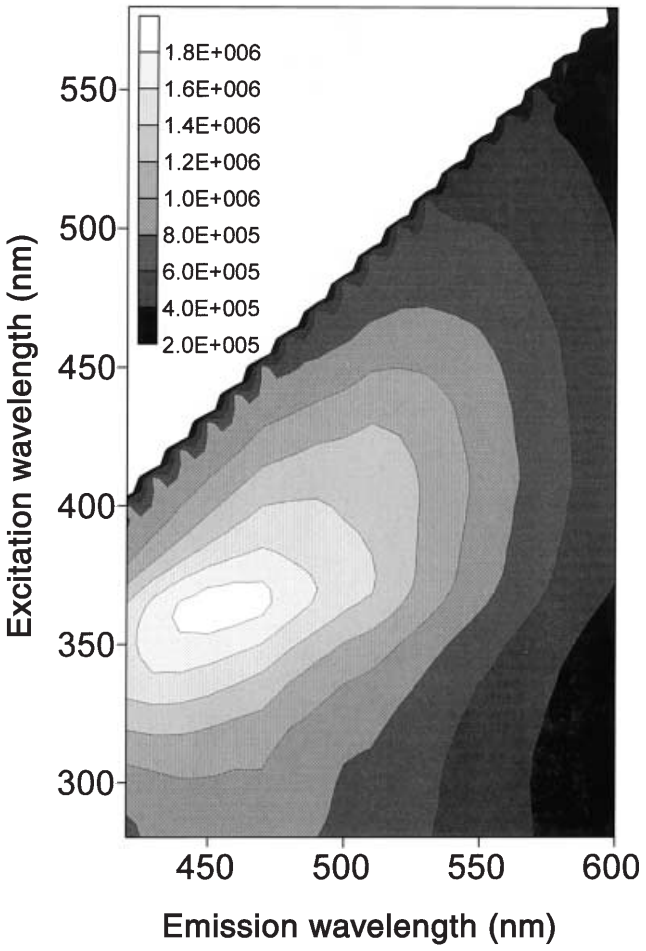

Fig. 6 Excitation-emission matrix of a bright band of solid coral from Laing Island, PNG, recorded using optical fibre bundles to deliver excitation and emission radiation. The contours are plotted between 2×10^5 and 1.8×10^6 cps, at intervals of 2×10^5 cps

By dividing the bright band EEM (Fig. 6) by the dull band EEM (Fig. 7), the relative intensity variation with wavelength can be seen (Fig. 8). A figure of one denotes an equal intensity in both bands, with regions of intensity greater than one having an increased intensity in the bright band. Clearly, the relative intensity of the bright and dull bands is not uniform across the studied wavelength region. There is an increase in the relative emission intensity of the bright band as the emission wavelength increases, with a peak difference at 450–500/550–600 nm. The relative intensity distribution shows that the presence of emitting species at longer wavelengths (lower energy) plays a significant role in characterising the banding pattern. This corresponds to green/yellow in the visible spectrum, and is seen as the characteristic colour of bright bands when illuminated under uv light. The eye is exceptionally sensitive to light in the region 500–650 nm (Bergmans 1960), and so any variation in the intensity of emission at these wavelengths will show an enhanced visual effect. At 350/550 nm, the bright band is between 15 and 20% more intense than the adjacent dull band. This compares

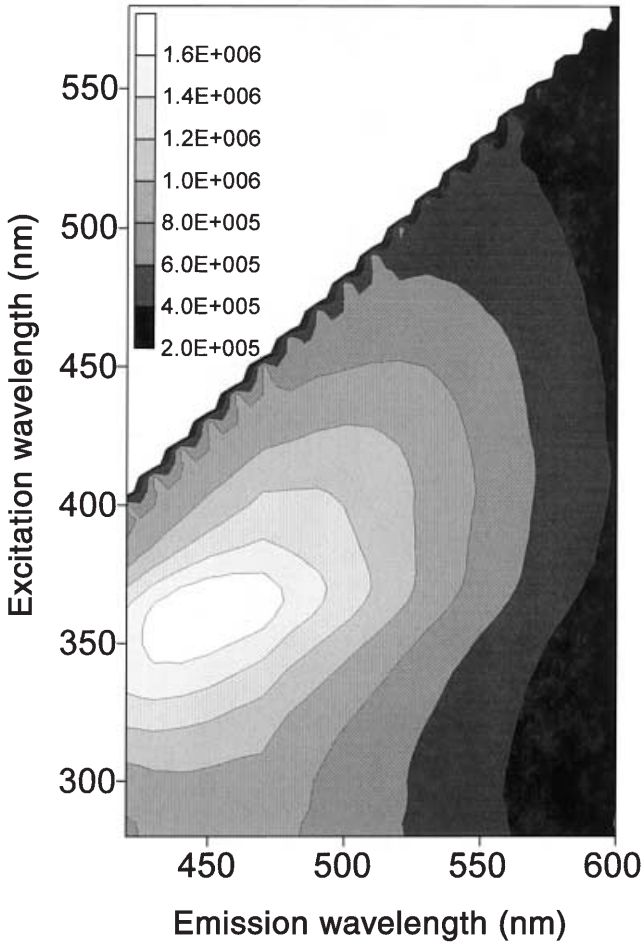

Fig. 7 Excitation-emission matrix of a dull band of solid coral recorded using optical fibre bundles for excitation and emission radiation. The contours are plotted between 2×10^5 and 1.6×10^6 cps, at intervals of 2×10^5 cps

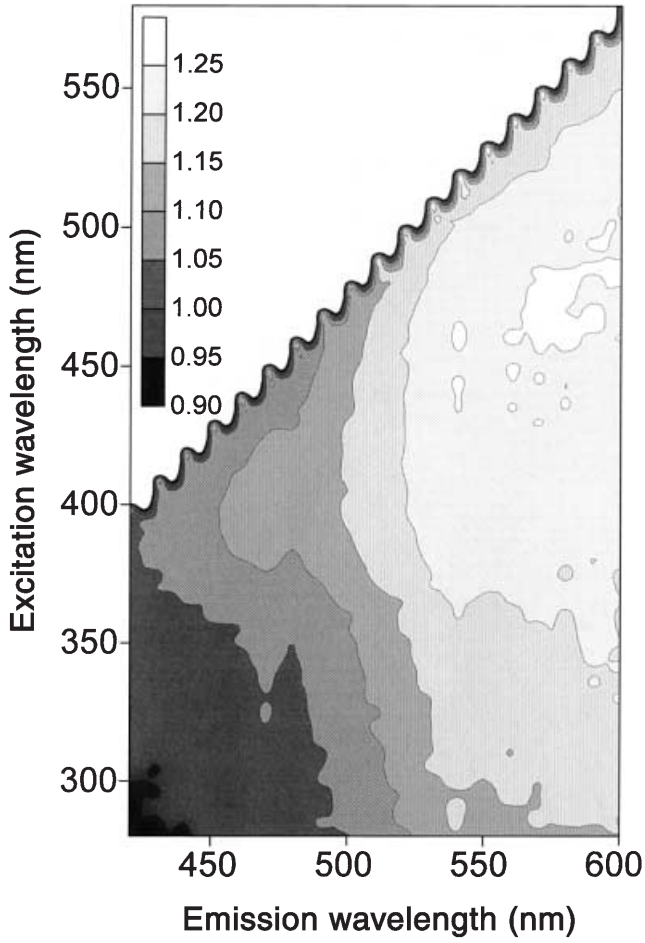

Fig. 8 Excitation-emission matrix obtained by dividing Fig. 6 by Fig. 7. This shows the relative intensity of the bright band to the dull band across the wavelength range. The contours are plotted between 0.90 and 1.25, at intervals of 0.05

favourably with the 15% difference observed when scanning across the surface of the coral sample.

There are several factors that may give rise to this enhanced emission at longer wavelengths in bright bands. There may be an increased concentration of lower-energy emitting luminophores, or an increased fluorescence or phosphorescence quantum yield for a particular luminophore that occurs in both bands. The environmental factors that affect the laying down of bright and dull bands may also cause variations in the skeletal structure of the coral. Fluorescence, and particularly phosphorescence, will be affected by the architecture of the skeleton and the relative pore size of the bands. There is some evidence to suggest that in corals on the outer Great Barrier Reef (GBR), luminescent bands coincide with regions of low density skeleton (Wild, unpublished). Scoffin et al. (1989) found that bright luminescent bands coincided with regions of low density skeleton in corals from Papua New Guinea and Indonesia. Klein (1990) found a similar relationship for corals from the Red Sea. However, Isdale (1984) found that in inshore reefs of the Great Barrier Reef, Australia, bright bands coincided with regions of high density skeleton. At this stage, investigations into the relationship between luminescence and density are inconclusive.

Phosphorescence decay

As the excitation shutter closes, fluorescing species cease to be detected at the PMT since fluorescence has a limited lifetime of up to 10^{-9} s. However, the lifetime of phosphorophores can be up to the order of seconds. Therefore, any phosphorescing species continue to emit light which can be detected. A decay signal is recorded that lasts for several seconds. This decay can be seen visibly as a greenish glow. A typical plot of luminescence intensity against time is shown in Fig. 9.

The first portion of the plot shows the initial total luminescence (fluorescence and phosphorescence) at 500 nm when excited at 300 nm. This combination of wavelengths was chosen as it corresponds to the greatest difference between bright and dull band phosphorescence contribution. Just before 3 s into the plot, the

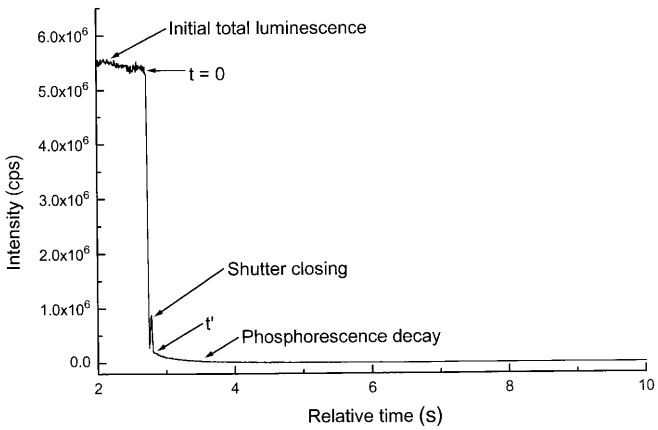

Fig. 9 Intensity variation with time during an experiment to record a phosphorescence decay. t = 0 is the time that the closing of the shutter was initiated; t′ is the time at which the shutter is closed

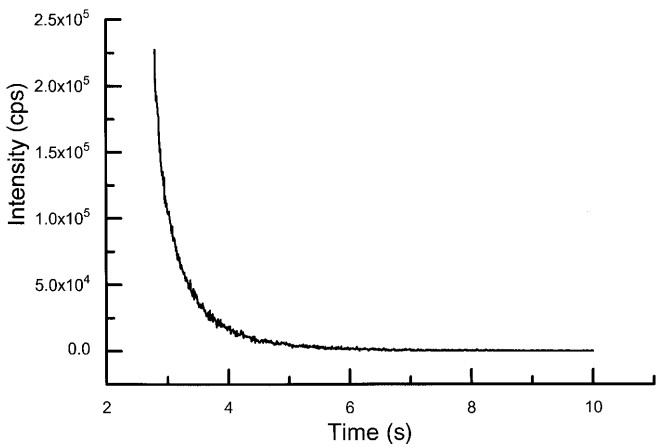

Fig. 10 Phosphorescence decay of a bright band of solid coral

shutter is closed and the intensity of the signal falls dramatically. There is a slight increase in intensity as the shutter 'bounces'; this can be seen as a small, sharp peak located at the base of the large drop in intensity. From this time onwards, all fluorescent emission ceases and the signal is due solely to the emission from long-lived phosphorophores. This emission lasts for several seconds, and is highlighted in Fig. 10.

By fitting this phosphorescent emission to an exponential decay function, we can gain an insight into the lifetimes of the phosphorophores giving rise to the signal. The phosphorescence decay was fitted from time t′, 100 ms after the shutter started to close. A triple exponential function of the form of Eq. (1) gave a significantly better fit to the data than a monoexponential or biexponential function.

$$I(t) = A_1 e^{(-(t-t')/\tau_1)} + A_2 e^{(-(t-t')/\tau_2)} + A_3 e^{(-(t-t')/\tau_3)} \quad , \tag{1}$$

where I(t) is the intensity of phosphorescence as a function of time after the shutter closes, t = 0 is the time at which the closing of the shutter was initiated, t′ is the time at which the shutter closed (t′ = 100 ms), and τ_n is the lifetime of the nth phosphorophore.

Typically, the values of the three fitted lifetime components were approximately 0.1, 0.5 and 1.5 s. We do not attribute any physical significance to the number of fitted lifetime components or the specific lifetime values, other than giving an indication of multiple phosphorescing species with a wide range of lifetimes.

To estimate the phosphorescence contribution to the total luminescence, the intensity of the decay function was extrapolated back to t = 0, the time at which the closing of the shutter was initiated. Coral samples from Laing Island show a marked difference in phosphorescence contribution from the bright and dull bands. Phosphorescence accounts for up to 8% of the total luminescence of the bright bands. Dull bands show a phosphorescence contribution of up to 3.5%. It is important to consider adjacent bands since there can be variation in percentage contribution between bright bands from different regions of the skeleton (minimum contribution for a bright band is 4% with the adjacent dull band only 2%). In all cases, the phosphorescence contribution to the total luminescence of the bright band is approximately twice that of the dull band. Samples from Madang, PNG show an increase in the total phosphorescence contribution to approximately 9% for the bright band and 7% for the dull band. However, the difference between dull and bright bands is not as marked. Samples from Oman show a phosphorescence contribution of approximately 15% for the bright bands and 12% for the dull bands. Again, the difference between the bright and dull bands is reduced. This is consistent with the observation that the banding pattern is less visible to the eye in the Oman samples.

It is likely that the present measurements underestimate the contribution of phosphorescence to the total luminescence of the system, since any phosphorophores with a lifetime of less than 100 ms are neglected as a result of the time taken for the shutter to close. The relative difference in phosphorescence contribution between bright and dull bands is significantly greater than the relative difference in total luminescence (phosphorescence + fluorescence) intensity. In Laing Island samples, bright bands show twice the phosphorescence contribution to the total luminescence intensity of dull bands. However, the bright bands show a maximum 15–20% increase in luminescence intensity compared with dull bands. Consequently, phosphorescence is an important indicator of the banding pattern. Since these studies underestimate the contribution of phosphorescence to the total luminescence of the system, further work taking into account phosphorescence lifetimes of less than 100 ms may also show phosphorescence to be an important contributor to the overall luminescence of the system.

In conclusion, this work has identified the contribution of phosphorescence to coral luminescence and suggests that in situ measurement and characterisation

of coral skeletal phosphorescence may be a more sensitive environmental proxy than total luminescence (i.e. what has been measured previously and generally called, incorrectly, 'fluorescence'). Our results demonstrate the role of phosphorescence in the dominant seasonal banding pattern; further work is in progress to quantify interannual variations in phosphorescence and their relationship to instrumentally recorded environmental parameters, such as local and regional rainfall.

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References

Boto K, Isdale P (1985) Fluorescent bands in massive corals result from terrestrial fulvic acid inputs to nearshore zone. Nature 315: 396–398

Bergmans J (1960) Seeing colours. Philips Technical Library, Eindhoven

Coble PG, Green SA, Blough NV, Gagosian R, (1990) Characterization of dissolved organic-matter in the Black-Sea by fluorescence spectroscopy. Nature 348: 432–435

Isdale P (1984) Fluorescent bands in massive corals record centuries of coastal rainfall. Nature 310: 578–579

Klein R, Loya Y, Gvirtzman G, Isdale PJ, Susic M (1990) Seasonal rainfall in the Sinai desert during the late quaternary inferred from fluorescent bands in fossil corals. Nature 345: 145–147

Matthews BJH, Jones AC, Theodorou NK, Tudhope, AW (1996) Excitation-emission-matrix fluorescence spectroscopy applied to humic acid bands in coral reefs. Mar Chem 55: 317–332

Miano TM, Sposito G, Martin JP (1988) Fluorescence spectroscopy of humic substances. Soil Sci Soc Am 52: 1016–1019

Milne PJ, Swart PK (1994) Fiber-optic-based sensing of banded luminescence in corals. Appl Spectrosc 48: 1282–1284

Scoffin TP, Tudhope AW, Brown BE (1989) Fluorescent and skeletal density banding in *Porites lutea* from Papua New Guinea and Indonesia. Coral Reefs 7: 169–178

Susic M, Boto K, Isdale P (1991) Fluorescent humic-acid bands in coral skeletons originate from terrestrial runoff. Mar Chem 33: 91–104

Theodorou NK (1995) The enigmatic properties of fluorescent banding in massive corals of the species *Porites lutea* from Phuket, Thailand. PhD Thesis, The University of Edinburgh

Tudhope AW, Lea DW, Shimmield GB, Chilcott CP, Head S (1996) Monsoon climate and Arabian Sea coastal upwelling recorded in massive corals from southern Oman. Palaios 11: 347–361

Tudhope AW, Shimmield GB, Chilcott CP, Jebb M, Fallick AE, Dalgleish AN (1995) Recent changes in climate in the far western equatorial Pacific and their relationship to the Southern Oscillation; oxygen isotope records from massive corals, Papua New Guinea. Earth Planet Sci Lett 136: 575–590