

# Turnitin-The measurements of calcification rates in reef corals using radioisotope $^{45}\text{Ca}$

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# The Measurements of Calcification Rates in Reef Corals Using Radioisotope $^{45}\text{Ca}$ at Pongok Sea, South Bangka

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## ABSTRACT

Coral reef ecosystem is one of the most important ecological systems in the Indonesian coastal zone. The aim of this study which was undertaken between August - October 2006, is to measure the calcification of corals in a reef coral in the Pongok Sea, South Bangka using  $^{45}\text{Ca}$ . The steps in conducting this study were surveying of the site, preparation, transplanting, incubation in the  $^{45}\text{Ca}$  - solution, and analysis of the coral fragments. The results showed that at the depth of 5 m different counts per minute (cpm) trend occurred. For the samples taken from the transplantation of the Artificial Colony (Ac) the cpm showed that with the progress of time the cpm declined, reaching its lowest cpm at 5 hours after retransplanting of the coral fragments. On the other hand the samples obtained from the natural (Nc) colony showed that the cpm increased with time progress. At the 10 m depth where only the coral fragment of the natural colony (Nc) was observed a different pattern showed up. Here with the progress of time up to 3 hours the cpm increased and after that it declined to reach a low cpm at 5 hours of observation. The cpm values were then transformed to disintegrations per minute (dpm),  $\mu\text{Ci}$  and at the end to  $^{45}\text{Ca}$  content. The same trend is shown for dpm,  $\mu\text{Ci}/0.5$  g sample and  $\mu\text{g Ca}/0.5$  g sample. The  $^{45}\text{Ca}$  content ( $\mu\text{g}/0.5$ g sample) were used to show the calcification rates of coral fragments. It showed clearly that  $^{45}\text{Ca}$  could be used to calculate the magnitude of calcification.

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## INTRODUCTION

Coral reef ecosystem is one of the most important ecological systems in the Indonesian coastal zone. Indonesian's coral reef is the most extensive in the Southeast Asia region. The area of reef corals in Indonesia is around 50,000 km<sup>2</sup> found on 841 sites, consist of 5.23% in very good condition, 24.26% on good condition, 37.34% at condition between good and worse and 33.17% at worse condition [1]. Eighty-two percent of this large area is in danger to be demolished by bombing and the use of potassium for reef fish catching and to take the corals for high sale prices. The rehabilitation of the reef corals will take time but is urgently needed to be done. The building up and the rehabilitation of the coral reef could be carried out using several methods, such as, building artificial reef [2]; reef transplantation [2,3]; use of electric stimulation to enhance reef growth [4].

The transplantation method is one of the most methods used, especially when material for artificial reef building is not available. The transplantation method is done by cutting off a part of donor reef corals and transplant at the rehabilitation site of the reef corals. The transplantation could be done but the measurement of the calcification rates of the corals transplanted is something more difficult to be done. The measurements of calcification in the past have been done by red alizarin and weighing methods. The weighing method is quite simple, the corals are taken from the reef and their volume and weight are measured from time to time. With the time development, it is expected that especially the weight of the corals will increase due to the calcium deposition. This method faced an obstacle which could not be removed which was that with the passing of time, holes will appear in the corals. These holes will of course lessen the coral weight regardless of time progress. These holes are speculated made by the sea creatures who live within the reef corals. So this method could not be relied on.

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Another method was developed which is the measurement of calcification rates of corals in reef corals colony. The calcification rate is a complex and dynamic process which is completely controlled by outside factors. The main factors are environment temperature and light. To be able to measure the calcification rate more accurately is using of  $^{45}\text{Ca}$ . The use of  $^{45}\text{Ca}$  is to determined the calcification rate in quantity in matter of hours. The  $^{45}\text{Ca}$  is used to measure calcification rates with the progress of time. The calcification process which is measured by its calcification rates is an assumption that there is growth in the coral reefs. This study was done to measure the calcification rates of corals in a reef coral in the Pongok Sea South Bangka using  $^{45}\text{Ca}$ .

## MATERIALS AND METHOD

### Site and time of the experiment

The study was carried out at coral reef ecosystem of Pongok Sea South Bangka, from August to October 2006. The coordinate of the site at sea is  $2^{\circ} 49' 48''$  S and  $107^{\circ} 02' 24''$  E as shown in Fig. 1. Figure 1 and 2 are the sites of the area where the coral fragments were taken from and the boat where the coral fragments were incubated in the  $^{45}\text{Ca}$ - solution.

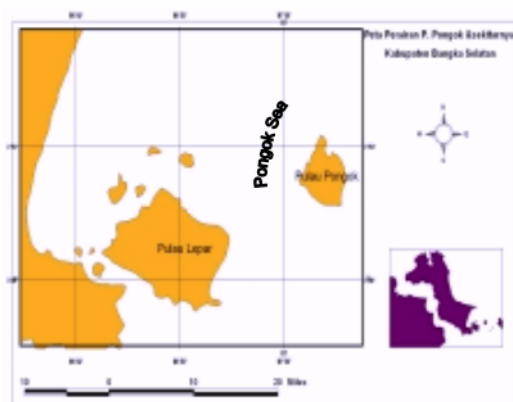


Fig. 1. The study site at Coral Reef Area of Pongok Sea, South Bangka.

The steps in conducting this experiment were done by starting with, survey of the site, preparation of the coral fragments, incubation of the coral fragments in the  $^{45}\text{Ca}$ -solution and ended by analyzing of the coral fragments.



Fig. 2. Preparation and transplantation of the coral fragment.

### Equipment used in the experiment

The main equipments used in this study is for incubation of the coral fragment in  $^{45}\text{Ca}$  solution were fluorescent lamps, lux meter, glass vials, aluminium foil, hand refractometer, counting vial, balances, gamma spectrometer, hot plate, sieve (2 mm diameter size), aerator, aquapro filter 5  $\mu\text{m}$ , stopwatch and incubator.

The materials used were radioisotope  $^{45}\text{CaCl}_2$  130  $\mu\text{Ci}/10\text{ml}$ , Aquadest (pH 7), and coral fragment.

### Coral fragments preparation

Prior to the incubation treatment the coral colony was transplanted on steel trays at sites which have been determined previously. The growth of the corals was observed visually two months before incubation with  $^{45}\text{Ca}$  took place. This was done to check whether the transplanted coral colony was still alive. The trays were put at a 5 and 10 m depth in the sea water. After cutting the coral fragments, they were put in glass vial with fresh sea water and then treated for  $^{45}\text{Ca}$  incubation.

### Coral fragments incubation in $^{45}\text{Ca}$ solution

The coral fragments collected were dissolved in fresh sea water for one hour prior to  $^{45}\text{Ca}$  incubation. Thereafter,  $^{45}\text{CaCl}_2$  at a rate of 13  $\mu\text{Ci}/\text{ml}$  was added to the sea water. The incubation

in the radioactive solution was done for 8 hours. The fluorescent lamp was put at a 1 m height above the incubation container. An aerometer was put into the incubation container to supply oxygen (O<sub>2</sub>) continuously during the whole incubation process.

The incubation process was done following the method of van der Meulen and Muscatine [5] with same alteration.

### Transplantation of the coral fragments

After the 8-hour incubation period in the <sup>45</sup>Ca solution, the coral fragments were transplanted to their original colony site. The coral sites were at a 5 and 10 m depth. At the 5 m depth, the colony consisted of an artificial (using trays) and natural site. On the other hand at the 10 m depth only a natural colony was maintained. The coral fragments were left for 1, 3, and 5 hours after transplanting, at the site where they were taken from, before the harvest was carried out.

### Harvesting of the coral fragments

As mentioned before the coral fragments which have been incubated in <sup>45</sup>Ca before transplanted at the original coral colony were harvested at 1, 3 and 5 hours after transplanting. Thereafter, the samples of the coral fragments were brought to the isotope lab of PATIR (The Center of Radioisotope and Radiation Technology), BATAN, Jakarta. Here the coral fragments samples, were analysis for their <sup>45</sup>Ca content. The calcification rates are based on the <sup>45</sup>Ca content in the coral fragments. Low or high <sup>45</sup>Ca content is used as an indication of the low or high calcification rates.

### Analysis for <sup>45</sup>Ca content

In the lab, the coral fragments were dried using an oven until they reach a constant dry weight. After drying the coral fragment were grinded to pass 2 mm sieve. A 0.5 g of coral fragments samples were taken and ashed in a furnace at 650°C for 12 hours. The coral samples were dissolved in 17 ml concentrated HCl and heated using a hot plate at 100°C till the solution becomes clear. A one ml solution was taken and put into counting vials and 14 ml water was added to each counting vials. The counting vials were then counted for the <sup>45</sup>Ca content of each sample. For counting a liquid scintillation counter was used. The counting vial consisting liquids of back ground, samples, and standard were put into the sample holder of the Liquid Scintillation Counter and was operated automatically, resulting in the cpm numbers of the

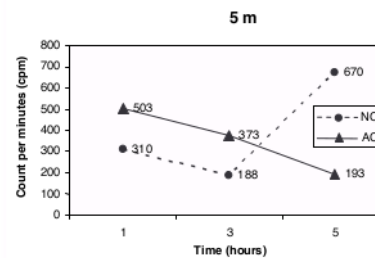
background, samples and standards. The analysis of <sup>45</sup>Ca from the counting vials was stated in cpm (counts per minute). The cpm values were transformed to dpm (disintegrations per minute), by dividing the number of cpm by the efficiency factor of the liquid scintillation counter which in this case was 0.5 and thereafter to  $\mu\text{Ci}/0.5$  g samples and at last to  $\mu\text{g Ca}/0.5$  g samples. Each sample is counted in duplo to get an average of the cpm.

## RESULTS AND DISCUSSION

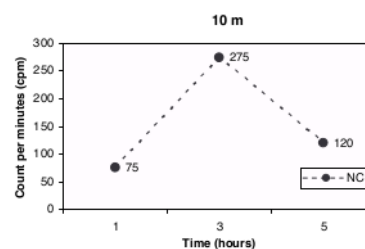
### Results

The results of this experiment are presented in Figs. 3,4,5 and 6. The counts per minute (cpm) of the coral fragment sample as one of the main parameter for calcification is the base for the other parameters. Fig. 3a and 3b show the cpm of the coral samples.

Figure 3a shows that at the depth of 5 m different cpm trend occurred. For the samples taken from the transplantation of the Artificial Colony (Ac) the cpm showed that with the progress of time the cpm declined, reaching it lowest cpm at 5 hours after retransplanting of the coral fragments. On the other hand the samples obtained from the natural (Nc) colony showed that the cpm increased with time progress.



(a)



(b)

Fig 3. Count per minutes (cpm) of the coral fragments at Natural (Nc) and Artificial Environment (Ac) at different depth (5 and 10 m).

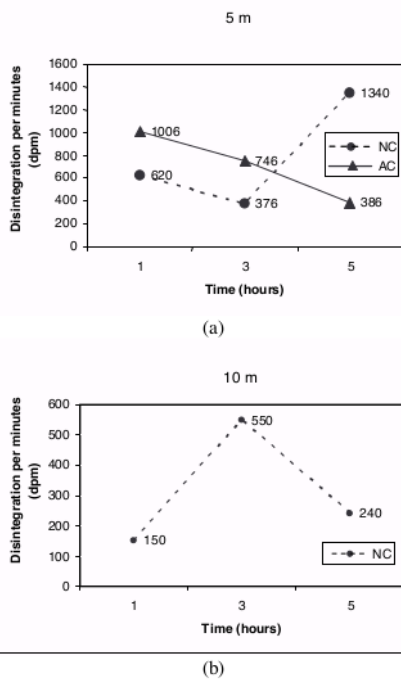


Fig. 4. Desintegration per minutes (dpm) of the coral fragments at Natural (Nc) and Artificial Environment (Ac) different depth (5 & 10 cm).

At the 10 m depth where only the coral fragment of the natural colony (Nc) was observed a different pattern showed up. Here with the progress of time up to 3 hours the cpm increased and after that in declined to reach a low cpm at 5 hours of observation. The cpm values of the coral samples were then transformed to dpm values, from there to  $\mu\text{Ci}/0.5 \text{ g}$  values, end ended with the content of  $^{45}\text{Ca}$  ( $\mu\text{g}/0.5 \text{ g}$ ).

The  $^{45}\text{Ca}$  values were used as an indicator of the calcification rates. In the discussion  $^{45}\text{Ca}$  content is used and as an indicator for the calcification rates. The same trend is shown for dpm,  $\mu\text{Ci}/0.5 \text{ g}$  sample and  $\mu\text{g Ca}/0.5 \text{ g}$  sample. These figures showed clearly that  $^{45}\text{Ca}$  could be used to calculate the magnitude of calcification.

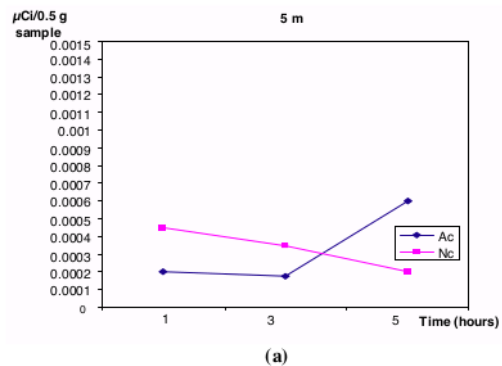
### Discussion

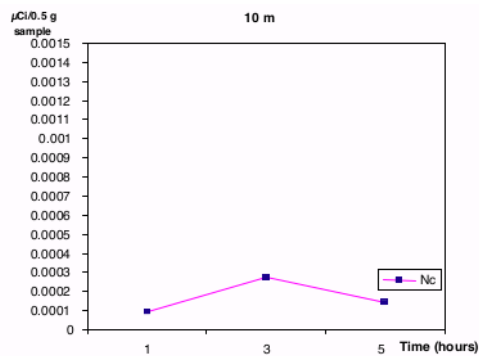
Theoretically, the content of calcium carbonate deposition increased with time and is irreversible as long as the coral colony is alive. According to Goreau [6], there is a positive correlation between photosynthesis and calcification. This is based on the statement by Goreau and Goreau [7] that the role of zooxantellae which play an important role in the process of

calcification. They do the photosynthesis for the corals and this activity play the main role to make the corals able to carry out the calcification process.

At the 5 m depth apparently the process of photosynthesis was inhibited. This inhibition could be due to the growth disturbance of the corals when taken for  $^{45}\text{Ca}$  incubation and after transplanted at their original site. It could be that the disturbances include the disturbance of the zooxantellae growth and this further disturbed the photosynthesis activity resulting in decreasing calcification rates for the artificial corals colony as shown by Figs. 3a, 4a, 5a and resulting in low  $^{45}\text{Ca}$  content (Fig. 6a). The opposite was shown for the natural corals at the same depth. As shown in Figs. 3a, 4a, 5a, 6a for the natural colony the increase in cpm, dpm,  $\mu\text{g}/0.5 \text{ g}$  and ended with  $^{45}\text{Ca}$  content as an indication of the calcification rates, which increased with the time progress. It might be that the coral fragments obtained from the natural colony could resist stress which was experienced by the artificial corals. This was shown by the increase calcification rates where apparently inhibition to their photosynthesis activity did not occur (Fig. 6a). For the 10 m depth, a different observation was obtained (Figs. 3, 4, 5, 6b). Here, it is shown that first there was an increase in calcification rate at the 3-hour observation and then declined at the 5-hour observation. The increase and decrease of the calcification rates did not differ from hour to hour similar as found by Idris [8]. In a way, that as found by Idris [8] similar trend of calcification rates was found in this study. He forwarded that the low calcification increase at a 10 m depth is mostly due to the coral morphology and not to the stress which could also occur in a 10 m depth.

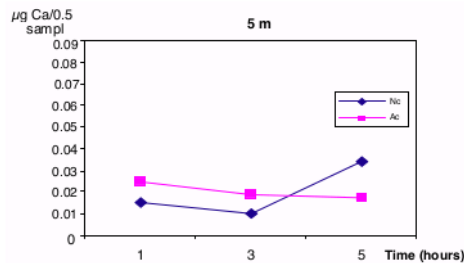
Further according to him [8] comparing depths, the corals in the 5 m depth will experience more stress than at 10 m depth, while for the 10 m depth the morphology at the coral will have a more significant influence on calcification compared to the corals in the 5 m depth.



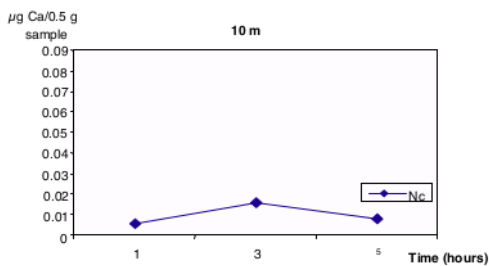


(b)

Fig. 5.  $\mu\text{Ci}$  of the coral fragments at Natural (Nc) and Artificial Environment (Ac) at different depth (5 and 10 m).



(a)



(b)

Fig. 6.  $\mu\text{g}$  Ca of the coral fragments at Natural (Nc) and Artificial Environment (Ac) at different depth (5 and 10 m).

In general it could be forwarded that in this study the  $^{45}\text{Ca}$  method was able to determine the calcification rates quantitatively.

## CONCLUSIONS

The  $^{45}\text{Ca}$  method has proved that it could be used satisfactorily to measure the calcification rates of the coral fragments under study. This method also showed that different calcification rates occur between coral fragments taken from artificial and natural coral colony at a 5 m depth. The natural

colony at the 10 m depth which contributed the coral fragment, to be used for calculating calcification rates showed different rates compared to that of the 5 m depth.

Some suggestion to further carry out this work is to have more different location and longer time (>5 hours) of observation and to determine the photosynthesis rates occurring in the reef corals.

## ACKNOWLEDGEMENTS

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