ON THE NATURE OF LUMINESCENCE IN CORAL SKELETONS

D.J. Barnes & R.B. Taylor
Australian Institute of Marine Science

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Foreword

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Coral researchers have long known that there is a wealth of information on past climatic conditions recorded within the skeletons of massive reef corals. Skeletal density, chemical characteristics, organic inclusions, isotopic composition and optical properties have all been identified as potential recorders of past events and conditions. The process of discovering the "language" in which the history of these coral colonies is recorded has been much more complicated that many scientists anticipated. From a management point of view, however the rewards are substantial. Long term records of climate variations and possible anthropogenic perturbations provide an essential background against which present day measurements and short-term trends can be compared. Such comparisons enable us to determine if an event is unprecedented in its severity or frequency when compared to a historical record which predates European influence on the system. In the absence of such records, managers must make informed guesses and run the risk of under or over estimated the significant of a perturbation event.

Fluorescent bands in near-shore massive corals are know to be well correlated with river run-off events, and until recently, the cause of these bands were thought to be directly attributable to the incorporation of humic acids associated with flood waters. In this carefully conducted series of experiments, Drs Barnes and Taylor demonstrate that this explanation is not correct, and that luminescence in skeletons is a function of variation in the skeletal architecture. This discovery both explains anomalous results of other researchers, and opens new opportunities for the application of luminescent banding work in non-coastal areas.

The clever scientific detective work described in this report is somewhat technical in nature, but it is vital to the development of effective tools for understanding how reefs respond to changes in the environment. It is commendable that the Cooperative Research Centre for the Ecologically Sustainable Development of the Great Barrier Reef has sponsored this research.

Jamie Oliver
Director, Information Support
Great Barrier Reef Marine Park Authority

EXECUTIVE SUMMARY

Work reported here was carried out as part of a CRC Reef project to use luminescent (fluorescent) bands in coral skeletons to provide information about the frequency, extent and magnitude of land influences on the Great Barrier Reef (GBR). We wanted to determine criteria for designing equipments to excite and record variations in luminescence in skeletal slices removed from Porites colonies collected at locations along the length and across the width of the GBR. Early results of this work did not accord with some of the generally accepted notions about coral skeletal luminescence. Accordingly, we investigated the nature and causes of this luminescence.

It was found that indentations in the surface of laboratory grade calcium carbonate powder could preproduce all features of coral luminescence. The yellow luminescence seen in slices of coral skeletons, and the blue luminescence measured in such slices, are properties of mineral calcium carbonate. In corals, enhanced luminescence is associated with regions with larger numbers of holes and indentations. The luminescent lines associated with monsoonal river flows in corals from the Great Barrier Reef are narrow regions of lower density skeleton ie, regions with greater amounts of holes and indentations. These narrow, low-density regions presumably result because significantly lower salinities reduce coral calcification without concomitant reduction in skeletal extension. Offshore corals, not subject to regular, periodically lowered saliniteis, show luminescent banding in which higher luminescence is associated with the lower density portion of the annual skeletal density banding pattern.

Long wavelength ultraviolet (UV) light from fluorescent tubes used to display coral fluorescent banding contains significant amounts of violet and blue light. Luminescence is excited in coral skeletons by UV, violet, blue and even green light. Light returning from indentations and holes in coral skeletons will have been subject to a greater number of reflections than light returning from the surface. Each bounce from a surface increases the probability of absorption of the light (UV, violet and blue) and its subsequent re-emission at longer wavelengths. Light returned from surface features of skeletal slices has been subject to far fewer reflections and contains relatively more short wavelengths and relatively less long wavelengths. Thus light returned from surfaces appears blue while light returned from holes appears yellow. Luminescent bands in coral skeletal slices are regions where less skeleton is exposed at the surface and there are more holes (which appear more yellow), relative to regions to either side (which appear more blue).

Luminescence in coral skeletal slices is, essentially, a measure of the density of nearsurface layer of a skeletal slice. Radiographic measurements (X-ray, gamma densitometry) of density in very thin slices tend to be noisy because, in very thin slices, information associated with skeletal architecture dominates over density information. Luminescence and reflectance are recorded when attempts are made to measure luminescence of skeletal slices. Allowance can be made for reflectance by repeating measurements at wavelengths at which the contribution of luminescence is very small. Thus, variations in luminescence can be a useful proxy for variations in near-surface density in coral skeletal slices because allowance can be made for architectural effects.

INTRODUCTION

The discovery of fluorescent bands in coral skeletons and their subsequent linking with organics in runoff from land offered an exceptionally important proxy record. Such fluorescent banding would have potential to provide several centuries of information about rainfall, regional variations in rainfall, river flow, changes in vegetation and land use and the impacts of runoff and land-use upon corals, coral communities and corals reefs. These records would have considerable use in a wide range of areas including climatology, environmental change, agriculture, engineering, water resources, land-use, pollution, oceanography, and coastal and reef management.

Isdale (1984) first reported fluorescent bands in the skeletons of massive corals from colonies of *Porites* on the Great Barrier Reef, Australia. The banding was made visible when sawn surfaces of corals sectioned along a growth axis were illuminated with long-wavelength UV light. The bands were discrete, bright, yellow or yellow-green lines. Isdale (1984) reported that such fluorescent lines were confined to corals growing within 20 km of the shore and were not present in corals from reefs further offshore. He reported that measurements of the intensity of fluorescence in corals from Pandora Reef closely correlated with the outflow of the nearby Burdekin River. Others have since confirmed this strong relationship between rainfall, river runoff and fluorescent lines in skeletons of inshore corals on the GBR (eg, Isdale & Kotwicki, 1987; Lough, 1991; Kotwicki & Isdale, 1991; Neil et al., 1995; Isdale et al, 1998). Further investigation of the source of the fluorescence led Boto & Isdale (1985), Susic & Boto (1989) and Susic et al. (1991) to suggest that the fluorescence resulted from incorporation of terrestrial humic acids into coral skeletons. Terrestrial humic acids are derived from breakdown of vegetation and they proposed that humic acids are carried to the nearshore marine environment during periods of seasonally high, monsoonal rainfall and runoff.

A relationship between fluorescent bands and river runoff was also found in corals from Papua New Guinea (Scoffin et al., 1989) and Florida (Smith et al., 1989) but, in corals from certain other regions, this relationship was poor or was not found. Scoffin et al. (1989) reported only a weak relationship between fluorescent bands and runoff in corals from Indonesia and Fang & Chou (1992) found only a weak relationship between local precipitation and amounts of fulvic (humic) acid in coral skeletons from Taiwan. Scoffin et al. (1992) found that the brightly fluorescent bands were formed during the dry season in corals from Phuket, Thailand. Fluorescent bands have also been reported in corals far

removed from land or from any source of freshwater (Susic et al., 1991, Smithers, 1996; Tudhope et al., 1996).

Humic materials are ubiquitous in terrestrial and marine environments. Marine humics were reported to impart a blue, background fluorescence to coral skeletons (Boto & Isdale, 1985; Susic et al., 1991; Milne & Swart, 1994; Isdale, 1995) and marine organic compounds have been suggested as a possible source of fluorescence bands found in corals distant from freshwater inputs (Smithers, 1996; Tudhope et al., 1996). Indeed, Jones (1990) suggested that fluorescent bands in inshore corals from the GBR result from humic materials created by breakdown of the blue-green alga, *Trichodesmium*, following seasonal blooms.

All reports of the fluorescent bands visible in corals describe their colour as yellow, yellow-green or off-white. Measurements of fluorescence in coral skeletons have been reported by 2 groups; Milne & Swart (1994) and Isdale and co-workers (Isdale, 1984; 1995; Boto & Isdale, 1985; Isdale & Kotwicki, 1987; Smith et al., 1989; Susic & Isdale, 1989, Jones, 1990; Klein et al, 1990; Kotwicki & Isdale, 1991; Neil et al., 1995). Milne & Swart (1994) reported that measurements of fluorescence in corals without fluorescent bands showed broad, featureless emission signals at the 450 nm wavelength characteristic of marine dissolved organic matter while fluorescent lines in the one specimen they analysed had an emission peak at 460 nm. Isdale and co-workers reported measuring skeletal fluorescence at wavelengths between 440 nm and 490 nm (Boto & Isdale, 1985; Smith et al.,1989; Neil et al., 1995).

Humic and fulvic acids have characteristic fluorescence emissions in the blue region (Larson & Stockwell, 1980) and humic materials extracted from coral skeletons also fluoresce in the blue (Boto & Isdale, 1985; Matthews et al., 1996). Data presented in Boto & Isdale (1985, Fig. 1) indicates that organics from fluorescent and non-fluorescent bands have broad emission peaks around 460-470 nm with maximum differences in emission around 490 nm. Thus, the literature indicates that the intensity of the yellow-green fluorescent bands visible in corals is best measured at wavelengths in the blue region of the spectrum. It has been suggested that blue emissions from fluorescent bands in corals are seen as yellow-green because the human eye favours the red end of the spectrum (Boto & Isdale, 1985; Isdale, 1995). This raises a problem that subtle differences in the fluorescence characteristics of marine and terrestrial humic materials measured by instruments (which are both reported to peak in the blue) must somehow transform into extremely wide differences (ie, a shift in wavelength equivalent to 25% of the visible spectrum) in fluorescence seen by the eye. It has also been suggested that the change in colour between bright fluorescent and dull non-fluorescent bands may be due to variable effects of quenching and energy transfer in different

concentrations of the same fluorophore (Matthews et al., 1996). However, the amounts of humics in fluorescent and non-fluorescent bands are not distinctly different (see Susic et al., 1991).

Susic et al. (1991) note that a faint yellow fluorescent banding pattern can be seen in offshore corals. The banding pattern in inshore corals described by Isdale and co-workers (ibid) is one of narrow, yellow-green fluorescent lines with wide non-fluorescent (or blue) regions. A distinction between lines and bands is not drawn in the literature, where all are termed bands. Some reports of fluorescent banding in corals which grew distant from terrestrial inputs could simply be descriptions of the banding pattern mentioned by Susic et al. (1991). For example, Tudhope et al. (1996, Fig. 2) show an annual banding pattern in coral skeletal fluorescence rather than more occasional occurrence of distinct, sharp lines of varying intensity. Measurements of fluorescence in inshore corals from the GBR do not show sharp lines that accord with its visual appearance. Isdale (1984) reported cubing the fluorescence values to make traces accord with the visual appearance[1]. Taylor et al. (1995) suggested that this procedure was necessary because yellow fluorescent lines are seen against a background yellow, skeletal fluorescence. They suggested that the brain discards the background and makes the lines appear sharp. They suggested that manipulation of fluorescence data was necessary because instruments cannot easily make such adjustments.

Boto & Isdale (1985) provided direct evidence for involvement of humics in coral skeletal fluorescence. They extracted fulvic acid from soil and added it to seawater in which they incubated the fast-growing staghorn coral, *Acropora formosa*. They reported that fluorescence was induced in the skeleton. Isdale (1995) reported that coral skeletal fluorescence is attenuated by photo-oxidation and physical destruction of coral skeletons. He pointed to these phenomena as evidence that skeletal fluorescence is a consequence of incorporation of the humics into the aragonitic crystals of coral skeletons.

Most workers have reported visible and measured emissions from coral skeletons as fluorescence. However, there are no published reports of measurements of emissions over the time following excitation.   Such measurements would resolve fluorescence from phosphorescence. Recent work indicates that emissions from coral skeletons involve both fluorescence and phosphorescence (Wild et al., in prep.). Accordingly, here we describe

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[1] A full description of procedures for processing fluorescence data is given in an internal report of the Queensland Water Resources Commission, dated February 1985, by Mr E.A Stewart in which he provides an account of a visit to the Australian Institute of Marine science. This report describes cubing of fluorescence values after subtraction of baseline values.

visible and measured emissions as luminescence; a term which covers both fluorescence and phosphorescence.

This report arises from preliminary work carried out to devise a reliable procedure for measuring luminescence in coral skeletons. It was intended to build upon unpublished work carried out by Drs W.C. Dunlap and D.J. Barnes in 1987. That work indicated that improved measurements of coral skeletal luminescence were obtained when values for reflected visible light (wavelength, 540 nm) were subtracted from values for luminescence (excitation = 390 nm; emission = 490 nm) obtained at the same point on a skeletal slice. It seemed that correction of luminescence by reflected visible light made some allowance for skeletal architecture and reflectance of light. Certainly, the correction provided much sharper luminescence peaks than were obtained by direct measurements. We began our preliminary work with an examination of some of the generally accepted notions regarding the nature and causes of coral skeletal luminescence. Initial results did not accord with some these accepted notions and, consequently, further investigations were carried out. This report describes those investigations and our conclusions regarding the nature and causes of luminescence in coral skeletons.

MATERIALS AND METHODS

Coral skeletal material came from colonies collected at Eel Reef (12.50^o S, 143.52^o E) and from the fringing reef around Pipon Island (14.12^o S, 144.52^o E). Eel reef is an inshore reef in the northern section of the Great Barrier Reef (GBR), 14 km from the mainland. Pipon Island is also in the northern section of the GBR, 6 km from the mainland. Dated skeletal material was obtained from 6-7 mm thick slices cut from colonies: dating was carried out using X-rays of, and densitometer tracks across, these slices (see Lough & Barnes, 1992). Colonies of *Porites* were also collected from the reef around Double Island (16.38^o S, 145.70^o E) and from 21-141 Reef (21.52^o S, 151.22^o E). Double Island is about 1 km off the coast and about 20 km north of Cairns. 21-141 Reef is a shelf-edge reef in the southern section of the GBR, 145 km from the mainland. Coral colonies and coral slices were identified by a code, which is also used here. For example, PIP_B05_S3 identifies the 3rd slice cut from coral colony B05 collected at Pipon Reef. The various slices, their associated X-radiographs and densitometric measurements are held at AIMS.

Luminescent and non-luminescent bands were sampled by lightly pencilling outlines of the bands on slices illuminated with an ultraviolet (UV) fluorescent tube (black light; see below). Luminescent and non-luminescent bands were removed by fret sawing along the pencil lines.

Coral skeleton was powdered using a KAD Humbolt Wedag ball mill. Pieces of skeleton were fractured into small pieces and placed in the ceramic mortar of the mill. The mill was run for about 15 min until the powdered skeleton had a similar consistency to laboratory grade calcium carbonate powder (May & Baker Ltd., Manchester, UK). The laboratory grade powder had an average grain size of 16 ± 5 μm. After milling, coral skeletal powder was passed through an 80 μm sieve (the smallest available) to remove any remaining larger particles.

Powdered activated charcoal (technical grade, Ajax chemicals, Auburn, NSW) was added to 10 g lots of the laboratory grade $CaCO_3$. Amounts of charcoal added were 1.2 mg, 10.0 mg, 20.0 mg, 30.0 mg, and 40.0 mg and the resulting mixtures contained 0.012%, 0.1%, 0.20, 0.3% and 0.4% charcoal by mass. Individual particles of charcoal were very small but they tended to form into clumps. The average size of the clumps was 20 ± 10 μm.

Laboratory grade $CaCO_3$ powder was coated with humic acid (sodium salt, HA 675-2, Sigma-Aldrich Pty Ltd, Castle Hill, NSW). The humic acid was dissolved in 20 ml distilled water and mixed into a paste with 75 g of powdered $CaCO_3$. The various pastes were returned to powders by drying them at 50 °C for 2½ days.

Powdered skeleton and laboratory grade $CaCO_3$ powder were loaded into plastic vials made of non-fluorescent polyethylene. The powder was gently pressed into a vial and its surface smoothed level with the top of the vial using the back of a spatula. Vials were 18.3 mm deep. They had an external diameter of 27.3 mm and an internal diameter of 24.6 mm. A hole was pushed into the powder at the centre of each vial with an unused, shiny, reversed, 4 mm drill bit. The drill bit was held in the chuck of a drill mounted in a drill press. It was gently and slowly inserted into and withdrawn from the powder to leave a hole 13 mm deep.

Powders and skeletons were illuminated by a 8 Watt UV fluorescent tube (black light; NEC, no. FL8BLB). The tube was 15 mm in diameter and 300 mm long. Holes in powders were photographed with the surface of the powder tilted 15° from the horizontal and with the camera held vertically. A strip of black card was positioned so that, although the surface of the UV fluorescent tube was only about 10 mm from the top of the hole in the powder, the

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camera could not "see" the tube directly. Vials were always positioned at the same point, which was about mid-way along the UV fluorescent tube. The camera was a Sony CCD/RGB colour video camera with a Fujinon-tv zoom lens fitted with a Fujinon close-up lens CL11052. Zoom was set to 75 mm and the f-stop was set to 2.0. Images were captured to the hard drive of an IBM-compatible personal computer.

Luminescence was measured with a Turner 430 spectrofluorimeter (Turner Associates, Palo Alto, California, USA). The sample chamber of this instrument was removed and the branched ends of a Y-shaped fibre optic were fitted to its excitation and emission ports (General Fibre Optic Inc., Cedar Grove, New Jersey, USA). The Y-shaped fibre optic was a UV-silica randomised bifurcated bundle with a 3.0 mm diameter common end and 0.32 x 14.0 mm branched ends. The branched ends conformed to the slits associated with the excitation and emission ports of the spectrofluorimeter. The operational range of the Turner 430 spectrofluorimeter was $300 - 700$ nm. The monochromator excitation slits had fixed half power bandwidth of 15 nm. The emission monochromator had a half power bandwidth of 60 nm.

For certain measurements, the 3 mm diameter common end of the Y-shaped fibre optic was restricted to a 1 mm diameter collecting area by a stainless steel sheath. The 1 mm diameter hole in the sheath passed through a 0.5 mm thick end on the collar. Thus, light coming from and returning to the common end of the Y-shaped fibre optic was slightly more collimated with the sheath than without it. The end of the stainless steel sheath was painted black to avoid gathering stray light.

Certain batches of $CaCO_3$ powder and coral skeletal slices were heated to 450 ^{o}C. Heating was carried out in 1700 W laboratory box furnace (Lindburg Equipment, Watertown, Wisconsin, USA). The internal dimensions of the furnace were 200 mm long x 110 mm high x 100 mm wide. Coral slices were initially heated to 100 ^{o}C and then stepped up to 450 ^{o}C in 50 ^{o}C intervals, each lasting about 30 min. After 2 h at 450 ^{o}C, the furnace was turned off and the coral slice allowed to cool overnight. The door to the furnace was not opened except to insert and remove the coral slice. Coral slices tended to fracture into several pieces without such careful treatment.

RESULTS

__Excitation wavelengths__. The simplest way to display coral luminescence is to illuminate lices with an ultraviolet (UV) fluorescent tube (black light). The Turner spectrofluorimeter showed that the spectrum of light emitted from this tube had a peak intensity around 350-360 nm and had a width of about 120 nm (Fig. 1). Thus, it produced significants amounts of visible light, mostly in the violet region but also grading into the blue region (Table I).

Illuminating luminescent bands in coral slices with a custom-built, high intensity, variable wavelength monochromator showed that most of the bands were most obviously displayed by excitation with instrument settings at around 390 nm. This monochromator and the Turner spectrofluorimeter produced equivalent wavelengths for equivalent settings. Thus, the excitation wavelength employed in measurements of luminescence with the Turner spectrofluorimeter was 390 nm.

The Turner spectrofluorimeter with the excitation wavelength set to 390 nm produced a peak with a width of around 80 nm (Fig. 2). Because 390 nm is only just outside the visible range (400-700 nm; Table I), considerable amounts of violet and lesser amounts of blue light were associated with this excitation wavelength.

__Luminescence seen by eye.__ Holes pushed into laboratory grade $CaCO_3$ powder gave off a yellow luminescence when illuminated by the UV fluorescent tube. The amount of yellow light appeared to increase as holes were deepened. Consequently, results described here are for a "standard", 4 mm diameter, 13 mm deep hole.

The surface of laboratory grade $CaCO_3$ powder illuminated by the UV fluorescent tube appeared light blue-grey, while a standard hole pushed into the powder appeared yellow. The yellow colour of the hole contrasted sharply with the blue-grey colour of the surface (Fig. 3a). Addition of charcoal to the $CaCO_3$ powder decreased the amount of yellow light apparent within the hole (Fig. 3; Table II). It also changed the colour of the surface of the powder towards violet.

Images of powders were made with a video camera (eg, Fig. 3). The camera did not register lower levels of yellow light that were apparent to the eye. The video camera "saw" no yellow light emerging from holes in $CaCO_3$ powder with 0.1% charcoal (cf. Fig. 3 & Table II). To the eye, holes in powder with 0.1% appeared to emit 10-20% of the amount of light emitted

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from powder without charcoal. Indeed, a glimmer of yellow light was visible in holes in powder containing as much as 0.4% charcoal (Table II).

The yellow colour within the hole remained strong after laboratory grade $CaCO_3$ powder was heated to 450 $^{\circ}$C for 2 h (Fig. 4). The colour at the surface of the powder and within the hole was slightly redder after heating. This treatment would have carbonised any organic materials within the $CaCO_3$ powder.

Powdered skeleton from a *Porites lobata* collected from 21-141 Reef (141_B05) was illuminated by the UV fluorescent tube. Being a shelf-edge reef 145 km from the mainland, 21-141 Reef is unlikely to be frequently influenced by coastal runoff. Yellow light was apparent within a hole in the powdered skeleton, although this luminescence was considerably less intense than within a hole in laboratory grade $CaCO_3$ powder (cf, Fig. 5a & 3a). Heating powdered skeleton to 450 $^{\circ}$C for 2 h caused it to become grey (under room light), in much the same way that addition of charcoal caused laboratory grade $CaCO_3$ powder to become grey. This might be expected because heating would have carbonised all organics associated with the skeleton. No yellow luminescence was seen within a hole in heat-treated skeletal powder and the surface of the powder was more purple than the surface of untreated skeletal powder (Fig. 5).

Skeletal powder was made from luminescent and non-luminescent bands in a colony of *Porites lutea* collected at Pipon Island (PIP_B05). Bands sawn from a skeletal slice encompassed the period 1981-85. Powder from the non-luminescent skeletal band provided slightly more light within the hole than powder from the luminescent band (Fig. 6). The amount of yellow light was considerably less than was obtained with laboratory grade $CaCO_3$ powder and slightly greater than was obtained with powder from a colony of *Porites lobata* from 21-141 Reef. The amount of yellow light was intermediate between that obtained with laboratory grade $CaCO_3$ powder with 0.012% and 0.1% charcoal (cf, Fig. 6 and Fig. 3).

Grains of laboratory grade $CaCO_3$ were coated with humic acid to test the effect of humic acid upon luminescence. This is a standard technique for measuring luminescence in a coating material (see Hurtubise, 1989). Final concentrations were 0, 25.5, 51.6 and 150.0 μg humic acid per g $CaCO_3$. There was very slightly less yellow light in the hole in the powder treated with distilled water alone (control) than was apparent in holes in untreated powder (cf, Figs. 3a & 4a). The amount of yellow light apparent within the holes decreased with increasing amounts of humic acid (Fig. 7). Distinct yellow light was still seen within the hole

in powder containing 150.0 μg humic acid per g $CaCO_3$ but the amount of light was not sufficient to register when the hole was photographed (Fig. 7d).

A skeletal slice from a colony of *Porites lutea* collected at Double Island (DOU_B02**)** was treated with bleach to remove any organic materials adhering to its surface. A segment was cut from the growth axis of this slice, gradually heated to 450 °C and then allowed to cool slowly. The segment became much darker due to carbonisation of organic materials remaining within the skeletal matrix. Its reflective characteristics were changed and individual calices and corallite fans became obvious as dark lines more or less aligned with the colony growth axis. These were more easily seen because holes and indentations within the surface of the slice appeared darker. A distinct feature within the segment was a series of dark lines lying across the growth axis. Microscopic examination of these lines suggested that they were regions where the surface contained more holes and indentations. The dark lines associated with calices and the dark lines across the growth axis were both largely obscured when talcum powder was gently rubbed into the slice, filling the holes and indentations at its surface. Some of the lines across the growth axis were not totally obscured by talcum powder. In these cases the lines were associated with skeleton that was slightly darker than the adjacent areas, ie, skeleton associated with these lines contained greater amounts of carbonised organics. Regardless of carbonised organics in the skeleton, the appearance of dark lines across the growth axis was mostly associated with more and larger holes and indentations within the surface of the slice. Thus, these dark lines represented narrow regions of lower density skeleton. The skeletal slice adjacent to the heat-treated segment was examined under light from the UV fluorescent tube. Luminescent lines within this slice aligned precisely with the dark lines of lower density skeleton in the heat-treated segment (Fig. 8). The sharpest fluorescent lines in the untreated portion were associated with the darkest, least dense lines in the heat-treated portion.

Measurements of luminescence and reflectance. Luminescence and reflectance were measured in powders and slices of coral skeleton using the Turner 420 spectrofluorimeter and Y-shaped fibre optic. Reflectance was measured across wavelengths in the range 390 nm to 650 nm. It was possible to correct such measurements for non-linearity in the sensitivity of the instrument. This was done by assuming that a glass mirror reflected equally all wavelengths across the range. It was then possible to derive and apply correction factors for each of the wavelengths at which reflectance measurements were made.

Luminescence was measured with the excitation wavelength set to 390 nm (see *Excitation wavelengths*, above) and with the emission wavelength ranging between 450 nm and 650 nm.

With an excitation setting of 390 nm, the instrument produced significant amounts of visible light (relative to the amount of light produced by luminescence) even at wavelengths as high as 550 nm (eg, Fig. 2). Thus, measurements of luminescence included light reflected from powders and coral skeletons as well as light due to their luminescence. It was not possible to correct such measurements of luminescence in the way reflectance was corrected because the relative contributions of luminescence and reflectance were not known. Thus, measurements of luminescence were always comparative. That is, measurements of "luminescence" emitted from holes in powders were compared with measurements of "luminescence" from the surface of the powder. Similarly, measurements of light emitted and returned from the visible, yellow luminescent bands in corals were compared with measurements of light emitted and returned from the adjacent, regions of skeleton. In making such measurements, the amount of light emitted and returned from the control surface was adjusted to an instrument reading of 100% at 450 nm.

Luminescence. Spectra of light (450-650 nm) returned from a glass mirror and from laboratory grade $CaCO_3$ powder were measured with the excitation wavelength set to 390 nm (Fig. 9). The Turner spectrofluorimeter data were adjusted so that measurements for the mirror and for the surface of the powder both gave a relative emission of 100% at 450 nm. Subsequent measurements from the hole in the powder were made without change to the calibration of the spectrofluorimeter.

Laboratory grade powder returned relatively more light (with instrument sensitivity set to 100% "emision" at 450 nm) between 460 nm and 600 nm than was relected from a glass mirror (Fig. 9). Although the hole returned less light than the surface at 450 nm (94% compared with 100%), it returned more light than the surface between 460 nm and 600 nm. The greatest difference between return from the surface of the powder and return from the hole was around 490 nm.

Similar measurements of emission spectra were made for laboratory grade $CaCO_3$ powder containing 0.012% and 0.1% charcoal (Fig. 10). The return from the surface was adjusted to 100% at 450 nm in each case. The return from the hole at 450 nm decreased from 94% with pure $CaCO_3$ (Fig. 9) to 71% with 0.012% charcoal (Fig 9a) to 55% with 0.1% charcoal (Fig. 10b). With 0.1% charcoal, the return from the hole decreased below the return from the surface at all wavelengths.

Emission spectra were also measured for laboratory grade powder which had been coated with 150 µg humic acid per g $CaCO_3$ (Fig. 11). As before, returns from the surface were set

to 100% relative emission at 450 nm. Treatment of laboratory grade $CaCO_3$ powder with distilled water (control) decreased emissions from the hole compared with untreated powder (cf, Figs. 9 & 11a). This decrease was probably real rather than due to differences in instrument settings and equipment set up because slightly less yellow light was visible within holes in powder treated with distilled water than in holes in untreated powder (Figs. 3a & 7a). With returns from surfaces set to 100%, the return from the hole dropped from 69% for powder treated with distilled water to 61% for powder coated with humic acid (Fig. 11). Powder treated with distilled water returned more light from the hole than from the surface over the range 470-570 nm (Fig. 11a). This was not the case for powder coated with humic acid where emissions from the hole fell below emissions from the surface at all wavelengths (Fig. 11b).

Emission spectra were measured in powder made from the 1981-85 non-luminescent and luminescent coral skeletal bands in a colony collected at Pipon Island (see above). Holes in powder made from non-luminescent bands returned more light than holes in powder made from luminescent bands (Fig. 12; cf, Fig. 6). As for previous emission spectra, maximum difference between surface and holes occurred around 490 nm. With the return from the surface set at 100% at 450 nm, the return from the hole in powder made from non-luminescent bands at 450 nm was 86% (Fig. 12a). The equivalent value for return from the hole in powder made from luminescent bands was 74% (Fig. 12b).

Measurements were also made of the light returned from intact coral skeletons containing luminescent bands. The tip of the fibre optic was positioned above the surface of skeletal slices in the same manner in which it had been positioned above the various powders. Measurements were made on a slice cut from a colony of *Porites lobata* collected at Pipon Island (PIP_B01_S3). They were made on the 1979 luminescent band and on the non-luminescent area immediately following this band. Measurements were also made on a slice cut from a colony of *Porites lutea* collected at Eel Reef (EEL_B10_S1). Measurements were made on the 1984 luminescent band and on the non-luminescent area immediately following this band.

In both coral slices, excitation at 390 nm returned less light from the luminescent band at 450 nm than from the non-luminescent band (Fig. 13). However, the luminescent band returned more light than the non-luminescent band over the range 470-570 nm. These spectra were similar to those obtained with the surfaces and holes in laboratory grade $CaCO_3$ powder, except that the curves for surfaces and holes were more separated than the curves for non-luminescent and luminescent bands (cf, Figs. 9 & 13).

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Reflectance. Reflectance of $CaCO_3$ powders and coral skeletal slices was measured over the range 390-650 nm. With the emission wavelength set to the same value as the excitation wavelength, what was measured was overwhelmingly reflectance from the surface. Such measurements of reflectance provided a relative measure of the amount of light absorbed by powder and coral skeletons at different wavelengths.

A hole in laboratory grade $CaCO_3$ powder reflected, on average, about a third of the light returned from the surface (Fig. 14). Returns from the surface declined slightly as wavelength was increased. A linear trend line fitted to the data showed a 4% drop in reflectance between 390 nm and 650 nm ($r^2 = 0.37$). Most of this fall occurred between about 390 nm and about 500 nm. Reflectance from the hole increased distinctly with increasing wavelength. A linear trend line showed a 13% increase in reflectance between 390 nm and 650 nm ($r^2 = 0.98$). Thus, some process within the hole was preferentially removing violet and blue wavelengths, relative to orange and red wavelengths.

Reflectance was measured from luminescent and non-luminescent bands a coral slices from Pipon Island and Eel Reef (PIP_B01_S3 and EEL_B10_S1; see above) The trends for reflectance with changing wavelength were similar to those obtained with the surface and hole in $CaCO_3$ powder (see above). The amount of reflectance was more-or-less the same for the different bands. In some cases, reflectance from luminescent bands was approximately similar at all wavelengths and reflectance from non-luminescent bands declined with increasing wavelengths (Fig. 15).   In other cases, reflectance from luminescent bands increased with increasing wavelength and reflectance from non-luminescent bands was approximately similar across the range of wavelengths (as in Fig. 14).

The surface and holes in powder made from luminescent and non-luminescent bands in a slice of *Porites lutea* collected at Pipon Island (PIP_B05; see above) gave results (Fig. 16) very similar to those obtained with laboratory grade $CaCO_3$.

Reflectance was measured from the surface and holes in laboratory grade $CaCO_3$ powders coated with humic acid to test whether humics could modify the reflective properties of coral skeletal powders and coral skeletons.   Powders were coated with 0, 25.5, 51.6 and 150.0 μg humic acid per g $CaCO_3$. Humic acid did not modify the way in which different wavelength were reflected from the powders. The only effect noted was that increasing amounts of humic acid very slightly decreased the amount of light reflected from the powders (Fig. 17).

Tracking across holes in CaCO₃ powders. Reflectance and luminescence were measured in laboratory grade $CaCO_3$ powder containing a "standard", 4 mm diameter, 13 mm deep hole. The powder was moved in 0.3 mm horizontal steps beneath the 3 mm diameter, common end of the Y-shaped fibre optic so that, in effect, the fibre optic tracked across the hole. At each step the powder was excited at both 390 nm and 490 nm and the emission was recorded at 490 nm (Fig. 18). The amount of light received with excitation set to 390 nm and emission set to 490 nm was around 300 times less than was received when both were set to 490 nm. In data presented here, values have been arbitrarily adjusted so that relative emission from the surface of the power was around 60% with the instrument set to 390 nm $\rightarrow$ 490 nm and around 40% with the instrument set to 490 nm $\rightarrow$ 490 nm.

Exciting the powder at 390 nm and recording emissions at 490 nm measured both luminescence emissions and reflectance from the powder. Exciting the powder at 490 nm and recording emissions at 490 nm measured essentially only reflectance.   Luminescence was obtained by subtracting the reflectance signal (490 nm $\rightarrow$ 490 nm) from the signal for reflectance plus luminescence (390 nm $\rightarrow$ 490 nm). This subtraction technique emphasised the luminescence component of the emission signal (but did not accurately remove the entire reflectance signal).

This procedure was repeated with the diameter of the fibre optic reduced to 1 mm with a stainless steel collar (Fig. 20). With a smaller collecting area for the fibre optic, the combined signal (reflectance + luminescence: 390 nm $\rightarrow$ 490 nm) was less from the hole than it was from the surface. Adjusting for reflectance gave a distinct luminescence signal from the hole (Fig. 21), whereas before subtracting a reflectance component there had been no peak (Fig. 20).

With a narrower diameter fibre optic collecting light, emissions from the hole accorded more with the shape of the hole. That is, there was less averaging of the signal with distance. However, the narrower fibre optic collected less of the light emerging from the hole and returns from the hole were less than returns from the surface. We cannot explain why there was a difference between measurements of returns from the surface relative to returns from the hole with 3 mm and 1 mm diameter fibre optics.

The effect of charcoal and humic acid on this luminescence signal was measured by tracking across laboratory grade $CaCO_3$ powder containing 0.1% charcoal (by mass) and powder coated with 150 μg humic acid per g $CaCO_3$ (Fig. 22). Instrument settings were the same as

for data presented in Figures 20 and 21. The additives altered both the signal for reflectance plus luminescence and the signal for reflectance (Fig. 23) and this compensated, to some extent, for the decrease in the reflectance plus luminescence signal relative to that returned by pure $CaCO_3$ powder.

DISCUSSION

Appearance of holes in inorganic powders under long-wavelength ultraviolet light.
Luminescence was apparent within holes pushed into a variety of relatively pure (analar; laboratory grade), white, crystalline powders illuminated with long-wavelength ultraviolet (UV) light from a fluorescent tube. Luminescence colour varied from blue through green to yellow. Yellow luminescence was notable within holes pushed into powdered $CaCO_3$, $MgCO_3$ and $NaHCO_3$ (Table III). This luminescence appears to be characteristic of certain inorganic powders and yellow luminescence is especially strong in powdered $CaCO_3$.

The yellow light apparent within holes in laboratory grade $CaCO_3$ powder contrasted with the blue-grey colour of the surface of the powder (Fig. 3a). Under a binocular microscope, holes within the architecture of coral skeletal slices illuminated with the UV fluorescent tube appeared yellow and this contrasted sharply with sawn and exposed skeletal surfaces, which appeared blue-grey to blue.

The problem of yellow versus blue luminescence.
Measurements of the light returning from the surface and holes in laboratory grade $CaCO_3$ powder illuminated with long-wavelength UV light showed that more blue than yellow or green light was returned from holes than from surfaces (eg, Fig. 9; cf Table I which lists colours associated with wavelengths). Consequently, the yellow (or, perhaps, yellow-green) luminescence seen by eye in holes does not accord with measurements, which indicate that holes return more blue light. A similar effect has been noted with coral luminescence, which is yellow or yellow-green to the eye but registers most strongly as blue light when measured (eg, Boto & Isdale, 1985; Isdale, 1995; see also Fig. 13). These measurements were not corrected for variations in instrument response with wavelength. Gratings used in spectrofluorimeters (ie, ranging across the UV and visible light range) and photomultiplier tubes are typically considerably more efficient in the blue than at longer wavelengths, such as green and yellow. The yellow versus blue problem may partly arise because most instruments operating in the UV-visible light range measure blue light more efficiently than yellow (see also below).

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Luminescence of CaCO₃ and organic inclusions. Holes in laboratory grade $CaCO_3$ showed a yellow or yellow-green luminescence very similar to that seen in natural holes in coral skeletons. Heating laboratory grade $CaCO_3$ to 450 ^{o}C for 2 h did not prevent luminescence in holes in the powder (Fig. 4), although it shifted its colour very slightly towards red. Since laboratory grade $CaCO_3$ is unlikely to contain significant amounts of organic contaminants, and since any organics associated with the $CaCO_3$ would have been carbonised by the heat treatment, the luminescence is not associated with organics. The shift in luminescence colour towards red was probably associated with a loss of water from the crystalline powder (in the same way that addition of water caused slight quenching of luminescence; see above; cf, Figs. 3a & 7a).

Visible, yellow luminescence (Fig. 7) and measured luminescence (Fig. 11) was quenched rather than enhanced by coating $CaCO_3$ powder with humic acid. Powder was coated with amounts of humic acid (25 & 50 μg humic acid.g $CaCO_3^{-1}$) equivalent to those found in coral skeletons and with amounts (150 μg humic acid.g $CaCO_3^{-1}$) considerably greater than those found in coral skeletons (Susic & Boto, 1989). Any significant luminescence of solid humic materials should have been displayed by this coating technique (see Hurtubise, 1989). It seems likely that humic materials in coral skeletons quench both visible yellow and measured blue luminescence.

Why do holes seem to luminesce yellow? Light returning from $CaCO_3$ powder illuminated by UV light has undergone reflections and/or absorptions and re-emissions (luminescence). Light from the UV fluorescent tube and light produced by the Turner spectrofluorimeter set with emission = 390 nm contains considerable amounts of violet and blue visible light (Figs. 1 & 2). Short wavelength visible light (violet and blue) is reflected slightly better from the surface of $CaCO_3$ powder than from holes in the powder (Fig. 14). The same occurs with powdered coral skeleton (Fig. 16). Similarly, non-luminescent bands in coral skeletal slices reflect short wavelength light better than luminescent bands (Fig. 15).

On average, light returning from a hole will have undergone more reflections and absorptions than light returning from a surface. Ramseyer et al. (1997) report that blue light excites luminescence in coral skeletons and Wild et al. (in prep.) report excitation by violet, blue and green light. Thus, the poorer return of shorter visible wavelengths from holes in $CaCO_3$ powder is probably be due to their absorption and subsequent re-emission at longer wavelengths. Consequently, multiple reflections within holes in $CaCO_3$ powder (and holes in coral skeletons) will tend to absorb UV, violet and blue light and shift emissions towards

longer wavelengths. Fewer reflections from the surface of powder (and the surface of coral skeletons) will result in return of relatively more violet and blue light and relatively less longer wavelength light. This effect is sufficiently great that holes visually appear yellow while surfaces appear blue (eg, Fig. 3a).

This explanation can be given in a slightly different way. Any white, non-luminescent surface will appear violet when illuminated by a UV fluorescent tube. If the surface is luminescent, reflected violet light will mask any weak luminescence while strong luminescence will moderate the colour of the light returning from the surface. The surface of $CaCO_3$ powder illuminated by a UV fluorescent tube appeared blue-grey rather than violet in colour suggesting that the powder was luminescing (in the same way that most white paper, and most white linens, appear bright white-blue rather than violet under a black light). Light returning from the hole must have been subject to many more reflections than light returning from the surface. Violet and blue light have a higher rate of absorption than longer wavelengths as light bounces around in a hole (eg, Fig. 14). Each such bounce involving absorption will produce luminescence. Thus, relative to returns from surfaces, multiple bounces within holes will remove more of the violet and blue light and produce more luminescent light of longer wavelengths. This is because the probability of absorption and re-emission (ie, the amount of luminescence) increases with the number of reflections.

Effects of charcoal upon luminescence. Some estimate of the number of reflections occurring in holes can be obtained from the observed and measured luminescence in $CaCO_3$ powder to which charcoal was added. The quenching of luminescence was apparently out of all proportion to the amount of charcoal added (Figs. 3 & 10; Table II). The small proportion of charcoal could only have such a massive effect where multiple reflections make it more likely that any one light ray will hit a charcoal particle and become absorbed.

The ratio of surface area of charcoal to surface area of $CaCO_3$ can be roughly estimated from the densities and sizes of the $CaCO_3$ and charcoal particles. Activated charcoal has a density around 2 g.cm^{-3} and $CaCO_3$ has density of approximately 3 g.cm^{-3}. Diameters of clumps of charcoal grains and $CaCO_3$ grains averaged 20 μm and 16 μm, respectively. Microscopic examination of the powder mixtures showed that the charcoal remained clumped after it was added to $CaCO_3$ powder. Consequently, in $CaCO_3$ powder containing 0.1% charcoal by mass, the ratio of surface area of charcoal to surface area of $CaCO_3$ was around 0.001. In this situation, an average of 999 photons will be reflected for every photon that hits a charcoal particle and is absorbed. Since 0.1% charcoal reduced visible, yellow luminescence by 10-20% (Table II), the number of reflections in the hole must have been 100-200. The number of

reflections involved in returning light from the surface must have been considerably less than this. Reflections may occur within and amongst crystalline particles of powdered $CaCO_3$, as well as back and forth across the hole.

Some conclusions. The appearance of luminescence in $CaCO_3$ powders and coral skeletons can be explained entirely in terms of the geometry of the $CaCO_3$. Heat-treatment of skeletal slices showed that luminescent lines are associated with narrow bands of lower density skeleton. Optical processes involved in this luminescence are not well understood. Optical processes occurring in coral skeletons and various white, crystalline powders deserve further study. Provided the level of skeletal inclusions does not significantly alter luminescence in coral skeletons - especially inclusions of terrigenous silt ($\equiv$ carbon particles) - low density regions within the annual density banding pattern are likely to be more luminescent than adjacent high density regions. Recent work by F.J. Wild at our laboratory confirms that luminescent regions and low density regions of annual density bands coincide in slices taken from corals at sites well removed from land influences, such as Ashmore and Myrmidon Reefs on the GBR, and southern Oman in the Arabian Sea (see Tudhope, 1996). Other workers have noted that luminescent bands coincide with the low density regions of annual density bands (Scoffin et al., 1989; Klein et al., 1990; Smithers, 1997).

In earlier measurements of density using a gamma densitometer, we have occasionally noted that prominent luminescent lines have been associated with a low density "line" in the skeleton. Narrow low density lines are not easily seen in X-radiographs (and not always seen in densitometer traces) because they are not aligned with the X-ray beam (eg, Barnes et al., 1989). There is strong evidence that luminescent lines in inshore corals from the GBR correlate well with coastal rainfall and river runoff (Isdale, 1984; Isdale & Kotwicki, 1987; Lough, 1991; Kotwicki & Isdale, 1991; Neil et al., 1995; Isdale et al, 1998). Consequently, it seems likely that the narrow bands of lower density skeleton, which result in luminescent lines, correspond to periods of reduced salinity that result in reduced calcification. Given that substantially reduced salinity is usually associated with river and coastal runoff, it is not surprising that some of these narrow bands of lower density skeleton are also associated with increased organics or sediments trapped within the skeletal matrix. However, work presented here demonstrates that luminescent lines result from lower density skeleton rather than from inclusions within the skeleton. This raises the possibility that luminescent lines may result from other factors that reduce calcification but do not reduce extension by an equivalent amount. For example, salinity may be reduced for extended periods by torrential rain on the enclosed lagoon of an atoll, or on other reefs not associated with islands. Calcification could be reduced while extension continues when corals experience periods of unusually elevated

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temperatures. Such effects may explain reports of luminescence in corals collected at sites far removed from land. On the other hand, luminescent banding may simply correspond with the annual density banding pattern, as reported here and by several other workers (Scoffin et al., 1989; Klein et al., 1990; Smithers, 1997).   In future reports of luminescent lines and luminescent banding in coral skeletons, attempts should be made to determine if this luminescence is associated with the annual density banding pattern or if it overlies the annual density banding pattern.

Ramseyer et al. (1997) found a correlation between luminescence and the architecture of speleotherms, marine cements and coral skeletons. Thus, they note (p. 365) that in speleotherms, "Rough surfaces correspond to highly fluorescing bands whereas flat surfaces correspond to darker zones with a lower fluorescence intensity". Later they note (p. 367) that in marine cements, "…the density of pits (holes) seems to reflect the degree of fluorophore abundance…". They similarly linked luminescence in the skeleton of *Porites solida* with regions where more organics were trapped between less densely packed crystals.

In the model presented here, luminescence is stronger where surface architecture is, essentially, less dense. Wild et al. (in prep.) show that luminescence in coral skeletons varies in intensity by only about 20% over the spectral range 470 – 620 nm. These measurements were corrected for variations in instrument response. Thus luminescence from $CaCO_3$ results in a broad band of emissions from blue through to orange (Table I). This broad band emission could appear yellow to the eye, especially where removal of shorter wavelengths is enhanced by geometry (ie, holes). It seems that luminescence is most easily measured in the blue region because most instruments are more sensitive in the blue than at longer wavelengths. It should be noted that results reported here, together with Wild's (Wild, 1996; Wild et al., in prep.) findings, suggest that the optimum emission wavelength for measurement of luminescence in coral skeletal slices may well vary with the equipment used. Moreover, increase in organic and, especially, increases in terrigenous silt (ie, dark particles) will emphasise reflectance at the expense of luminescence and shift light returned by skeletons towards the blue. Thus, the emission wavelength may vary with skeletal density and between collection sites for coral colonies.

The light returned from holes in $CaCO_3$ powder and coral skeleton will depend upon the geometry of the holes and the physical set-up of the illumination and detection systems (eg, size and depth of holes, area illuminated and area "inspected"). For example, the signal obtained from a 4 mm diameter hole using 1 mm diameter detector was different from the signal obtained from the same hole using a 3 mm diameter detector (cf, Figs. 18 & 19 with

Figs. 20 & 21). Subtraction of a reflected signal (490 nm $\rightarrow$ 490 nm) from a reflectance plus luminescence signal (390 nm $\rightarrow$ 490 nm) is a way of making allowance for effects due to the geometry of the illumination and detection systems (cf, Fig. 18 with 19 & Fig. 20 with 21). This subtraction leaves a signal that depends, mostly, upon the ratio of holes to (sawn) surface in a skeletal slice. That is, a signal which is a direct measure of density in the near-surface layer, say upper 0.1-0.2 mm, of the slice.

Suggested system for measuring luminescence. Luminescence in coral skeletons is a proxy for density in the near-surface layer of a skeletal slice. A variety of techniques might be employed to measure this near-surface density. However, it does not seem appropriate to explore other techniques until the linkage of luminescence with near-surface density and geometry is better understood and accepted. Most importantly, optical techniques appear to offer the best procedures for measuring near-surface density along tracks of useful length on skeletal slices. By useful length we mean tracks decimetres to metres long representing tens to hundreds of years of coral growth.

Variations in near-surface density could be measured as luminescence (ie. luminescence + reflectance) or as simple reflectance. All such measurements will encounter the same sort of problems that have been encountered with density measurements. Problems with density measurements involve cutting slices sufficiently thick and using beam sizes of sufficient diameter that skeletal meso-architecture and macro-architecture are averaged out without compromising the basic density signal (see Barnes et al., 1989; Barnes & Lough, 1990; Lough & Barnes, 1990a). Annual density variations can be measured with a relatively large beam size. Lough & Barnes (1990a) suggest that the optimum gamma beam will have a diameter approximating about half the width of the annual density bands. In practice, these workers have standardised on a 4 mm diameter beam (although they employ narrower beams for very slow growing corals collected at the extreme of their range). This optimum is a compromise between a large diameter beam which would smear the annual density signal and a small diameter beam which would tend to emphasis variations in skeletal architecture at the expense of variations in skeletal density (see Lough & Barnes, 1990a). Applying the same principals, the optimum size for a light beam for measuring luminescent lines (ie, near-surface density) would appear to be less than 1 mm in diameter because such lines are normally 1-2 mm wide. Skeletal slices for X-radiography and densitometry are normally cut 5-10 mm thick. A light beam probably penetrates much less than 1 mm below the surface. Consequently, attempts to recover surface density information using a light beam about 1 mm in diameter will encounter severe problems with skeletal meso-architecture and macro-architecture. Indeed, drawing on

previous experience (Barnes et al., 1989; Barnes & Lough, 1990; Lough & Barnes, 1990a), the ratio of sawn surface to holes and indentations in a skeletal slice inspected by a 1 mm diameter light beam is likely to have a considerable variability due to chance (ie, Lough & Barnes, 1990b).

Unpublished work carried out by Drs W.C. Dunlap and D.J. Barnes in 1987 indicated that improved measurements of coral skeletal luminescence were obtained when values for reflected visible light were subtracted from values for luminescence obtained at the same point on a skeletal slice. It seemed that correction of luminescence by reflected visible light made allowance for skeletal architecture and reflectance of light. Certainly, the correction provided much sharper luminescence peaks than were obtained by direct measurements. The present work indicates that architectural noise in a luminescence signal is anti-correlated with architectural noise in a reflected signal. That is, the luminescence signal will go down when the reflected signal goes up because of greater reflections from a more even surface. Thus, it would initially appear that subtraction technique should not remove or decrease architectural noise. However, Dunlap and Barnes found that a subtraction procedure decreased noise and improved the luminescence signal.

In fact, the subtraction technique works because the luminescence signal is smeared due to the multiple reflections and absorptions necessary to create it. These tend to spread luminescence through the coral skeleton and, hence, smooth out the architectural contribution to variations in luminescence. Reflectance involves only a few bounces (otherwise it would become luminescence) and consequently the reflectance signal is not smeared. As a result, the reflectance signal defines the architectural structure of the skeleton. The luminescence plus reflectance signal will include architectural noise due only to the reflectance component. Thus, when a reflectance signal is subtracted from a luminescence plus reflectance signal, the resultant has only a small component, or no component, due to architectural noise. The smearing of luminescence can be observed when holes in $CaCO_3$ powder are observed with transmitted UV light rather than under direct illumination with UV light. The hole observed with transmitted UV light is seen mainly because of luminescence and appears blurred, whilst the hole appears sharp when directly illuminated with UV light because what is then seen is mostly reflected blue light.

It is apparent that, if we are to measure near-surface skeletal density from luminescence, we need to correct for variations in the luminescence plus reflectance signal associated with skeletal architecture, and for a large background signal. Results presented here offer a way to make these corrections. It is not possible to measure luminescence without also measuring

reflectance with techniques described here. Thus, a measurement of luminescence actually records luminescence plus reflectance. If the same point on a coral slice is then illuminated with visible light, the light returned will be (overwhelmingly) due to reflectance. A corrected luminescence signal can then be obtained by subtracting such a reflectance signal from the luminescence plus reflectance signal. This procedure offers a way of substantially allowing for the effects of coral skeletal architecture because any architectural effects will be the same in the luminescence plus reflectance signal and the reflectance signal. The procedure also diminishes the large background signal. Additionally, it makes some allowance for differences in reflectance between different regions of the skeleton. Such differences may be associated with differences in colour of the skeleton due to, say, varying skeletal inclusions.

Computer-driven variable monochromators are available that would allow control of both emission and excitation wavelengths in equipment intended to be flexible in measuring skeletal luminescence. Such computer-driven variable monochromators are expensive (around $8000 each) and the need for them is questionable. Fluorimetry is not nearly as wavelength-dependent as spectrometry. Settings away from the excitation and emission peaks give reduced sensitivity but the reduction would be even across all measurements. Obviously it is desirable to obtain maximum sensitivity – and this could be most easily done by using variable monochromators to obtain data for the excitation and emission peaks in measurements on different coral slices. A cheaper, simpler option would be to use fixed excitation and emission wavelengths. The simplest option would be to record light intensity at the wavelength close to known emission peaks and to illuminate the sample at the same wavelength (=reflectance) and at wavelengths close to the excitation peak (= reflectance + luminescence).

Emission wavelengths previously employed have varied from 440 nm to 490 nm (Isdale, 1984; 1995; Boto & Isdale, 1985; Isdale & Kotwicki, 1987; Smith et al., 1989; Susic & Isdale, 1989, Jones, 1990; Klein et al, 1990; Kotwicki & Isdale, 1991; Milne & Swart, 1994; Neil et al., 1995). Wavelengths that have been most often employed are in the range 460 nm to 490 nm. In a systematic and detailed investigation of luminescence of coral skeletons, Wild et al. (in prep.) found that optimum excitation wavelengths were in the range 360-430 nm and these resulted in emission maxima over a broad band from 465 nm up to, at least, 600 nm. These data suggest that, when the excitation maximum is around 370-390 nm (ie, long-wavelength UV light), peak emissions will occur around 490-500 nm.

Accordingly, it seems appropriate to use narrow wavelength filters to control the excitation wavelengths at, say, 380 nm and 490 nm with narrow band pass filters. Computer-driven

wheels are available to allow automatic changing from one filter to the other. Light would be directed onto the coral slice via a bifurcated (Y-shaped) fibre optic. It would be returned to a photomultiplier via another 490 nm narrow band pass filter. In actuality, the optimum wavelength for measurement of emissions will depend upon the variation in response of the detector with wavelength. Consequently, the choice of the higher wavelength filters will be set by the characteristics of the detector.

Suggestions for future research. A prime requirement is for investigations of the optical processes that occur in crystalline powders and result in luminescence and other unexpected optical effects. This seems to be an area of (19th century) physics that has not been explored. We feel uncomfortable because we are not able to explain fully all of the results that we obtained.

There is a need to examine slices taken from a range of corals that grew far removed the influence of land and rivers. Questions here would relate to other environmental factors that might be recorded as luminescent banding imposed on top of the luminescence pattern associated with annual density banding. For example, we have arranged to recover *Porites* colonies from Rowley Shoals and Scott Reef, off the north coast of Western Australia. We wish to see if monsoonal and cyclonic rains over shallow, well-enclosed lagoons far removed from land can lower salinity sufficiently to introduce luminescent lines. Corals from these and other sites might indicate if luminescent lines can also be caused by factors, other than lowered salinity, that may affect calcification, such as periods of unusual temperature.

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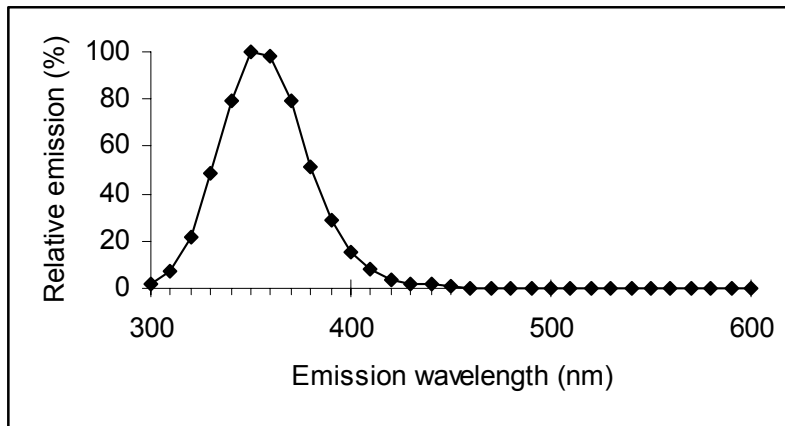
Fig. 1. Emission spectrum of ultraviolet fluorescent tube used to display luminescence in coral skeletons and skeletal and $CaCO_3$ powder.

Colour	Central wavelength (nm)	Range in wavelength (nm)
Violet	410	400 - 424
Blue	470	424 - 491
Green	520	491 - 575
Yellow	580	575 - 585
Orange	600	585 - 647
Red	650	647 - 700

Table I. Wavelengths of light associated with colours in the visible spectrum. From the CRC Handbook of Chemistry and Physics, (60th Edition, 1980), p. E214.
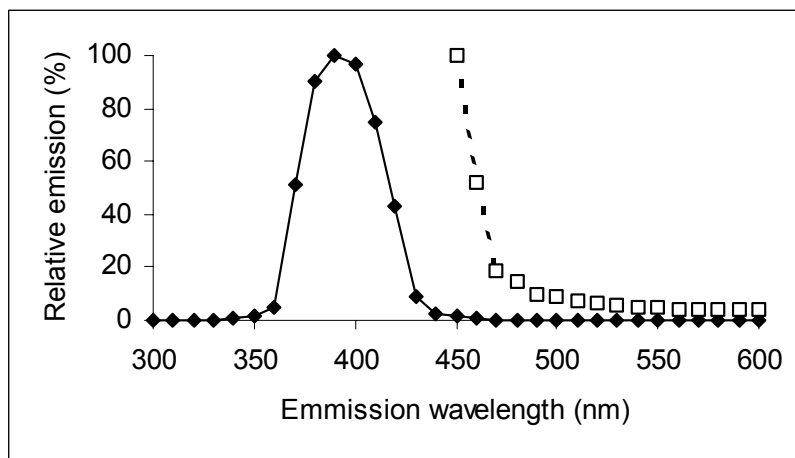
Fig. 2. Emission spectrum of Turner spectrofluorimeter used to measure fluorescence in coral skeletons and skeletal and $CaCO_3$ powder. Excitation wavelength set to 390 nm. Dotted line shows emission spectrum from 450-600 nm with intensity at 450 nm set to 100%
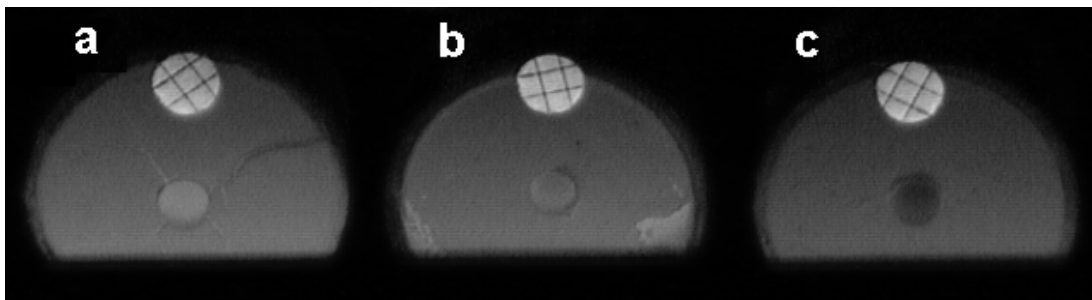
Fig. 3. Images of laboratory grade $CaCO_3$ powder under UV light. Illumination provided by a ?? W UV fluorescent tube. The dark area at the bottom of each image is a shade above the UV fluorescent tube. Each vial of powder has an identical 4 mm diameter, 13 mm deep hole at its centre. These are separate images of the powder at the same position under the centre of the UV fluorescent tube. Images captured with a video camera. (a) $CaCO_3$ powder alone, (b) and (c) $CaCO_3$ powder with addition of 0.012% and 0.1% by weight, respectively, of activated charcoal powder. The surface graded from blue-grey (a) to purple (c) and the lighter region within the holes (a & b) was yellow. The disc at the top of each image is cut from graph paper with 2 mm squares and confirms even exposure between the images.

Mass of charcoal: mass of $CaCO_3$ (%)	Yellow light apparent in hole (%)
0	100
0.012	50 -70
0.1	10 -20
0.2	5
0.3	< 5
0.4	< 1

Table II. Subjective assessment of the amount of yellow light emerging from 4 mm diameter, 13 mm deep holes in laboratory grade $CaCO_3$ powder.
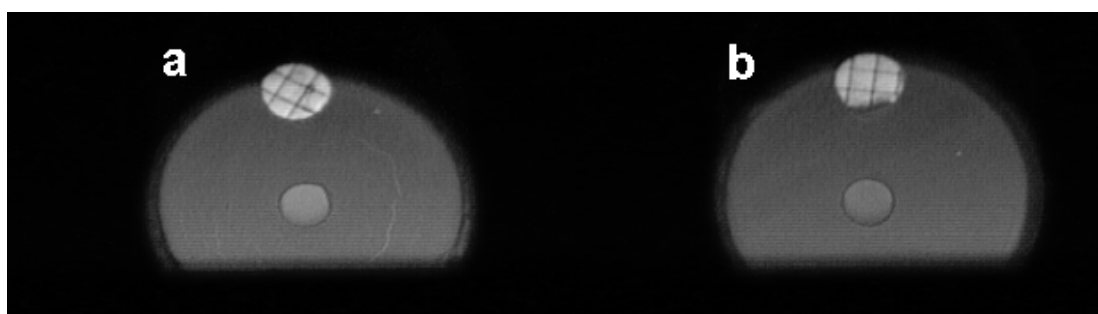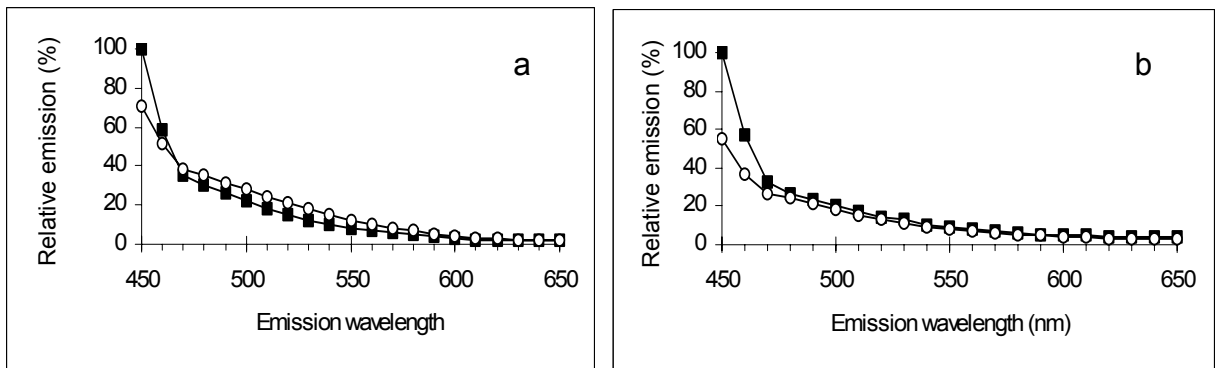

Fig 4. Images of laboratory grade $CaCO_3$ powder under UV light. Each vial of powder has an identical 4 mm diameter, 13 mm deep hole at its centre. (a) Untreated powder. (b) Powder heated to 450 $^{\circ}$C for 2 h. Other details as for Fig. 3.
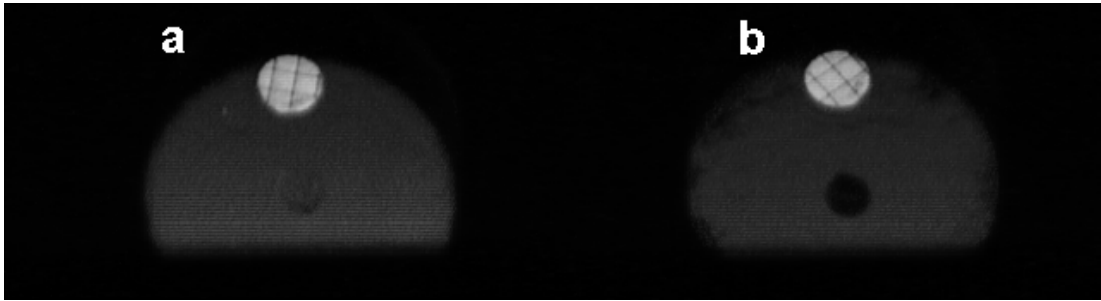
Fig 5. Images of powdered coral skeleton under UV light. Each vial of powder has an identical 4 mm diameter, 13 mm deep hole at its centre. Skeleton came from an offshore coral collected at Reef 21-141 (see text). (a) Untreated skeletal powder. (b) Skeletal powder heated to 450 °C for 2 h. Other details as for Fig. 3.
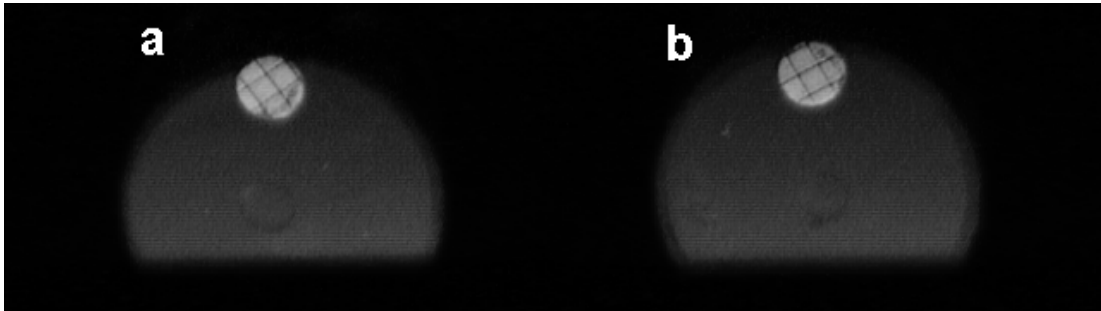
Fig 6. Images of powdered coral skeleton under UV light. Each vial of powder has an identical 4 mm diameter, 13 mm deep hole at its centre. Skeleton used represented growth from 1981-85 bands in a coral from a reef close to the mainland at Pipon Island (see text). (a) Powder made from non-luminescent bands. (b) Powder made from luminescent bands. Other details as for Fig. 3.
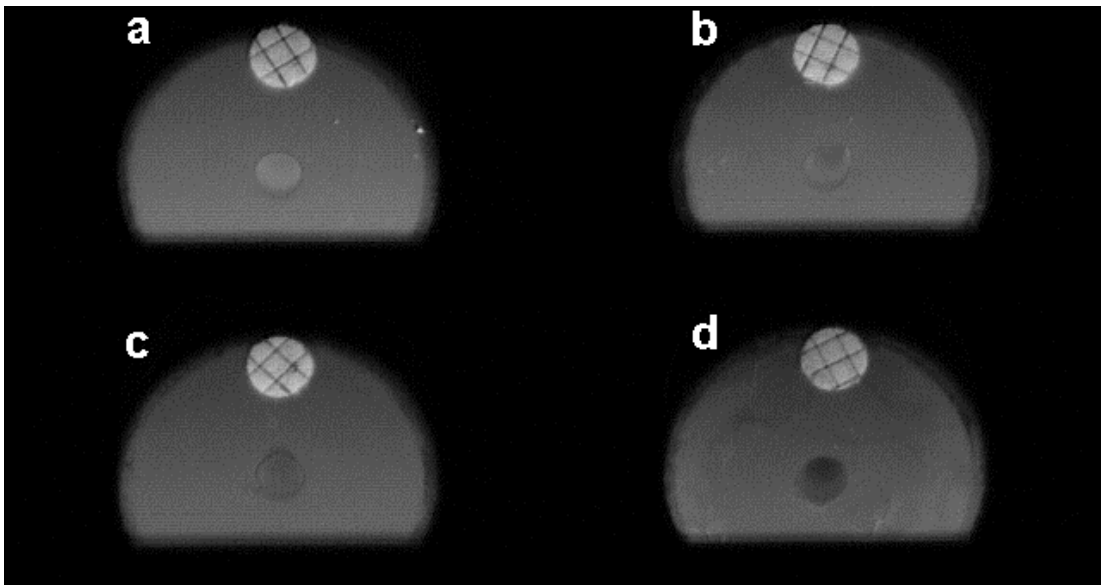
Fig. 7. Images of laboratory grade $CaCO_3$ powder under UV light. Each vial of powder has an identical 4 mm diameter, 13 mm deep hole at its centre. Sets of powder previously made into a paste and dried. (a) Paste made with distilled water alone. (b, c, & d) pastes made with humic acid dissolved in distilled water to give a mass of humic acid ($\mu g/g$ $CaCO_3$ after drying) of 25.5 $\mu g/g$, 51.6 $\mu g/g$ and 150 $\mu g/g$. Other details as for Fig. 3.
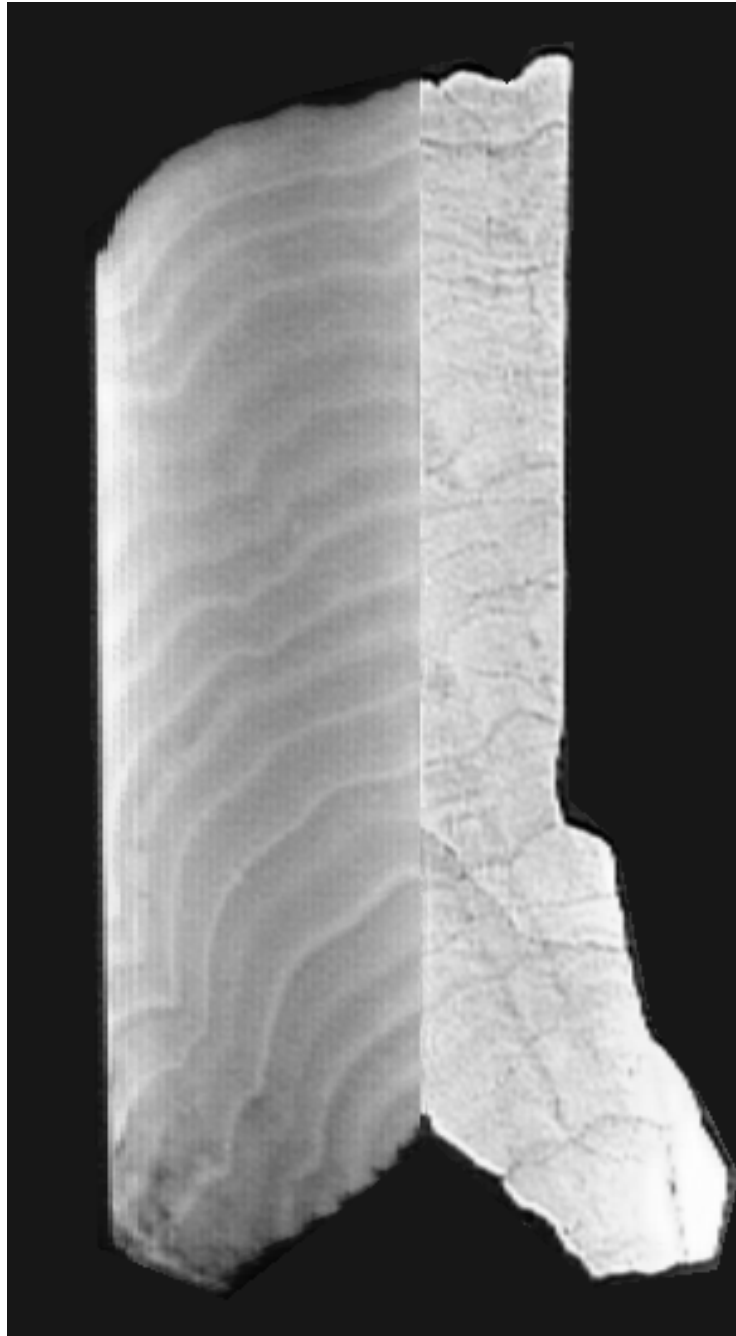
Fig. 8. Composite image of adjacent regions of a skeletal slice cut from a colony collected at Double Island. The skeletal section of the left was photographed under long wavelength ultraviolet light and shows luminescent lines. The section on the right was heated to 450 $^{\circ}$C to carbonise organics and alter the reflective properties of the skeleton. Dark bands in the section on right align with luminescent lines in the section on the left. The dark bands are mostly due to these areas having lower skeletal density. They appear dark because of greater numbers of bigger holes and cavities.
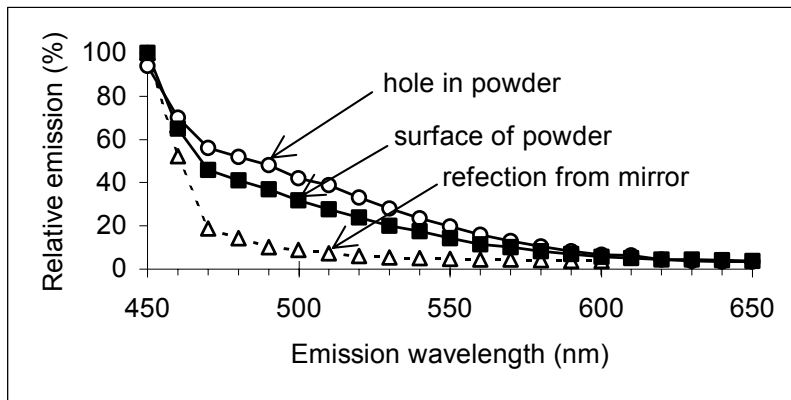
Fig. 9. Emission spectrum of laboratory grade $CaCO_3$ powder excited at 390 nm compared with the spectrum of light reflected from a glass mirror (cf, Fig. 2). Measured with a 3 mm diameter fibre optic. Black squares = surface of powder; open circles = hole in powder; open triangles = reflection from a glass mirror.

Fig. 10. Emission spectra of laboratory grade $CaCO_3$ powder containing (a) 0.012% charcoal and (b) 0.1% charcoal. Excited at 390 nm. Black squares = surface of powder; open circles = hole in powder.
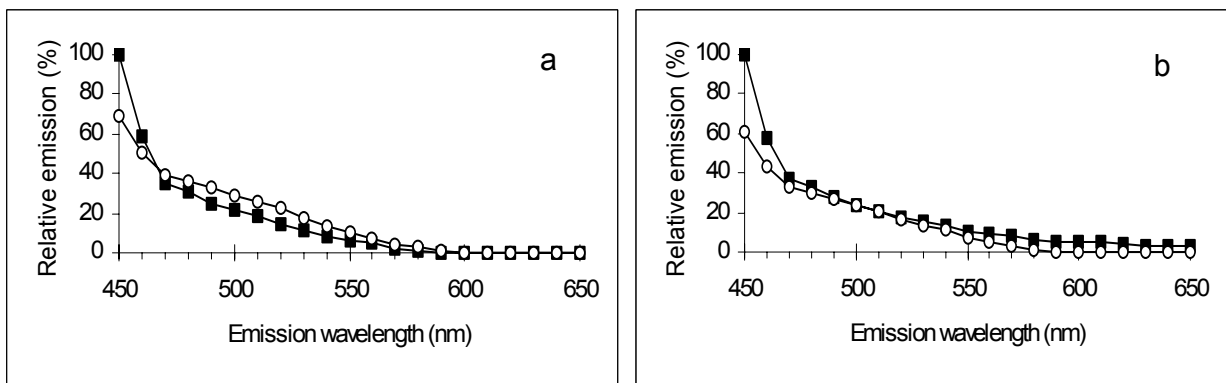
Fig. 11. Emission spectrum of laboratory grade $CaCO_3$ powder treated with (a) distilled water and then dried, and (b) powder treated with humic acid solution and then dried. Powder coated with humic acid to give a final concentration of 150 μg humic acid per g $CaCO_3$. Excited at 390 nm. Black squares = surface of powder; open circles = hole in powder.
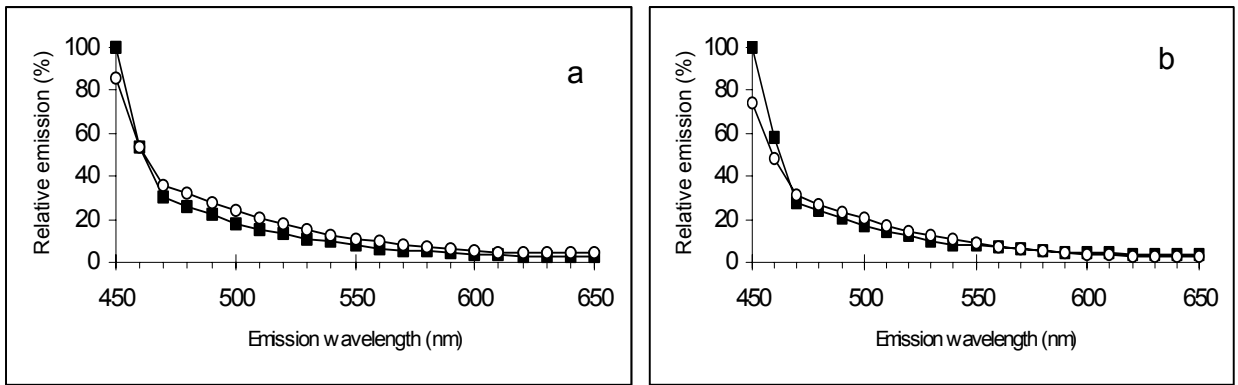
Fig. 12. Emission spectra of powders made from (a) non-luminescent bands and (b) luminescent bands cut from a slice of *Porites* sp. from Pipon Reef. Excited at 390 nm. Black squares = surface of powder; open circles = holes on powder.
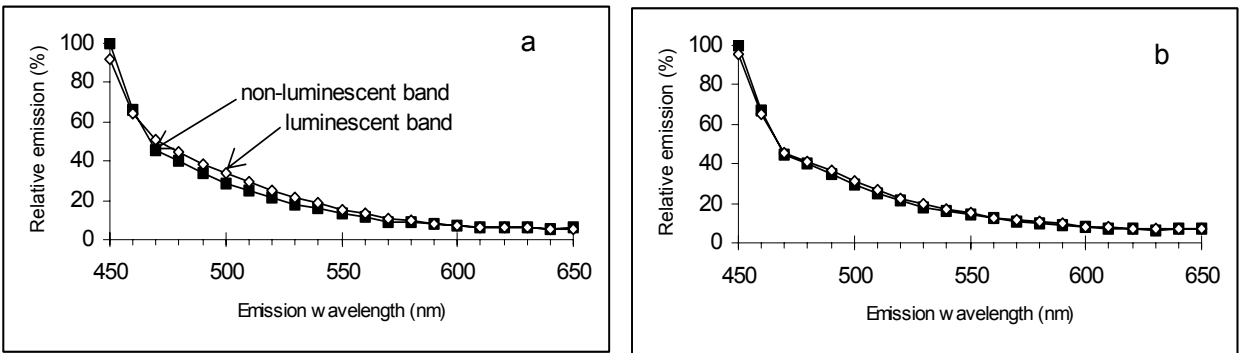
Fig. 13. Emission spectra of non-luminescent bands (black squares) and luminescent bands (open diamonds) in coral slices; (a) 1979 bands in a slice from a coral at Pipon Reef (PIP_B01_S3), (b) 1984 bands in a slice from a colony collected from Eel Reef (EEL_B10_S1). Excited at 390 nm.
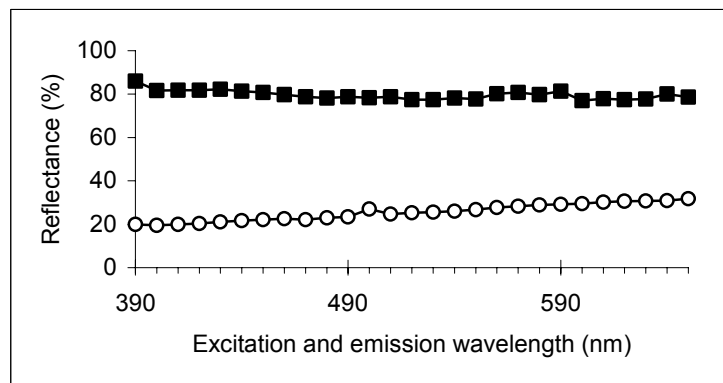
Fig. 14. Reflectance from the surface (black squares) and a hole (open circles) in laboratory grade $CaCO_3$.
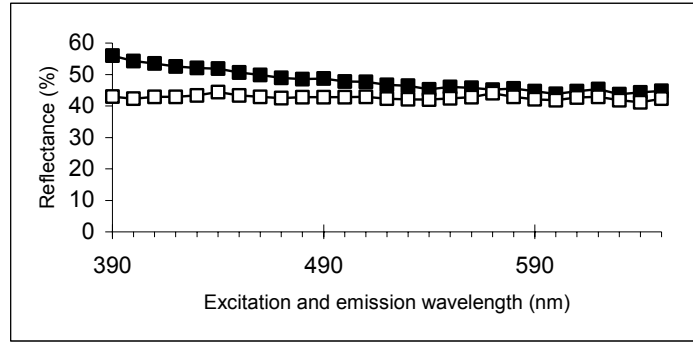
Fig. 15. Reflectance from the 1979 luminescent band (open squares) and the following non-luminescent area (black squares) in a slice of *Porites lobata* from Pipon Island (PIP_B10_S3). Measured with the combined end of the Y-shaped fibre optic reduced to 1 mm diameter.
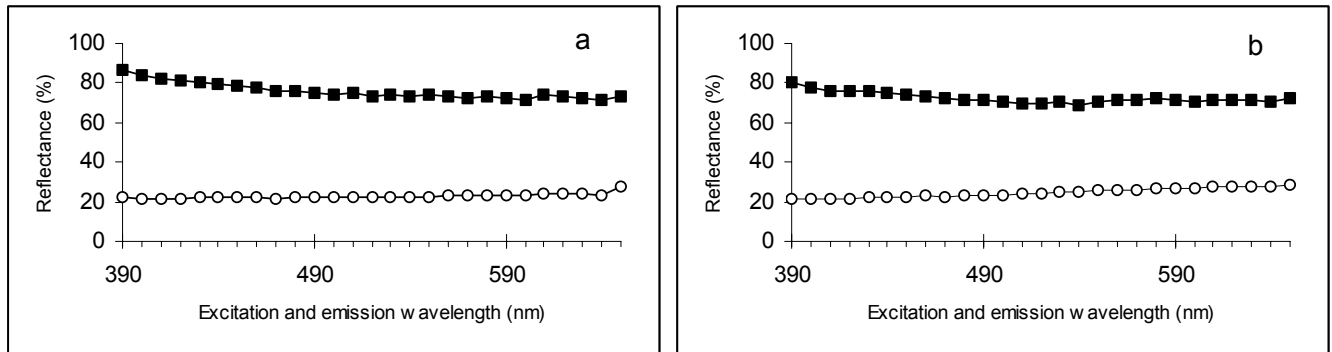

Fig 16. Reflectance from the surface (black squares) and a hole (open circles) in powder made from luminescent bands (a) and non-luminescent bands (b) from a skeletal slice from a colony of *Porites lutea* collected at Pipon Island (PIP_B05).
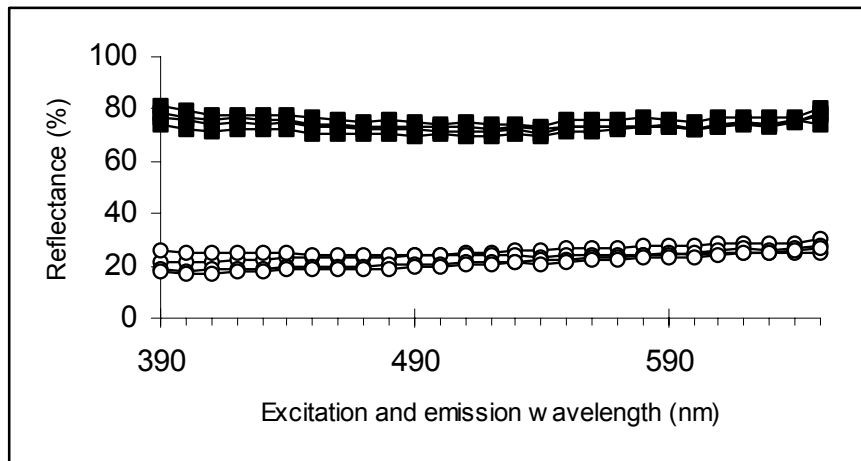
Fig. 17. Reflectance of surface (black squares) and holes (open circles) in laboratory grade CaCO3 powders coated with 0, 25.5, 51.6 & 150.0 μg humic acid per g $CaCO_3$.
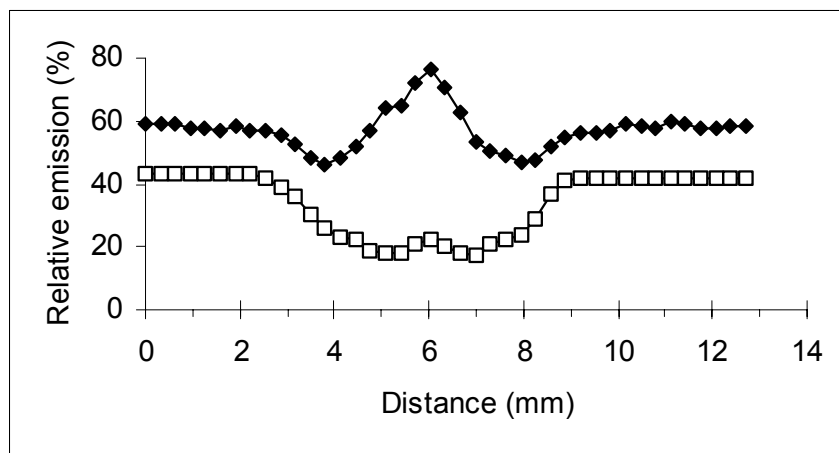
Fig. 18. Emissions recorded at 490 nm with the excitation wavelength set to 390 nm (black diamonds) and 490 nm (open squares). The 3mm diameter combined end of the Y-shaped fibre optic was tracked across a 4 mm diameter, 13 mm deep hole in laboratory grade $CaCO_3$ powder. The values have been adjusted so that relative emission from the surface of the powder is around 60% at 390-490 nm and around 40% at 490-490 nm.
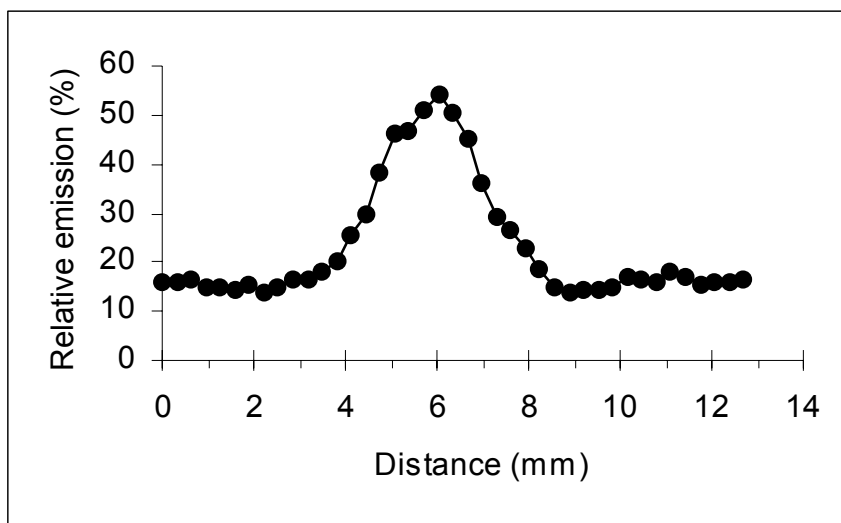
Fig. 19. Luminescence along a track across laboratory grade $CaCO_3$ powder containing a 4 mm diameter, 13 mm deep hole.
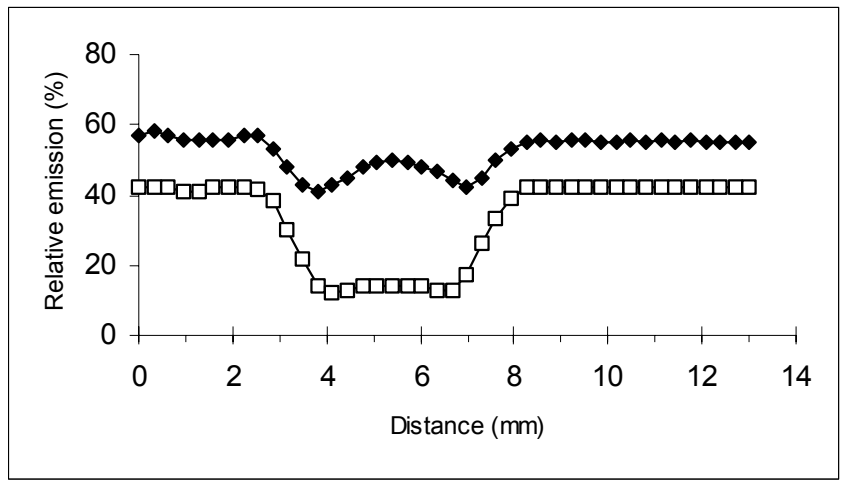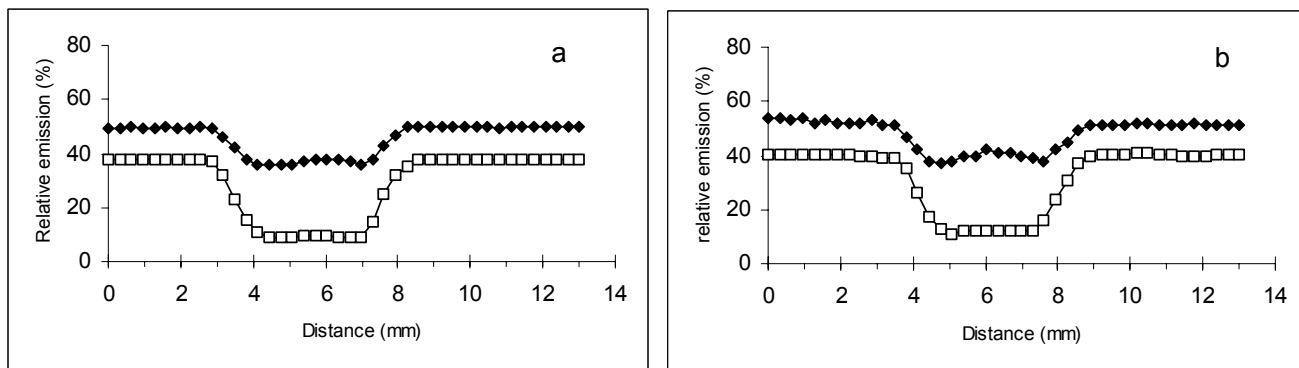
Fig. 20. Emissions from laboratory grade CaCO$_3$ powder with a 4 mm diameter times 13 mm deep hole. The combined end of the Y-shaped fibre optic was 1mm in diameter. See Fig. 2-back for other details.
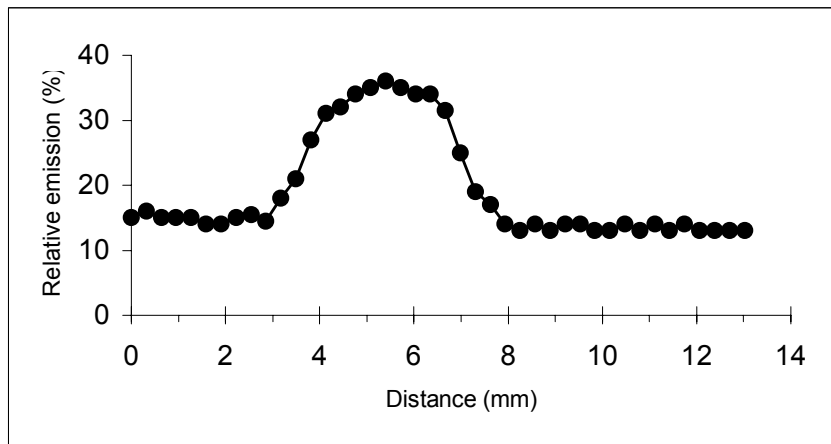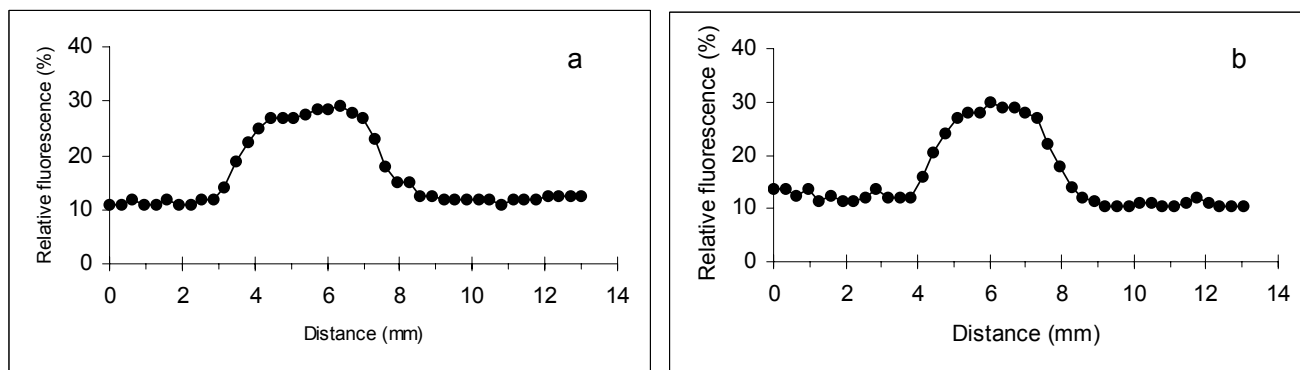

Fig. 21. Luminescence measured with a 1 mm diameter fibre optic along a track across laboratory grade CaCO$_3$ powder containing a 4 mm diameter, 13 mm deep hole.

Fig. 22. Emissions from laboratory grade CaCO$_3$ powder with a 4 mm diameter times 13 mm deep hole. (a) powder containing 0.1% charcoal by mass. (b) powder coated with humic acid to give a concentration of 0.015% by mass The combined end of the Y-shaped fibre optic was 1mm in diameter. See Fig. 12 for other details.

Fig. 23. Fluorescence emission measured with a 1 mm diameter fibre optic along a track across laboratory grade CaCO$_3$ powder containing a 4 mm diameter times 13 mm deep hole. (a) powder containing 0.1% charcoal by mass. (b) powder coated with humic acid to give a concentration of 0.015% by mass.

White powder	Luminescence in hole
$CaCO_3$	Strong yellow
$CaCl_2.2H_2O$	Weak yellow
CaO	None
$MgCO_3$	Strong yellow
$MgSO_4.7H_2O$	Weak blue-green
$MgNO_3.6H_2O$	Weak blue-green
Na_2CO_3	Faint yellow
Na_2SO_4	Faint light blue
$NaNO_2$	None
$NaHCO_3$	Yellow
$Na_2B_4O_7$	Blue-green
Na_2MoO_4	Green-yellow
$Na_2H_2PO_4.2H_2O$	Blue-green
$Na_3PO_4.12H_2O$	None
K_2HPO_4	Blue-green
K_2CO_3	Trace
K_2NO_3	Trace
K_2SO_4	Trace
$KHCO_3$	Trace

Table III. Luminescence displayed in holes pressed into the white powders of some common laboratory chemicals illuminated by long wavelength ultraviolet light.