

Determination of seasonal skeletal growth layers in *Porites lutea* colonies from Teluk Nyior reefs, Langkawi Island, Malaysia

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Abstract. This study includes seasonal layer study of *Porites lutea* using combination of alizarin staining and UV illumination methods and the correlation with climate data. The growth layers were recognized by using alizarin staining method, which was marked on live tissue of corals *P. lutea*. Under the UV-light, dark fluorescent layers represented the wet season (June to November) and light fluorescent layers corresponded to dry season (December to May). The results revealed that the alizarins integrated into newly forming skeleton which looks as pink color. The color remains as a permanent implant indicating the place of calcification during the experiment. Seasonal layers (wet and dry season) can be used to determine the skeletal growth thickness yearly, since the season cycles are relative regular in whole year. Recognizing and ascertaining the dark and light fluorescent layers were useful for retrospectives analysis of coral age prediction.

Key Words: alizarin, fluorescent, coral growth, *Porites lutea*, season.

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Introduction

Long-lived massive corals can contribute to the monitoring of coral reef environment through measurable growth records in the annual growth layer thickness. Annual growth layer pattern in massive coral skeletons provide means to “retrospectively monitor”. These can provide a historical perspective, which is able to determine environmental changes and can help to establish limits of coral growth. Furthermore, records from corals may provide information about long-term variability in the performance of coral reefs, allowing unnatural changes to be distinguished from natural variability.

The studies of skeletal growth in massive coral have been intensively reported (Buddemeier & Kinzie 1975; Barnes *et al* 2003; Carricart-Ganivet 2004; Gourbert *et al* 2001; Lough & Cooper 2011). Coral growth determinations using alizarin staining method have been investigated by Lamberts (1978). Technically, the annual increments of growth of shallow-water of *Porites lutea* were calculated using seasonal fluorescent banding (revealed by UV-light) and alizarin staining. The timing and seasonality of skeleton construction has been calibrated using alizarin marked coral (Mizrachi *et al* 2010). There have been written many publications employing alizarin for coral growth staining. However, there are scarce reports on combination of the alizarin staining method with UV artificial luminescence for determination of coral seasonal layers particularly

on *Porites lutea*. Hence, the objective of the present study was to investigate the coral growth layer and also intends to ascertain seasonal layers in *P. lutea* skeletal using alizarin staining. This paper is contributing the advance techniques to study the annual coral growth pattern of massive corals.

Material and methods

Study area

The study was carried out at Teluk Nyior reef flat, Langkawi Island, Malaysia. The island is situated in Strait of Malacca, at latitude 6° 26' N; longitude 99° 45' E on the west coast of Peninsular Malaysia (Figure 1) and the detail map of the sampling location was presented in Figure 2. The site is approximately 40 km from the main town of Kuah, Langkawi Island. The reef slope, where the studies have been made is visibly flat.

Alizarin staining

Samples of *P. lutea* with size 20-30 cm diameter were collected from study sites seasonally. Approximately 22 colonies were stained with 10-ppm alizarin red for 24 hours (Lamberts 1978; Harriot 1999) during for both wet (17 October 2001) and dry season (17 January 2002). Staining *Porites* colonies during wet and dry season were done to recognize characteristic of growth layers. After staining the corals were placed on their original location and allowed to grow until completely one year.

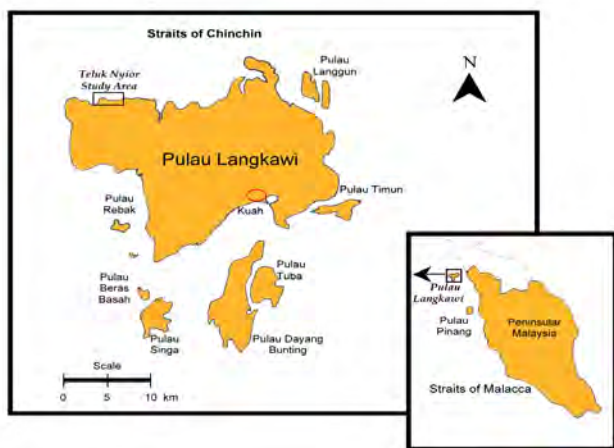


Figure 1. Map of Pulau Langkawi, showing the Teluk Nyior study area.

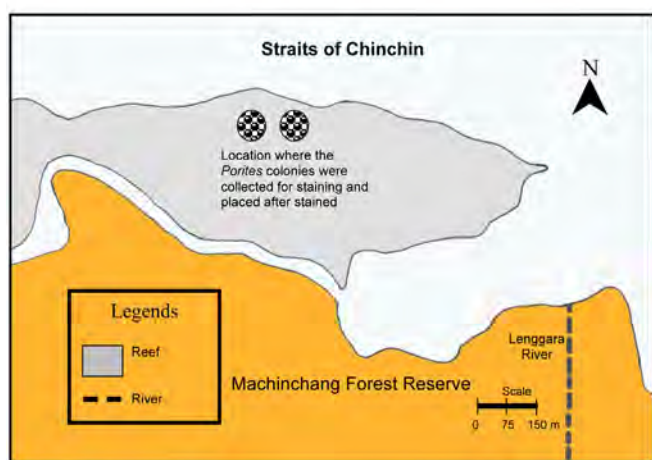


Figure 2. Description of place where *Porites* colonies were collected on Teluk Nyior reef, Langkawi Island.

Coral sample cutting

One year after staining, the samples were collected and sent to the laboratory. The samples were cut vertically using an electric saw (Model L80P, Light outer, S. D. K. Co. Ltd) in a dark room to avoid light oxidation reaction on organic material in the coral skeletal. At least 2 cm thick slices were removed from the center of the colony (Figure 3). The corals slices were washed by distilled water and then air-dried. UV artificial luminescent was used to observe seasonal growth layers pattern using UV-lamps (4 black tube x 20 watt) (Figure 4).

Results and Discussion

Seasonal layers

Fifty-two colonies of *Porites lutea* have been alizarin stained, twenty-two colonies of which were stained during wet season and the thirty colonies were stained during dry season. The samples were placed in Teluk Nyior reef for one-year growing from the date of staining, then after one year the samples were re-collected. However, only thirty-six colonies were taken, which were thirteen colonies of wet season treatment and twenty-three colonies of dry season samples. Sixteen colonies have been lost in the sea, there were moved by sea current. Annual growth layers of *P. lutea* have been observed in skeletal slices. The growth

layers were recognized by using Alizarin staining method, which was marked on live tissue of corals *P. lutea* (Lamberts 1978; Mizrahi *et al* 2010; Swart *et al* 2002). The alizarin was used to definite seasonal layer in *P. lutea* skeletal.

Alizarin in *P. lutea* skeletal was marker permanently, which can be directly observed in skeletal slices of *P. lutea* samples and photographed. One year after staining, the pink line of alizarin was clearly observable in *P. lutea* skeletal. Under the UV-light, dark and light fluorescent layers can be differentiated. Alizarin line stained on coral colonies during wet season (17 October 2001) appears in dark fluorescent layers as shown in Figure 5. Unlike in wet season treatment sample, alizarin line stained during dry season (17 January 2002) appears in light fluorescent layers as shown in Figure 6. These results indicated that dark layers in *P. lutea* skeletal from Teluk Nyior reef were deposited during wet season (June to November) and light layers form during the dry season (December to May).

It is interesting to note that the alizarin stain stained in wet season exhibited the line mark on the dark area (under UV-light) in coral skeletal as shown in Figure 5. This line marks are consistent in other samples. Seventy percent of the samples in this treatment show a consistent position of alizarin line mark, which was on dark area. Unlike wet seasonal samples, the alizarin mark

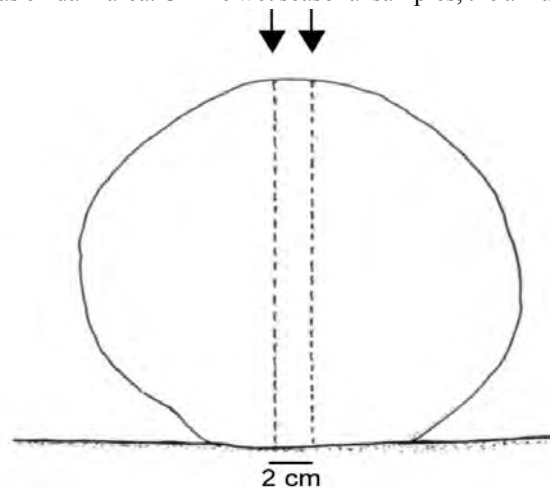


Figure 3. Illustration of a *Porites* colony sliced vertically.

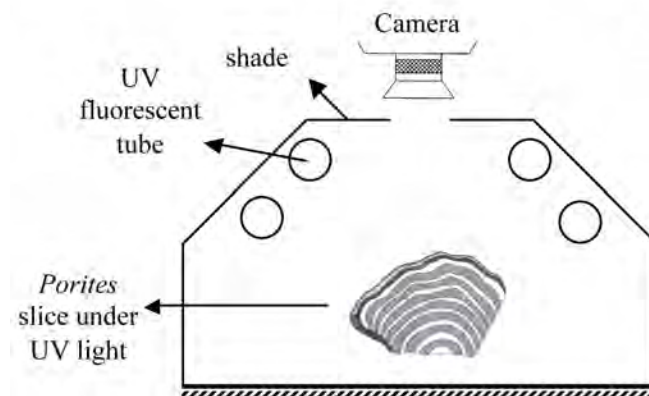


Figure 4. Illustration of mechanism to determine seasonal Layers using UV-light.

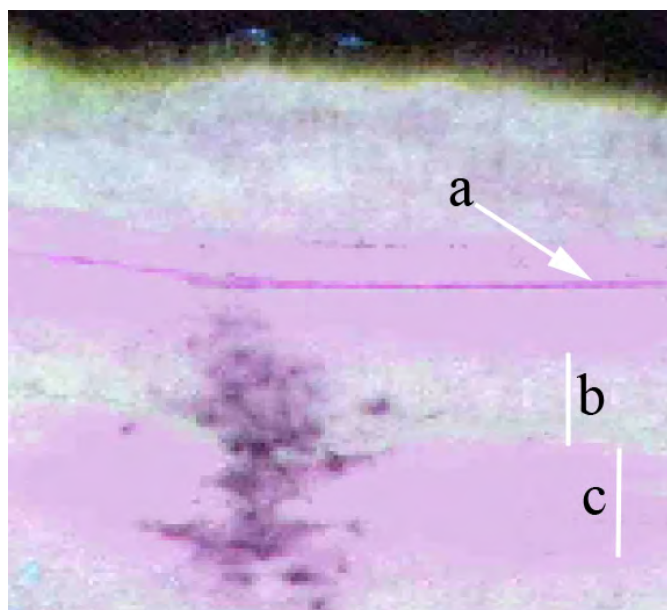


Figure 5. The *Porites* skeletal slice showing alizarin-stained line on wet season (a). The stained line was located in dark area of fluorescent layers; (b) Light fluorescent layer, (c) Dark fluorescent layers.

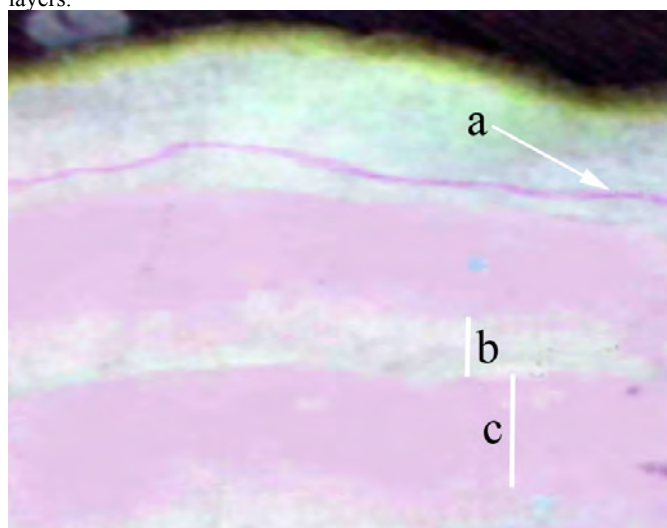


Figure 6. The *Porites* skeletal slice showing alizarin-stained line on dry season. (a). The stained line was located in light area of fluorescent layers; (b) Light fluorescent layer, (c) Dark fluorescent layers.

of coral stained in dried season however appeared on the light area (under UV light) of coral skeletal (Figure 6). This is also consistent on the other dried seasonal treatment samples. About eighty percent of samples consistently showed the alizarin mark on the light area as shown in Figure 6. This finding is consistent with previous report employing X-ray diffraction to observe the skeletal layers. As state by Scoffin *et al* (1989) that the dark area of fluorescence bands were formed on the wet season.

Living corals *P. lutea* were subjected to alizarin stain, which is dissolved in seawater appear as purple color. The alizarins integrated into newly forming skeletons, which look as pink color. The color remains as a permanent implant indicating the zone of calcification during the experiment. The pink line in *P.*

lutea skeletal samples was varying in evident, 70% of samples colonies were bright clear and the rest samples have blur pink line in their skeletal. As Lamberts (1978) suggested that alizarin coloration is influenced by the total amount of living tissue depositing the dye, the concentration of the dye in solution, length of exposure to the dye, and biological activeness of the calcifying tissue. Dark layer in *P. lutea* skeletal might be correlated to the absorption of humic acids produced by plants and flowed by fresh water run-off to the sea as reported by Isdale (1984).

Having identified in this study that the dark area represents coral growth in the wet season and the light area is in dry season, it is possible to predict the age of corals on the basis of dark and light layer. Since the season cycles are relative regular in whole year. Several authors including Buddemeier & Kinzie (1975), Barnes & Lough (1993), Hudson *et al* (1994) and Priess *et al* (2002) have introduced these predictions. Furthermore, Wolanski *et al* (2004) predicted changes in salinity, nutrient, pH, incident light, temperature or various changes in the chemical constituents of seawater on the basis of seasonal layer.

Recognizing and ascertaining the dark and light fluorescent layers were useful for retrospectives analysis of coral age predicting. In addition, dark and light fluorescent layers in *P. lutea* skeletal from Teluk Nyior reef may be the effect by terrestrial runoff. These layers also have the potential to provide information of regional variations in rainfall, river flow and changes in land. They also provide a means to assess the risk to corals, coral communities and corals reefs from runoff and changes in land-use, pollution, oceanography, and coastal and reef management (Al-Rousan *et al* 2007; Ahmad *et al* 2011; Schiedek *et al* 2007).

Conclusions

The alizarins integrated into newly forming skeletons, which look as pink color. The color remains as a permanent implant indicating the calcification place during the experiment. The pink line in *P. lutea* skeletal samples was varying in evident, 70% of samples colonies were bright clear and the rest samples have blur pink line in their skeletal. The alizarin stained line can be used to determine the skeletal growth thickness, to ascertain seasonal layers (wet and dry season). Dark fluorescent layers represent coral growth in wet season and light fluorescent layers correspond to the growth in dry season.

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