

Original paper

## GROWTH RATES OF THE MASSIVE CORAL *Porites lutea* EDWARD AND HAIME, ON THE COAST OF BONTANG, EAST KALIMANTAN, INDONESIA

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### ABSTRACT

Growth rates (linear skeletal extension) and the timing of skeletal band formation were measured in eight specimens of the massive coral *Porites lutea* at three sites (BK1, BK2, and BK3) and three depths, i.e. 1 m, 3 m, and 5 m in each site. The sites were located in Bontang Kuala Regency, located about 7.5 km from the fertilizing industry, PT Pupuk Kaltim Tbk, Bontang. Growth rates were measured by using two techniques, i.e. X-radiograph and UV-light.

Result of the study indicates that the timing of the high density (HD) and low density (LD) bands is synchronous at the three locations. A one year growth is characterized by three HD bands, one of which is usually very dense. Illumination of the coral slabs by UV-light revealed a distinct fluorescent banding pattern on all coral specimens. The data indicate that the fluorescent bands are usually associated with the high density bands which are accreted during the wet season period. It is characterized by high land run-off containing elevated concentrations of fulvic and humic acid compounds, and this apparently occurred almost through out the year. However fluorescent bands were absent from a number of density couplets, known as "stress bands". The results suggest that in the present study the linear skeletal extension rates, based on X-ray radiographic techniques, are a more accurate measure of *P. lutea* growth rates than fluorescence banding.

Comparisons of the skeletal extension rates indicate that the growth rates of *P. lutea* are not significantly difference ( $p > 0.05$ ) either between sites or depths. The average of coral growth rates ranged from 0.8-1.2 cm/year. These are significantly correlated ( $p < 0.01$ ) with the amount of rainfall. While the amount of rainfalls is not correlated with urea production of fertilizing industry, P.T.Pupuk Kaltim Tbk, which some of them are loss as dust (a core for water vapour) during process production.

**Key words:** Coral growth rate, massive coral's growth

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### INTRODUCTION

*Porites lutea* is one of the most common scleractinian coral species on Indonesian reefs. This species, *P. lutea*, is known very

tolerant to high sedimentation rates and/or turbid waters (Hudson *et al*, 1982; Supriharyono, 1986,1987). Therefore, the growth form (coral morphology) is usually affected by the environmental factor, that

exhibits nodular growth form (Chappell, 1980; Supriharyono, 1986).

The skeletons of massive corals have been used by several workers (Hudson *et al*, 1982; Isdale, 1984; Boto and Isdale, 1985; Supriharyono, 1986; 1987; 1998) as tools for measuring environmental changes over their growth history. Accretion of calcium carbonate by reef building corals depends on a number of environmental factors (i.e. such as sun hour, light transparency, and temperature). These environmental factors affect the accretion of high and low density bands within the skeletal matrix of coral colonies. The high density (HD) and low density (LD) bands are revealed by X-ray radiographic techniques. Generally one year growth consist of two density bands, one HD band and the other LD band. However, some corals may have more than two high density bands during a one year growth period. Similar results were reported for the massive coral *Porites lutea* from Ko Phuket, Thailand (Charuchinda and Chansang, 1985; Brown *et al*, 1986), and North and South coast of Central Java, Indonesia (Supriharyono, 1986; 1987; 1998).

Boto and Isdale (1985) have suggested that organic compounds produced by freshwater plants in soil, such as fulvic and humic acids, may be incorporated into the coral skeleton. These compounds fluoresce under ultra-violet

light produce an alternate bright/dull banding pattern in corals (Isdale, 1984). Moreover, Isdale (1984) suggested that UV light may be used to analyze coral growth rates. The fluorescent banding pattern may provide additional information on the environmental conditions which affect the growth history of the coral colony.

The study reports on the growth characteristics of *Porites lutea* on the coast of Bontang, East Kalimantan, Indonesia.

## MATERIALS AND METHODS

### Site Description

The sites of the study were located in Bontang Kuala Regency, about 7.5 km from the fertilizet industry, PT Pupuk Kaltim Tbk, Bontang (**Figure 1**). Reefs on the coast of Bontang are characterized by poorly developed fringing reefs. The coastal waters receive heavy sediment loads from terrigenous run-off, during rainy days which occurred almost of the year. As well, the fringing reefs are also directly and/or indirectly affected by a number of anthropogenic activities, e.g. dredging, fishing with bomb and poison materials.

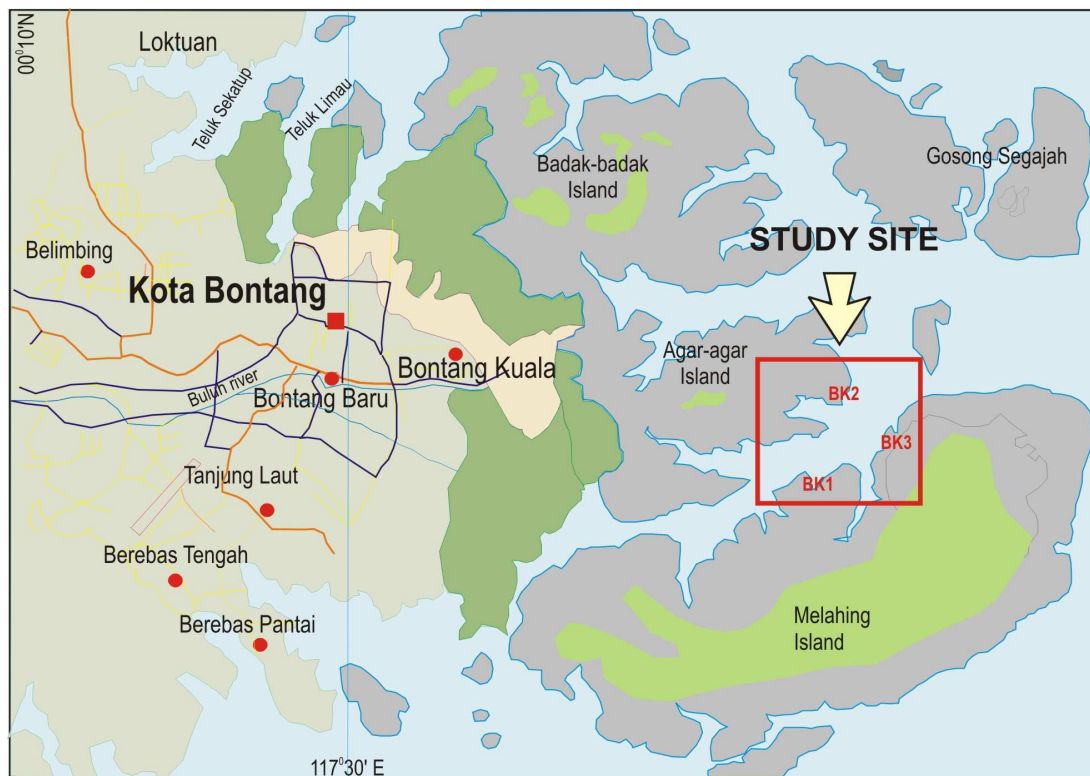


Fig. 1. Map of the study site, Bontang Kuala, Bontang

### Coral Collection and Analysis

Eight colonies of *Porites lutea* were collected from the reef flat in April 2004. Coral specimens were taken from a depth of 1 m (BK11, BK21, and BK31), 3 m (BK1, BK23, and BK33), and 5 m (BK15, BK25, and BK35). After being air dried, each coral head was then cut (with a hack saw machine) parallel to the major growth axis to produce a slab 5-10 mm thick. The coral specimens (slabs) were, then, X-rayed on a medical X-ray machine unit, TOSHIBA, Model DC-12MB-1 using Kodak film at the "Pupuk Kaltim" Hospital, Bontang, East Kalimantan. Exposures were made at 50 kv, 150 mA for 0.03 sec with a source to film distance of 90 cm. X-ray negatives were contact printed on to photographic paper and the positives were used for analysis of annual

bands. Fluorescent banding pattern was analyzed by illuminating all coral slabs with of UV-black light (350 nm). The latter technique was used to compare the banding patterns under the UV-light and X-rays, and to investigate whether land run-off (associate with fluorescent bands) is associated with a high skeletal density bands (i.e. HD bands are formed during the rainy season). In addition, annual growth rates (linear skeletal extension) were measured using the techniques described by several workers (e.g. Dodge and Vaisnys, 1977). One year growth refers to the distant of two HD bands (annual HD bands).

### Environmental Assessment

The most of environmental factors may affect on reef corals, among others are

amount of rainfall, sun shine duration, salinity and seawater temperature. These parameters, mainly climatological data, were collected from the nearest climatolog station, located at PT Pupuk Kaltim Tbk, about 7.5 km from the study sites. While other parameters, such as seawater temperature, water transparency, suspended solid, and salinity were adopted from the secondary or previous data collection.

## **RESULTS AND DISCUSSION**

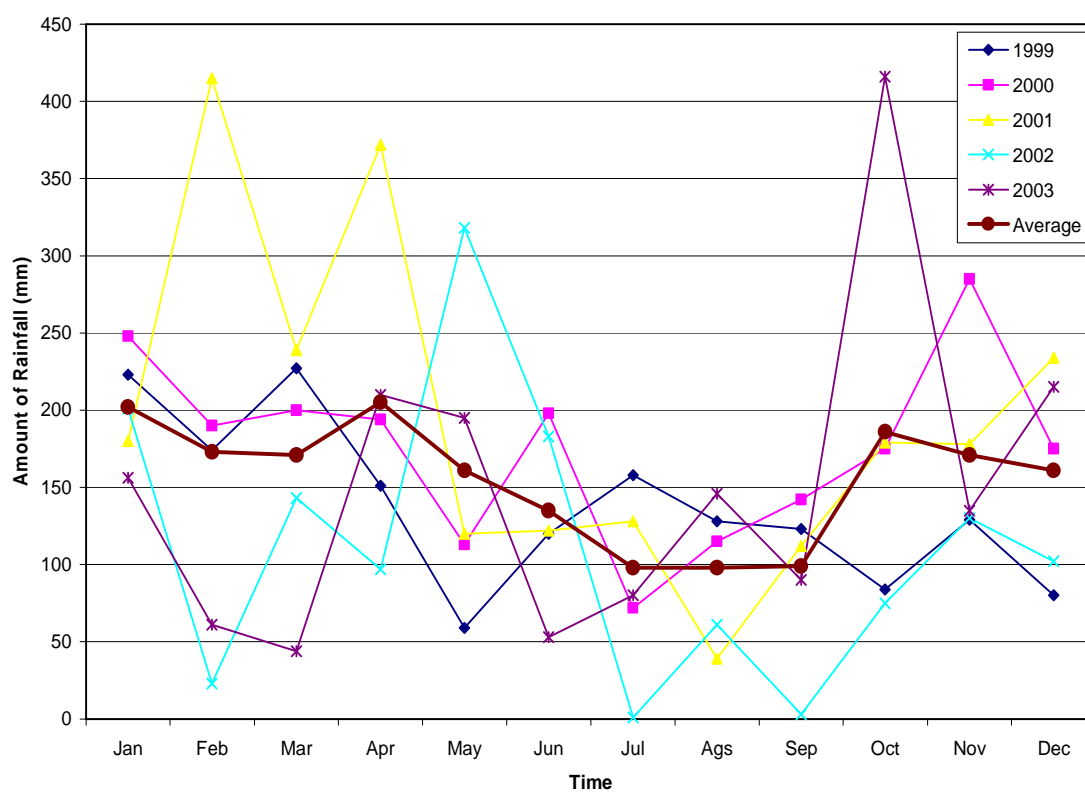
### **Environmental Information**

The sites are under the influence of a tropical monsoon climate. Generally, the northwest monsoon lasts from about December to February and the southeast monsoon from June to August. The rest of the year consists of two transition periods, from the northwest to the southeast monsoon (March-May), and from the southeast to the northwest monsoon (September- November). The northwest monsoon is also called the wet monsoon, since the wind, blowing from the northwest to southeast, brings heavy rainfall. Conversely, the southeast monsoon is characterized by dry conditions and it is called the dry monsoon, with the wind blowing from

southeast to northwest. Apparently, this phenomenon may not occur in the study sites. It is no distinct seasons between the dry and the wet, since generally the rain almost occurred through out the year (**Table 1**). Moreover, monthly amount of rainfall fluctuated from year to year, however, in average the amount of rainfall is slightly lower during July-September (**Figure 2**). This phenomenon of uncertainly rainfall, may be related to the production of urea by PT Pupuk Kaltim Tbk, Bontang in the study sites. Sasongko *et al* (2004) suggests that during process, the fertilized industry of PT Pupuk Kaltim Tbk, which produces urea and ammonia may release these components to the air. While, these components, particularly urea (as dust) is a good particle as a core for condensation of water vapour to become precipitation. Since the production of those fertilizers are going continuously throughout the year, the highly rain, then, always occurs in the study sites. In addition, the monthly amount of rainfall, therefore depending on the activity of monthly urea production of PT Pupuk Kaltim Tbk or meteorological condition (distribution of dust Urea). Moreover, this condition will affect to other climatology parameters, such as sun shine duration and temperature, known also affect on reef corals in the study sites.

**Table 1.** The mount of rainfall in the study sites, Bontang, 1999-2003

Year	Amount of Rainfall (mm)					Average
	1999	2000	2001	2002	2003	
January	223	248	180	201	156	202
February	174	190	415	23	61	173
March	227	200	239	143	44	171
April	151	194	372	97	210	205
May	59	113	120	318	195	161
June	120	198	122	183	53	135
July	158	72	128	0.9	80	98
August	128	115	39	61	146	98
September	123	142	112	3	90	99
October	84	175	179	75	416	186
November	129	285	178	130	135	171
December	80	175	234	102	215	161



**Fig. 2.** Fluctuation monthly amount of rainfall in the study sites

The high amount of rain means that the desalinization may be occurred, and this affects on the decreasing of seawater salinity, with the sequent that the salinity will be lower than the usual. The salinity is recorded about 29.5-32.80‰ in the study sites (PKT-PPLH-UNDIP, 2001), but it is possible that the minimum level will be lower than 29.5‰. As well, the amount of rainfall is also related to the number of land water runoff, with the sequence of sedimentation in coastal areas. Since, the water colour is brown, just looked like “coco” during rainy season. It is reported that the suspended solid went up to more than 50 mg/l, particularly after heavy rain, while in normal condition is only around 5-10 mg/l (PKT-PPLH-UNDIP, 2001). As well, water transparency is recorded less than 3 m after heavy rain, although normally is about 5-7 m. Both the decrease of seawater salinity, high sedimentation (suspended solids), and water transparency will affect on marine organisms, including reef corals. Although some coral may withstand in lower salinity, for the long exposure they will die (Supriharyono, 1986; Hariyadi, 2004). As well, some corals may survive on high sedimentation, but the growth rate will be very low (Hubbard and Pocock, 1972; Pastorok and Bilyard, 1985; Supriharyono, 1986).

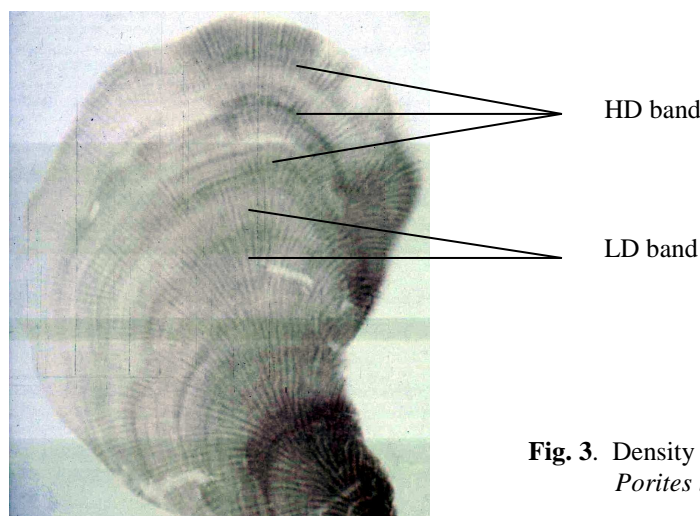
Average seawater temperature ranges from 25-29°C in the study sites, it is

normal for reef corals. With exception, seawater temperature goes to about 39-42°C in the area close to the outlet of cooling system PT. Pupuk Kaltim Tbk. This may be dead point for living marine organisms, including reef corals (Neudecker, 1981; Supriharyono, 1996). However, it will not be dangerous for the living coral in the study sites, since the location is far enough from the study sites.

As previously informed, that other that amount of rainfall and sea water temperature, the sun shine duration is also important factor to affect of coral growth.

### **Banding Pattern**

X-ray radiography revealed distinct density banding patterns in a number of specimen of the massive coral *Porites lutea*. High and low density increments, in the coral skeletal matrix, appear as dark and light bands on the black and white positive prints of X-radiographs. The dark band is considered as the high density (HD) band, while the light band is considered the low density (LD) band (**Figure 3**). Whereas UV-light illuminates high density band as a bright, and low density banding is considered as dull in colour. In the present study not all the HD bands to be fluoresced under the UV-light. This figure suggests that the X-radiography is a more sensitive method to determine massive coral growth rates than UV-light.



**Fig. 3.** Density banding pattern of massive coral *Porites lutea*, revealed by X-radiograph

In general the HD band is a production of  $\text{CaCO}_3$  during the wet season period (Dodge dan Thomson, 1974; Hudson *et al*, 1976; Supriharyono, 1986). In case for central Java's reefs, Supriharyono (1986) suggested that high density bands were deposited during the wet season (probably between November and March) when the amount of rainfall and sedimentation were very high and the number of sun hours low. Low density bands were probably deposited between April and October, since the amount of rainfall and sedimentation were very low and the number of sun hours were very high. However, some workers, e.g. Macintyre and Smith (1974); Weber *et al* (1975a); Weber *et al* (1975b), found that HD bands were produced during the hot season period. Therefore, Highsmith (1979) suggested coral density is depending on light intensity (hot) and seawater temperature. Moreover, he claims that HD bands may be able to be produced either during high or low temperature. As well, HD bands are possibly accreted when light intensity high or low. While the LD band is generally accreted during medium temperature, 24- 29°C. Therefore, it may be concluded that high density (HD) bands

are produced as a response from various environmental factors, while the low density (LD) bands are accreted during the period, when light intensity is very high and range of seawater temperature is low. It is different, with Wellington and Glynn (1983), who worked on coral growth in Eastern Pacific (Panama), they found that various light intensity is more important than seawater temperature in order to affect coral density. Moreover, they concluded that HD bands formation is determined by low level of light intensity.

In the present study, the *Porites lutea* produced high density bands either in the dry season and in the wet season. It is also reported that the coral specimens accreted three high density bands (one band wider than the others) and three low density bands. The dominant wider high density band is considered as the wet season band, while the other two HD bands are considered as *stress bands* (transition seasons production). This HD band formation apparently happened not only in the study sites. It also occurred in other areas, subject high sedimentation rates, e.g. Bandengan Bay, Jepara, central Java (Supriharyono, 1986), Ko Phuket, Thailand (Charuchinda and Chansang,

1985; and Brown *et al*, 1986). It proved that the HD band formation is very determined by water transparency and turbidity.

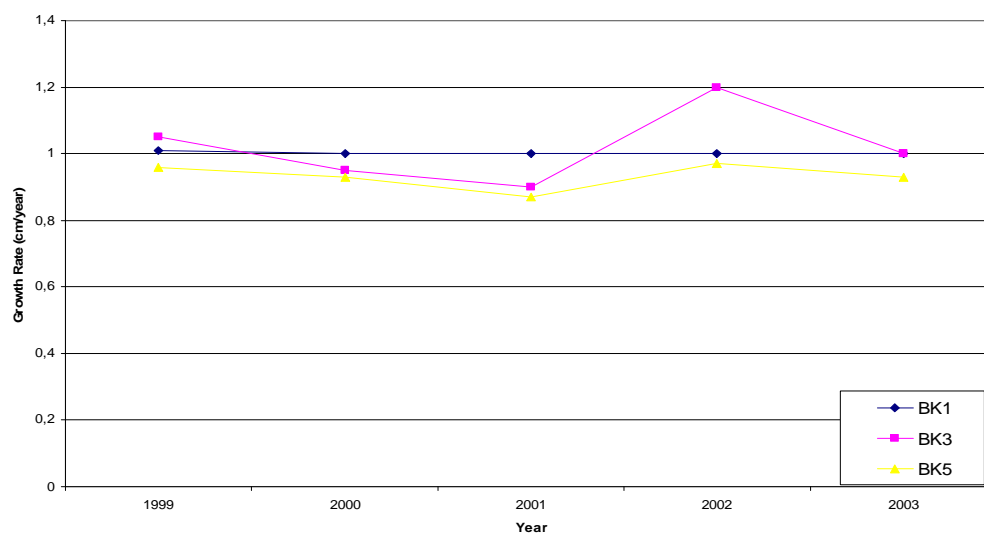
### Coral Growth Rate

Growth rates of *Porites lutea* vary from the minimum of 0,92-1,05 cm/year, with average of about 0,98 cm/year (Table 2; Figure 4) in the study sites. The annual growth rate is getting lower with the

increase of depth, although according to variance analysis (ANOVA) for the last three years growth rate of coral specimens collected from the depths of 1 m, 3 m and 5 m are not significantly different ( $p > 0.05$ ). As well, the linear skeletal extension growth rates are also not significantly different between sites, BK1, BK2 and BK3 ( $p > 0.05$ ). Table 2 shows the growth rates of linier extension coral specimens in the study sites.

**Table 2.** Growth rates of *Porites lutea* (cm) in the study sites, Bontang, East Kalimantan

Sites	Growth Rate					Average
	1999/00	2000/01	2001/02	2002/03	2003/04	
BK11	1,0	0,8	0,8	0,8	1,0	0,88
BK21	1,2	1,2	1,2	1,0	1,0	1,12
BK31	1,1	1,0	1,0			1,03
<b>Average</b>	<b>1,01</b>	<b>1,00</b>	<b>1,00</b>	<b>1,00</b>	<b>1,00</b>	<b>1,00</b>
BK13	-	-	-	-	-	-
BK23	1,0	0,8	1,0			0,93
BK33	1,1	1,1	0,8	1,2	1,1	1,06
<b>Average</b>	<b>1,05</b>	<b>0,95</b>	<b>0,90</b>	<b>1,20</b>	<b>1,0</b>	<b>1,02</b>
BK15	0,9	0,7	0,9	0,9	0,9	0,86
BK25	1,0	1,3	1,0	1,0	1,3	1,12
BK35	1,0	0,8	0,7	1,0	0,6	0,82
<b>Average</b>	<b>0,96</b>	<b>0,93</b>	<b>0,87</b>	<b>0,97</b>	<b>0,93</b>	<b>0,93</b>
<b>Average (Total)</b>	<b>1,00</b>	<b>0,96</b>	<b>0,92</b>	<b>1,05</b>	<b>0,98</b>	<b>0,98</b>



**Fig. 4.** Fluctuation of linier skeletal extension rate of massive corals, *Porites lutea*, in different depth.



In addition, it is informed that environmental condition is suitable for living corals, therefore the coral growth rates are not significantly different either sites or depths. **Table 3** shows environmental condition in the study sites. This Table exhibits that some “key” environmental parameters, e.g. salinity, suspended solids, water transparency, and sea water temperature, are in optimum condition for

living corals (Supriharyono, 2000). As well, other environmental factors, which affect those parameters, e.g. amount of rainfall (salinity) and sun shine duration (water temperature) are the same. It is due very closely to the distance inter sites, therefore their effects on reef coral growth are also the same in the study sites. However, there is a correlation between amount of rainfall on coral growth rates.

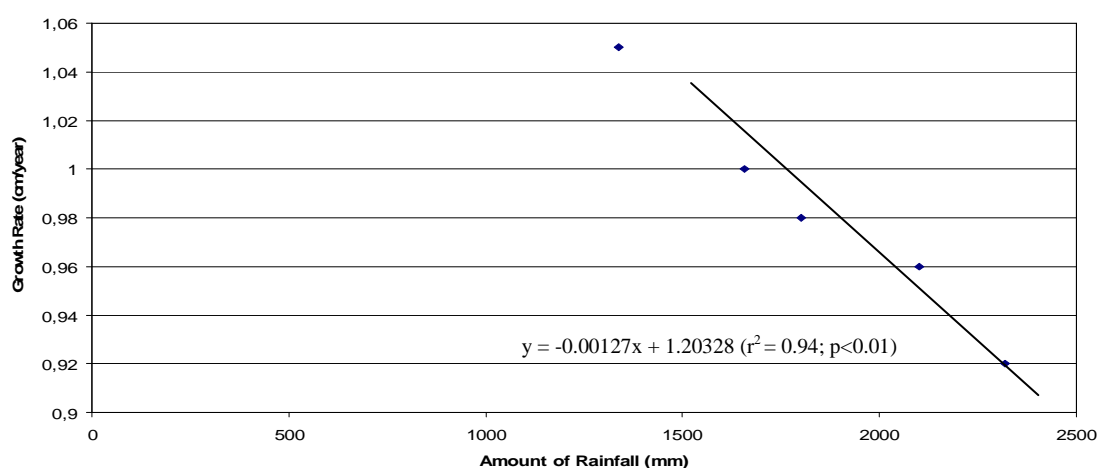
**Table 3.** Seawater quality in the study sites

Parameter	Unit	Ranges <sup>1)</sup>	Optimum level for coral growth rate <sup>2)</sup>
Salinity	‰	29,5-32,80	34-36
Suspended solid	mg/l	5-10	< 10 mg/l
Water transparency	m	5-7	bottom
Water temperature	°C	25-29	25-29

Source: 1) PKT-PPLH, UNDIP (2001); 2) Supriharyono (2000)

The coral growth rate tends to decrease with increasing the amount of rainfall (**Figure 5**). According to analysis of correlation, it is proved that relationship between those variables, is highly significant correlated ( $r^2 = 0.94$ ;  $p < 0.01$ ), with regression equation,  $y = -0.00127x + 1.20328$ . This proved that amount of rainfall affects on the coral growth rate,

increasing amount of rainfall resulted the decrease of linier skeletal extension rate of corals in the study sites. This is also proved that the amount of rainfall may be dominant factor affects on coral growth rates in the study sites, together with other environmental factors, i.e. sun shine duration, light intensity (Supriharyono, 1986).



**Fig 5.** Relationship between amount of rainfall and coral growth rates in the study sites

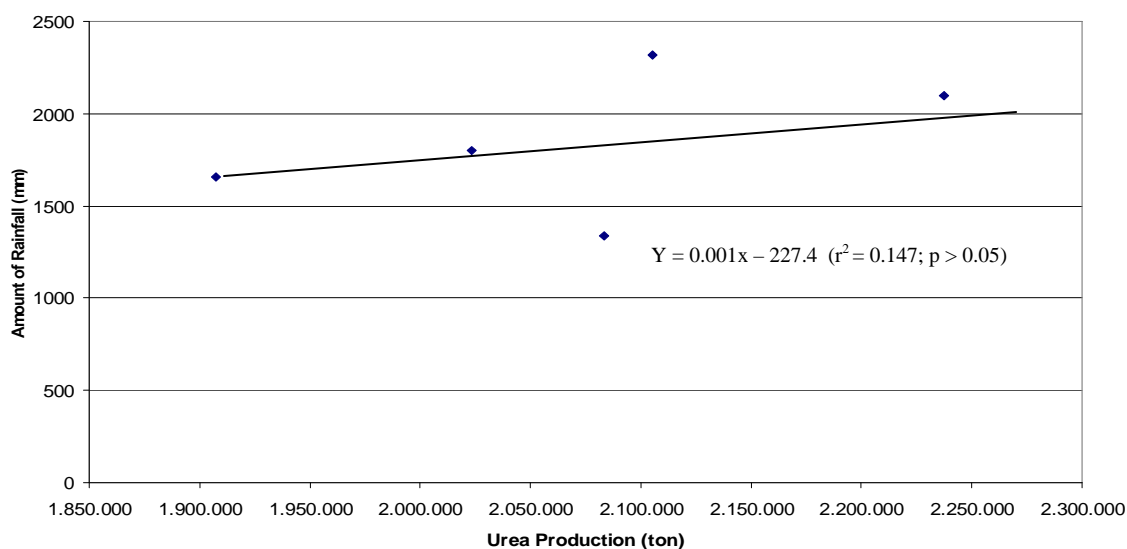
As mentioned previously, that the loss production of urea (as dust) may affect precipitation. Since the production of those fertilizers are going continuously throughout the year, the rainfall may always occur in the study sites. While, the amount of rainfall will depend on the amount of (dust) urea, that may be released in the atmosphere. Consequently that the amount of rainfall will be correlated with the number of urea production. Unfortunately, the loss urea as dust from PT Pupuk Kaltim Tbk is distributed to all

area, surrounding this industry, depending on the wind direction. Therefore the number of urea production is not automatically reflected to number of dust urea in each area, including study sites. This phenomenon, may result that no correlation between the number of urea production and the amount of rainfall in the study sites ( $r^2 = 0.147$ ;  $p > 0.05$ ). While the amount of rainfall affects significantly on coral growth rates. The data of these variables, moreover, are presented in **Table 4** and **Figure 6**.

**Table 4.** Relationship between the number of urea production, amount of rainfall, and the growth rate of corals in the study sites.

Year	Urea Production (ton/year) <sup>1)</sup>	Amount of rainfall (mm/year) <sup>2)</sup>	Coral growth rates (cm/year)
1999	1,907,551	1,656	1.00
2000	2,237,593	2,107	0.96
2001	2,105,270	2,318	0.92
2002	2,083,587	1,337	1.05
2003	2,023,321	1,801	0.98

Note: 1) PKT. 2003. Performance Pabrik Amoniak dan Urea Tahun 1991-2003.  
 2) Sasongko *et al* (2004)



**Fig. 6.** Relationship between urea production of P.T. Pupuk Kaltim Tbk and the amount of rainfall in the study sites

This figure proves that not all loss urea productions were spread through out the area, it is depending on the wind direction. Some areas may receive a lot of dust urea, while the others may not in the same time. This reflects that no correlation between the number of urea production and the amount of rainfall in the study sites.

## CONCLUSION

### Conclusion

Based on the results of the study, it may be concluded as follows:

1. Coral growth rates fluctuated with the changes of amount of rainfall in the study sites occurred almost through out the year;
2. The average of coral growth rates ranged from minimum of 0.8 cm/year to maximum of 1.2 cm/year. There is no significantly difference ( $p > 0,05$ ) of the coral specimens, both between the depths and between the sites;
3. Some chemical compounds of fertilizing industry, P.T. Pupuk Kaltim Tbk, may be loss, particularly urea as dust during process of production. The consequence of this may affect on uncertainty rainfall in the study site;
4. No correlation between total production of urea and the amount of rainfall ( $p > 0,05$ ); and
5. Amount of rainfall affects significantly on coral growth rate ( $p < 0,01$ ).

### Suggestion

Based on the result that may release (loss) of chemical compounds as a dust, particularly urea, during process production of fertilizing industry, P.T. Pupuk Kaltim Tbk, on the atmosphere,

some activities may be suggested as follows.

1. Reprocess management to anticipate the loss of chemical compounds during process of production;
2. Need assessment of the loss the chemical compounds, mainly urea and ammonia, both in water column, including coral skeleton in reef, and in terrestrial, particularly for human health, in surrounding P.T. Pupuk Kaltim Tbk;

## ACKNOWLEDGEMENTS

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This figure proves that not all loss urea productions were spread through out the area, it is depending on the wind direction. Some areas may received a lot of dust urea, while the others may not in the same time. This reflected that no correlation between the number of urea production and the amount of rainfall in the study sites

## **CONCLUSION AND SUGGESTION**

### **Conclusion**

Based on the results of the study, it may be concluded as follows:

6. Coral growth rates fluctuated with the changes of amount of rainfall in the study sites, which occurred almost trough out the year;
7. The average of coral growth rates ranged from minimum of 0.8 cm/year to maximum of 1.2 cm/year. There is no significantly difference ( $p > 0,05$ ) of the coral specimens, both between the depths and between the sites;
8. Some chemical compounds of fertilizing industry, P.T. Pupuk Kaltim Tbk, may be loss, particularly urea as dust during process production. The consequence of this may affects on uncertainty rainfall in the study site;
9. No correlation between total production of urea and the amount of rainfall ( $p > 0,05$ ); and
10. Amount of rainfall affects significantly on coral growth rate ( $p < 0,01$ ).

### **4.2. Suggestion**

Based on the result that may releasing (loss) of chemical compounds as a dust, particularly urea, during process production of fertilizing industry, P.T. Pupuk Kaltim Tbk, on the atmosphere, some activity may be suggested :

3. Reprocess management to anticipate the loss of chemical compounds during process production;
4. Need assessment of the loss the chemical compounds, mainly urea and ammonia, both in water column, included coral skeleton in reef, and in terrestrial, particularly for human health, in surrounding P.T. Pupuk Katim Tbk;

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## ABSTRACT

*Mucus is one of a non-specific defense mechanism, since this is the first element of aquatic organisms, which contact physically, chemically, or biologically with the environment. The mucus self defense mechanism investigation was carried out on fresh water fish tilapia (*Oreochromis mosambicus*). Eight (8) types of lectine were used to examine residual carbohydrate-based protein from mucous component based on histological and histochemical observation method. The review was directed as basic information for detail review about physiology adaptation aspects.*

*The results showed that mucous in goblet cells from palatal, gills primary lamella, esophagus and skin reacted with WGA (Wheat Germ Agglutinin) lectine. In another part, mucous from the goblet cells in palatal and esophagus cells reacted with PNA (Peanut Agglutinin). Based on these results, therefore, it can be concluded that mucous from goblet cells in esophagus contains residual of N-asetil glucosamine and/or similar acid  $\beta$ -galactose and  $\alpha$ -N-acetyl galactomine. Mucous from goblet cell in palatal contains residual of X-acetyl glucosamine and/or sialat acid and galactose. While mucous in the gills lamella contains carbohydrate residual, namely N-acetyl glucosamine and/or sialat acid.*

**Key words:** Mucus, Tilapia, histochemical analyzes

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## INTRODUCTION

Mucous which coats or covers the outer side of fish and other aquatic invertebrate is known as a non-specific mechanical and chemical defense against environmental

change and any pathogenous agent. Mucous from each species of fish is different in its physical and chemical natures, and quantitatively, the mucous production is also different in each species of fish.

Mucous has several functions; many of these are as a mechanical protection, osmoregulation, and barrier to colonization of parasites, fungus and bacteria. Mucous contains several substances, such as immunoglobulin (Rombout *et al*, 1995), lysozyme (Fletcher and White, 1973), C-reactive protein (CRP) (Ramos and Smith, 1978) and lectine (Suzuki, 1995). The main component of mucous is glycoprotein produced by goblet cells/mucous cell (Pickering, 1974). Glycoprotein in mucous varies depends on the fish species. Histochemical painting by lectine at superficial skin tissue showed the existence of glycoprotein mucous in the goblet cells (Asakawa, 1970).

Tilapia (*Oreochromis mosambicus*) is one of fresh water fish that can survive in brackish water or in relative muddy water, and has a good adaptation level and resistance in the unfavorable environment. The investigation was aimed at examining goblet/mucous cells distribution in producing mucous and biochemical nature of lectine in Tilapia.

## MATERIALS AND METHODS

Tilapia (*Oreochromis mosambicus*) of 15.2 – 25.0 cm total body lengths and 145 – 250 grams of body weight was used in this experiment.

After anesthesia by 0.01% 2-b-phenoxyethanol, samples from palatal epithelium, gills, esophagus and skin were drawn. These samples were fixed in Bouin liquid for 24 hours at room temperature, and then dehydrated through series of ethanol, infiltrated with paraffin and embedded in hystoparaffin. The embedded tissue was cutted in 5-cm thickness and painted with haemotoxylin-eosin. The tissue observed using microscope.

**Table 1.** The figures of mucous cell histology from several tissues of Tilapia

Histochemical painting was done in which each sample was fixed in 4% paraformaldehyde liquid in 0.1 M phosphate buffer pH 7.2 and stored several days at temperature at 4° C. These samples were embedded and cut with thickness aforementioned. The lectine was conjugated by FITC (Floresence Immuno Thiocyanat) that was used for histochemical painting. In this test, 8 lectine were used. Painting technique orders were done after deparafination process by xylene continued by dehydration to series ethanol concentrat from 100% to 70%. Sample was washed 3 times in PBS at pH 7.4, and then incubated by FITC-lectine that was diluted by PBS (1: 1000) in room temperature for 1 hour in dark condition, and then washed 3 times by PBS. After the slides were closed by glass object using 1.4 Diazabicyclo (2-2-2) octane solution (Sigma, St. Louis, MO) liquid that mixed with glycerol (1:1) was then observed by florescent microscope. The sample incubated by PBS without FITC-lectine at room temperature for 1 hour was used as a control.

## RESULTS AND DISCUSSION

### Mucous cell histology

The figures of mucous cell histology from several tissues of Tilapia were shown in **Table 1**. The epithelia surface from palatal cells of Tilapia (**Figure 1a**) indicates many goblet/mucous cells in different shapes and sizes. Several mucous found in this area has cylindrical shape, but no broken cells in proximal were found. The mucous cells density was approximately 47 cells / 0.01 mm<sup>2</sup>, and the biggest mucous cell size was 60 µm height and 40 µm widths.

No	Variable	Palatal epithelium	Gills	Esophagus	Skin
1	Type	Oval, ellipse	Globular, small	Oval, ellipse	Oval
2	Biggest size (µm)	Height 60, width 40	Diameter 10	Height 70, width 50	Small
3	Total (0,01 mm <sup>3</sup> )	47	12	23	4

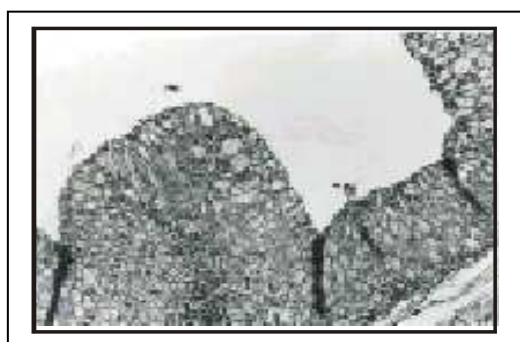
In the gills (**Figure 1b**), mucous cells were relatively low and distributed at apical area. Several cells contain mucous in vesicle shape and swell to oval cells. These mucous cells were relatively small in their shape at the diameter of about 10 µm. The number of mucous cells approximately 12 cells / 001 mm<sup>2</sup>.

In esophagus (**Figure 1c**), the epidermis was constructed by cells layer in cuboids shape. In this area, the mucous cells were well developed and varied in their shape, from circle to oval. Mucous cells density about 23 cells / 0.01 mm<sup>2</sup>, and the biggest cell was 70 µm height and 50 µm width respectively.

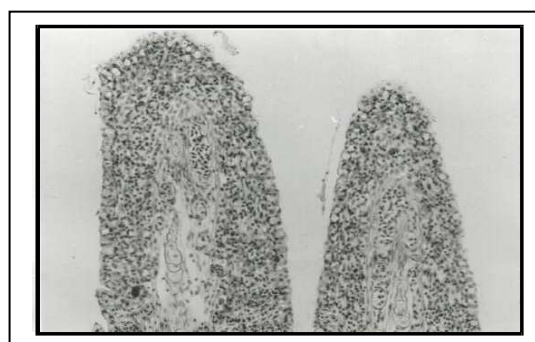
Many mucous cells were found in palatal epitheliums cell and esophagus. According to Drenner *et al* (1984), tilapia

(*Oreochromis esculeritus*) was reported as a type of “size-selective suspension feeder” fish and the mucous that disposed from digestion duct was related to feeding digestion activity. Furthermore, Sanderson *et al* (1996) reported that in Tilapia (*Oreochromis niloticus*) the mucous secretion speed was determined by stimulus response toward particle size of feed.

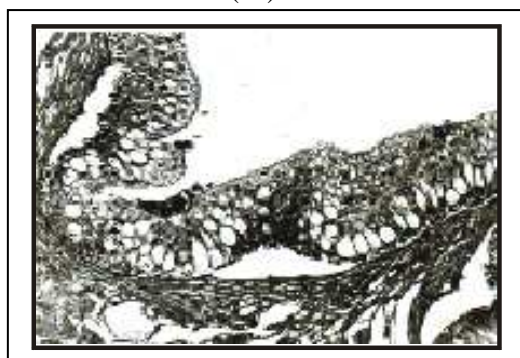
A thin layer from several epithelia cells built the skin epidermis of tilapia (**Figure 1d**). The epidermis surface was relatively smooth in its shape covered by several epithelia squamous cells. The number of mucous cells was relatively small, i.e. about 4 cells / 0.01 mm<sup>2</sup> which were distributed on the surface of epidermis layers.



( a )



( b )



( c )



( d )

- Fig. 1.** Observation histological from several tissues of *Tillapia (Oreochromis mosambicus)*
- Goblet cell epithelium from palatal
  - Goblet cell from gills
  - Goblet cell from esophagus
  - Goblet cell from skin

**Histochemical of goblet/mucous cells**

From 8 types of lectine used in the test (**Table 2**), WGA (Wheat Germ Aglutinine) reacted with epithelium of palatal cells, gills primary lamella, esophagus and skin of *Tilapia*. Lectine of PNA (Peanut Aglutinine) type reacted with surface of palatal epithelium and esophagus cells. Lectine from DBA (*Dilichos Biflofus*) type reacted only with esophagus. Painting/reaction intensity shows that WGA was stronger than PNA and DBA toward mucous cells. The other types of lectine

such as LCA, RCA, OHA, Con-A and UEA showed no reaction with mucous cells.

The epithelium cells of palatal, lectine of WGA type was different from PNA such as painting intensity or the number of mucus cells that positively reacted (**Figure 2**). Lectine of WGA type was stronger in their reaction intensity with mucous cells than PNA. Also lectine of WGA type reacted with mucous cells from the gills, esophagus and skin. Both PNA and DBA lectines weakly reacted with mucous cells from esophagus.

**Table 2.** Painting FITC-lectine on several tissues of *Tillapia*

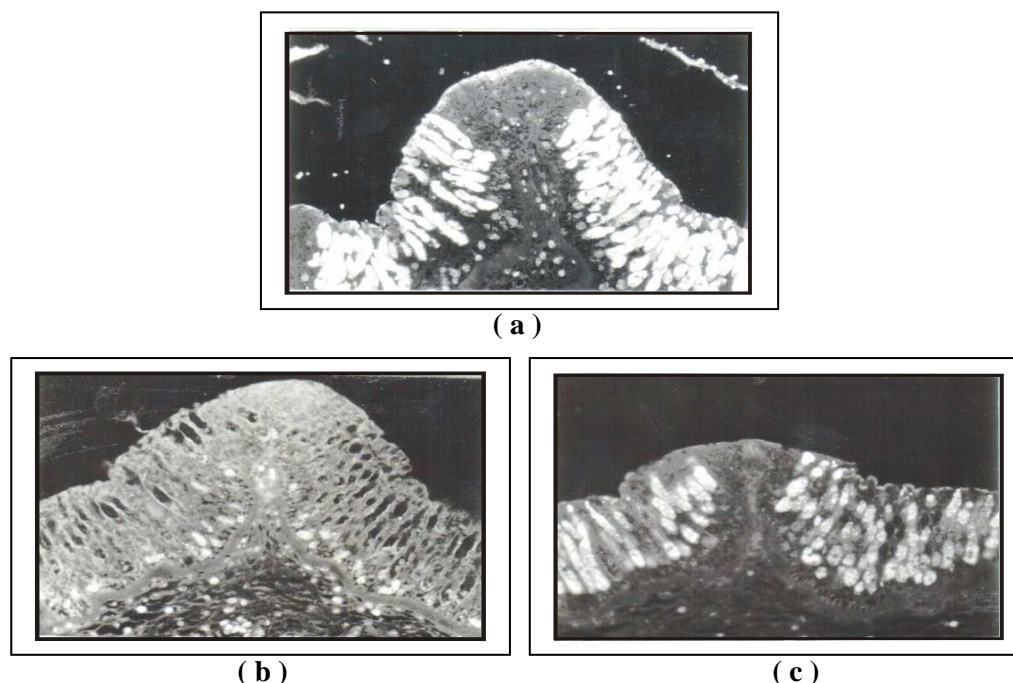
No	Kind of lectine	Palatal epithelium	Gills	Esophagus	Skin
1	WGA	++	++	++	+
2	PNA	+	-	±	-
3	LCA	-	-	-	-
4	RCA	-	-	-	-
5	PHA	-	-	-	-
6	Con-A	-	-	-	-
7	UEA	-	-	-	-
8	DBA	-	-	±	-

Explanation:

- ++: Strongly reacted
- : Weakly reacted
- ± : Fairly reacted

According to Gona (1979) the function of mucous layer was very related to type of glycoprotein produced by mucous cells. Lectine of WGA type was found more in mucous cells in rat, monkey, ma, and guinea pig. The lectine

of WGA type was specific to residual of carbohydrate N-acetyl glucosamine and sialat acid. These meant that residual of protein-based carbohydrate was found more in this tested *tilapia*.



**Fig. 2.** Observation of FITC-lectine on epithelium palatal cell from *Tilapia*  
a) Epithelium cells from palatal positive reaction with WGA. Goblet cells strong reaction with WGA (arrow);  
b) Epithelia cells positive reaction with PNA. Painting intensity is not strong with WGA (arrow);  
c) Epithelia cells of palatal as control. No reaction with goblet cells (arrow).

Lectine of PNA type found in mucous cells of *Tilapia* intestine according to Pajak and Danguy (1993) was contained residual of  $\beta$ -galactose and  $\beta$ -N-acetylgalactosamine that plays as a viscoelastic barrier that covers mucous from acid and proteolyses environment. Lectine of DBA type was also found in mucous cells of intestine according to Menghi *et al* (1996), lectine of DBA type was generally found in epithelium and glandule of *tilapia's* side, frog and turtle intestine.

## CONCLUSION

1. The mucous cells in *tilapia* were found in palatal cells, gill primary lamellae and esophagus or skin.
2. Lectine of WGA type was found in mucous cells from palatal cells, gill primary lamella, esophagus and skin. Lectine of PNA and DBA types were found only in mucous cells from *tilapia's* esophagus.

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