CHAPTER 2

MATERIALS AND METHOD

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1. Location and study site

Kang Kao Island is a small island in the inner part of the Upper Gulf of Thailand, south of Si Chang Island, at latitude 12° 6′ 35″ N to 12° 7′ 0″ N and longitude 100° 48′ 50″ E to 100° 80′ 20″. The area is approximately 0.25 km^2 and the total length of shoreline is about 5 km (Figure 1). The study site has general configuration of small bay which is affected directly by the north-east monsoon, The patch reef is at the distance of about 50-90 metres from shoreline.

2. Profile and permanent line transects

The profile of the coral reef topography was carried out by setting up a perpendicular measured line which was fixed the direction by underwater compass from shore. The line was 100 metres long. Reef zones were noted and live coral cover in each zone were estimated as described later. The monitoring programme was planned by using the permanent line transect at shallow and deep zones to differentiate the composition of borers and their effects on bioerosion. Figure 2 illustrates pattern and sizes of 8 permanent parallel transect lines. Each line was

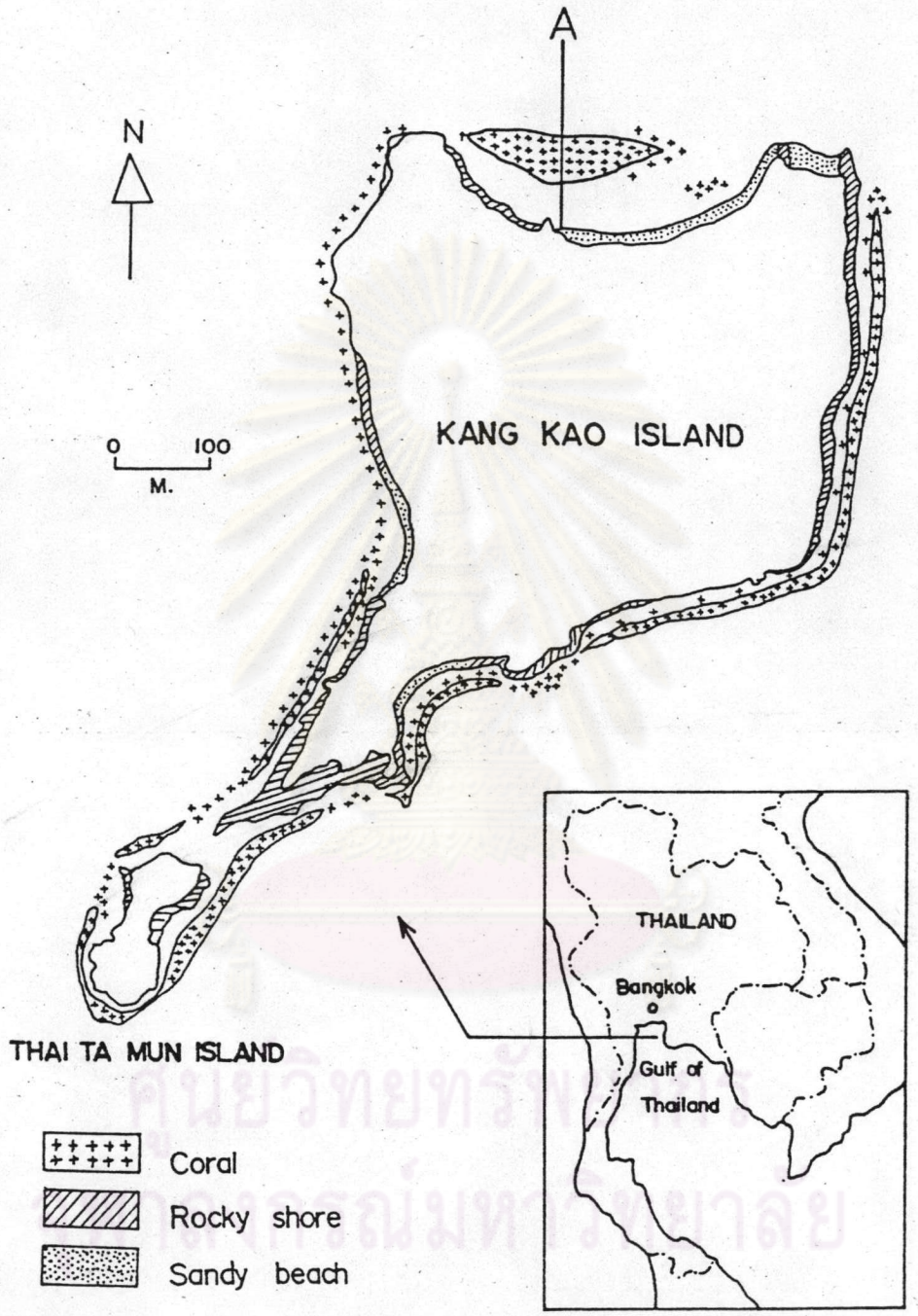

Figure 1 Map of Kang Kao Island, showing the location of the study area.

FIELD EXPERIMENTAL PLANNING

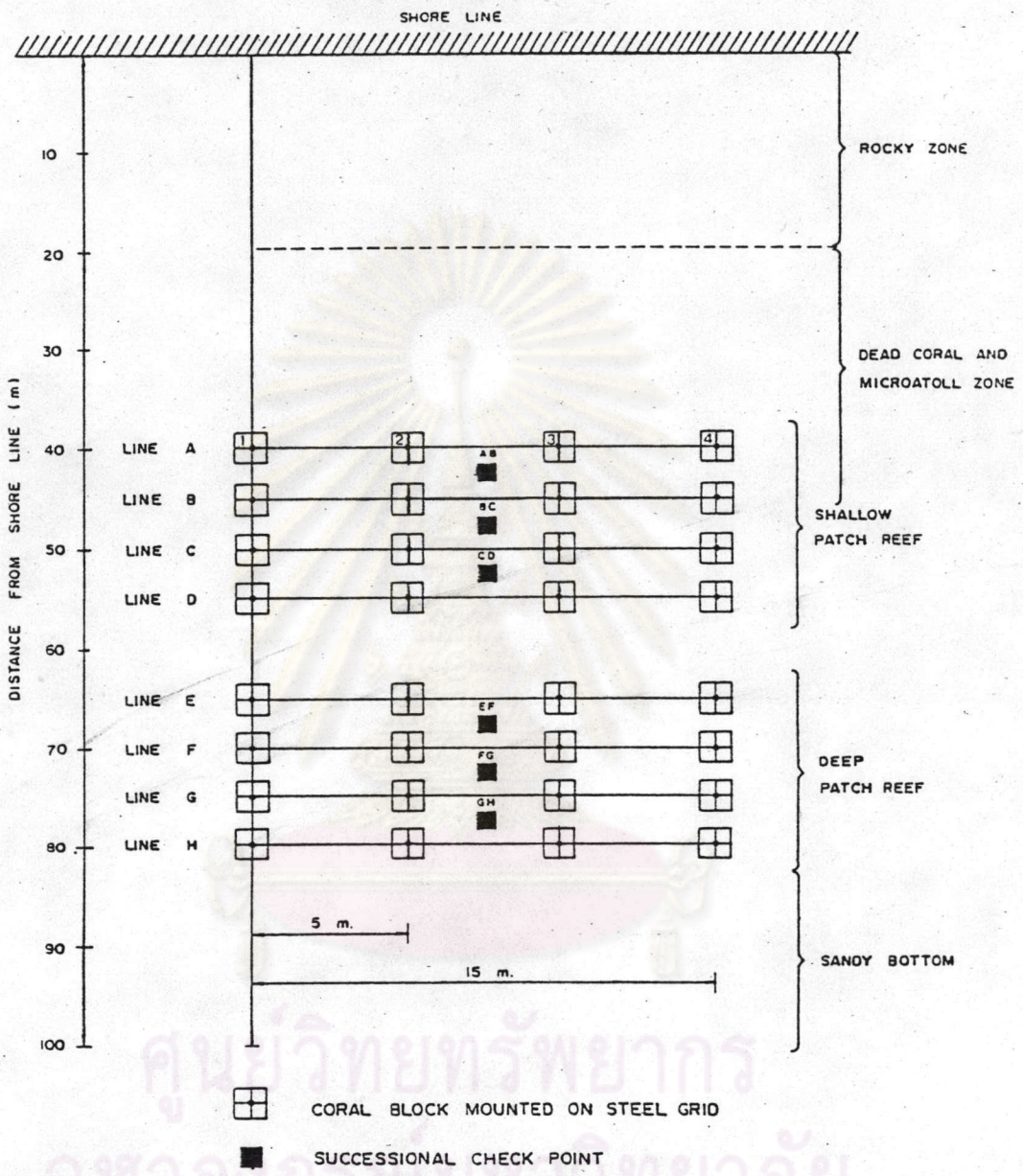

Figure 2 Diagramme indicated the position of permanent transect lines and coral blocks on reef transect.

15 metres long, 5 metres apart, and the distance between zones was 10 metres.

3. Preliminary survey on coral reef condition

The coverage area of coral reef substratum was estimated by photographing each quadrat ($0.25\ m^2$) along the permanent transect lines. Subsequently, the photographs were identified as dead coral, live coral, sand, Porites lutea and were duplicated in tracing papers. The areas of each component was figured out by using planimeter $0.1\ cm^2$, then converted to percentage of the total.

4. Environmental factors measurement

4.1 Meteorological and oceanographic data :-

Meteorological data include air temperature, precipitation (rain), wind direction and speed were obtained from the Department of Meteorology. Data of predicted sea level at Si Chang Island was obtained from Department of Hydrology. They were used to determine monthly and yearly mean sea level during the study period.

4.2 Salinity and temperature

Both of them were detected in situ by using S-C-T meter, YSI Yellow spring Instrument, Model 33 at surface and bottom in shallow zone, deep zone including mid depth.

4.3 Light intensity

The light intensity was detected by combination of

Whitney underwater relative photometer and laboratory light meter (2500 lux unit in full scale). Due to high sunlight intensity in tropics which is sometimes up to 10 kilolux unit, four modify blue filters were used to decreasing lowersunlight intensity and then converted by multiplying the calibration values (26.9792)

The calibration method of filtered efficeincy of filters which decreases the light intensity from source (2500 lux) in percentage as follows :-

Filter no.	detected light intensity	calculation	decreasing %
1	700	$\frac{700 \times 100}{2500}$	28.00
2	250	$\frac{250 \times 100}{700}$	35.70
3	170	$\frac{170 \times 100}{250}$	68.00
4	92	$\frac{92 \times 100}{170}$	54.53

Thus, one lux of the intensity of sunlight determined by light meter is actually equal to 26.9792 lux which was the accurate light intensity in reality . Underwater light intensity was detected by underwater photometer and converted from percentage to lux unit, formula is as follow :-

Sunlight intensity = 26.9792 x detected value from filtered light meter (lux or kilolux)

Underwater light intensity

$$= \frac{\text{sunlight intensity x relative percentage}}{100}$$

The underwater light intensity was detected at surface, mid depth and bottom at the deep zone at noon

4.4 Suspended solids

The seawater were collected by Van Dorn water sampler at surface (1.0 m depth) to investigate suspended solids. The known volumes of seawater was filtered through the GF/C glass filter and then converted to mg/litre unit.

4.5 Sedimentation rate : The sediment traps as designed by followed Hargrave (1979), were used in this study. They were made from PVC pipes which were attached on cement blocks with screws and then painted with copper oxide to prevent the growth of fouling organisms. They were 1.5 cm in diameter and 30 cm high. Hargrave (1979) founded that such sediment traps possessed efficiency up to 100 % of their volume. The sediment trap was fixed at each study point.

These sediment traps were usually collected and replaced every 2 months with some exception 1 month period. After being collected, they were frozen for analysis in the laboratory. The analysis was carried out by wet sieving

method (mesh size 63 μm) to seperate into larger than 63 μm and smaller than 63 μm grain size classes. Prior to the sieving, it had to be rinsed with tap water to remove contaminating salt. Then both fraction were dried in oven at 100-110 ^{o}C for 24 hours, weighed and converted to sedimentation rates mg/cm^{2}/day which were calculated as follows :-

$$\text{sedimentation rate} = \frac{\text{sediment wt. (gm or mg)/time}}{\pi r^{2}}$$

r = 0.75 cm

time = day

4.6 Plankton primary productivity

The oxygen production method, using dark and light bottles were adopted for the determination of primary productivity. Firstly, sea water was samplinged by Van Dorn bottle in shallow and deep zones. In shallow zone surface and bottom water were collected but in deep zone the mid depth was added. The water sample was splitted into 2 portions : one for initial dissolved oxygen determination, the other for dark and light bottles analysis. For the latter the water samples had to be filtered through zooplankton net (mesh size 300 μm) before filled up in BOD bottles. Caution was made not be allow the air bubble appear while the filter was done. The dark one was covered by aluminium foil. Both were hung vertically on rope at a

fixed length equal to the depth of sampling and were incubated in situ for 3-6 hours in shallow and deep zones.

After incubation, all of the samples were collected and was dissolved oxygen determined by method of Strickland and Parson (1969). Consequently, the quantity of oxygen evolution could be determined by using the formula of primary productivity as follows :-

$$\text{Gross primary production (GDP)} = \frac{375\ (Vl-Vd)\ X}{PQ}$$

Community respiration (CR) = 375 (VI-Vd) RQX

Net primary production (NPP) = GPP - CR

VI = Initial dissolved oxygen (mg/l)

VP = Dissolved oxygen in dark bottle (mg/l)

RQ = Respiratory quotient = 0.83

PQ = Photosynthesis quotient = 1.20

375 = The values use to change mg-O to mg-C unit

X = The values changed from hour to day unit

The values of net primary production could be converted to the total net primary production in the water column as a whole by plotting curve and then determining curvation area. So the net unit of total net primary production was gm or mg C/m^2 /day.

5. Analysis of bioerosion in live coral (Porites lutea)

5.1 Analysis of coral heads

The coral colonies were collected and taken to the laboratory to determine wet weight by using 0.02 kg spring balance and then measured the volume by water replacement method. Density of each coral colony was determined and estimated by using the calculation as follows.

Density

$$Dw = Ww/V$$

when Dw = wet density (gm/cm^3)

Ww = wet weight (gm)

V = volume (cm^3)

The 5 coral heads of each zone were collected and cut vertically at the center of colonies into approximately 1 cm thick slabs. Then, using the technique of coral x-radiography (modified from McGeachy and Stearn, 1976), the coral slabbs were analysed by Portable x-ray unit mode F-10, the potential voltage is 65 KV, 2.5 second of time, ORWO x-ray film HS 90, 12 x 15 $inch^2$. The analysed x-ray films were then developed into photographs for estimation the eroded area and converting to percentage of excavated skeleton in gm/m^3 unit. maximum bioerosion was also calculated in gm/m^3 unit.

5.2 Quantitative and qualitative study on infaunal animals

Sorting and relaxation methods :- 25 coral heads

of Porites lutea in each zone were collected by covering with cloth bag. The chisel and hammer method was used to expose all infaunal animals. After that particular groups of organisms :- sipunculids, polychaetes and bivalves, which were specified as the causative agents of the erosion were sorted out. These organisms were relaxed in 7 % $MgCl_2$ in sea water for 10 - 12 hours, prior to preservation.

Preservation; The specimens were preserved in 7 % neutralized formalin for 24 hours, and then changed into 70 % ethyl alcohol for permanent preservation.

Analysis of specimen; The individuals of each group were counted and identified as to family level unless genera or species are identifiable.

6. Field experiment

6.1 Methods and materials

Coral block :- from the preliminary study, it was found that coral colonies in shallow water at Samui Island were highly qualified for this experiment as they had few or very few borers. Thus, large heads of living Porites lutea from there were collected and cut into rectangular blocks approximately 6 x 5 x 3 cm. Nevertheless, special care was taken in selection of blocks i.e. those showing any traces of boring were discarded. The size of blocks was measured by using calipers. Porosity, bulk volume, bulk density and dry wieght were determined later in the laboratory. Selected coral blocks were cleaned by

immersing in 15 % H_2O_2 for 24 hours and then rinsed with distilled water twice. Afterwards, they were dried in oven at 100-110 ^{o}C for 24 hours. Small holes were drilled near the corners of each block for attachment horizontally on steel grids.

Porosity, bulk volume and bulk density determination ; The Ruska Universal Porometer at Department of Mining Engineering (Figure 3) was used to determine these parameters. The coral chip form each coral block after being processed had to be weighed before determination.

Description of the instrument :- The Ruska Universal Porometer consists of a 100 cc volumetric mercury pump, to which a pycnometer is attached. The pump has a precision ground. The chamber of the stainless steel pycnometer has a volume of approximately 50 cc and admits coral chips with size 1.5 inches long and 1.5 inches in diameter. The porometer is furnished with one or two test quality pressure guages. The hand wheel dial is graduated in 0.01 cc. subdivisions and permits estimation of plunger displacement of 0.001 cc. The right hand scale is used for all volume measurement during determination of porosity by mercury injection. Additionally, the right hand scale is used to provide bulk volume readings when the mercury injection method of porosity measurement is used. The right hand and left hand scales are respectively refered to as the volume scale and pore space scale.

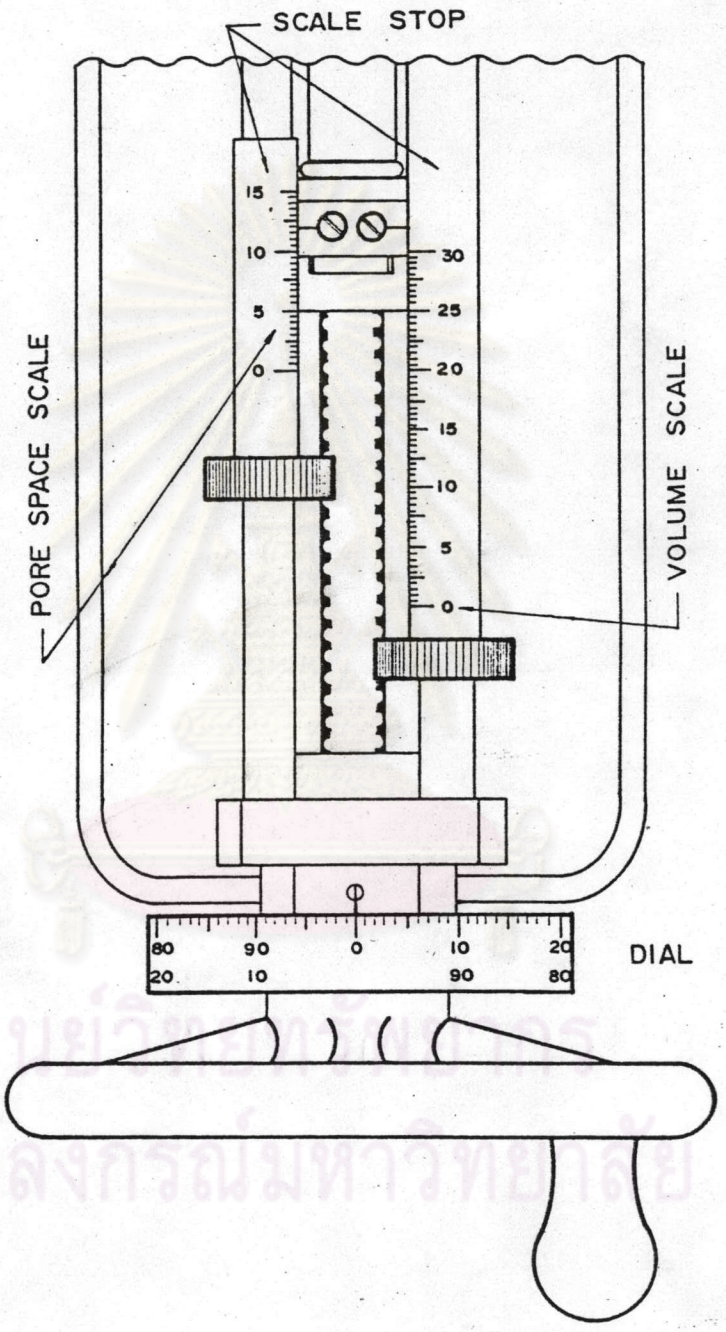

Figure 3 The Ruska Universal Porometer.

The bulk volume was determined directly by reading the volume scale whilst the porosity was determined by mercury injection with pressure at 750 psi. The calculation was achieved by the formula as follows :-

$$\text{Porosity} = (1 - \frac{Pv - FC}{Bv}) \times 100$$

Pv = Pore volume (cm^3)

Fc = Correction factors according to pressure

Bv = Bulk volume (cm3)

$$\text{Bulk density (Bd)} = \frac{Wd}{Bv} \quad (gm/cm^3)$$

Wd = Dry weight at 100-110 ^{o}C (gm)

6.2 Coral blocks experiment :- All of coral blocks which were mounted on steel grids as shown in Figure 4 and were placed 32 study points. The succession of borers in coral blocks, at 6 checked point, were investigated by bi-monthly one coral block collection per point. All coral blocks at 32 study points had to be collected after leaving at various points for 10 months periods. The coral blocks were collected by using plastic boxes and then preserved in 7 % neutralized formalin.

6.3 Analysis of coral blocks

The surface examination was performed by using binocular steriomicroscope. For the interior, 7 % nitric

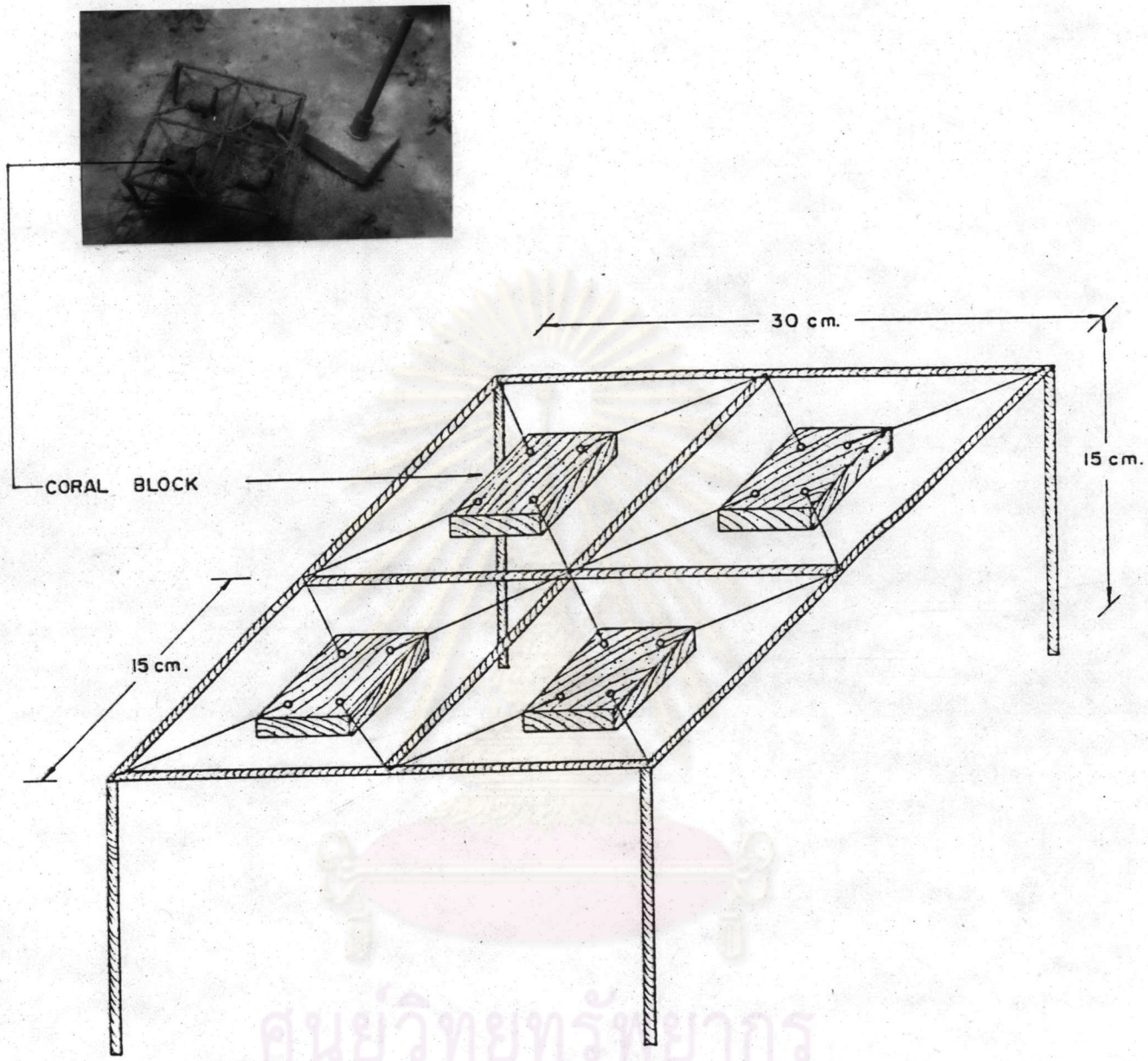

Figure 4 The steel grids with mounted coral blocks which were placed at 32 points in the study area.

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acid was used to dissolve the exoskeleton (Hutchings and Weate 1978). In case of undissoluable coral blocks, they had to be extracted by chisel and hammer. With these techniques, the study on succession of epicryptofauna and endocryptofauna were satisfactorily accomplished. The definitions of these specific names are as follows :- epicryptofauna are nestlers, encrusters, and endocryptofauna. However, some endocryptofauna are opportunistic invaders which inhabit in initial boreholes and crevices in coral substrates for feeding, reproduction and predator avoidance advantages. These animals particularly macroborers would occupied coral blocks rapidly.

In analysis the cryptofaunal composition on coral blocks, four of coral blocks were set into two groups : one for short term examination during 10 months period and the other for long term study. This research emphasized on short term which aimed at 2 objectives : taxonomic study of borers and bioerosion rate estimation.

The coral blocks for taxonomic study after being examined under the binocular steriomicroscope were extracted by dissloving in 7 % nitric acid (in 70 % neutralized formalin) and also in combination with chisel and hammer method. Cryptofauna was released from coral blocks could be sorted by using 300 Um sieve. The animals length longer than 1 mm were sorted for identification. These were counted and identified especially the boring groups.

The bioerosion estimation was done by assuming the borers as cylindical shape and measured the space diameter directly by calipers, then converted to volume and weight of coral substrates which were excavated by those borers.

Each coral block was examined at the outer surface by binocular steriomicroscope and then were oxidised with 15 % H_2O_2 for 24-48 hours depend upon the organic material content; oven dried at 100-110 ^{o}C for 24 hours, weighed. Subsequently, it was cut vertically and horizontally to make a serial thin section approximately 4.0 mm thick. The maximum bioerosional estimation was obtained by using the standard tracing paper grid 0.01 cm^2 (Figure 5) which was attached permanently on a slide. Then, bring against the slabbed coral and draw the shape of bored holes. This had to be done on both sides of the slab to figure out the excavated volume, all perforated holes were assumed 2 mm deep equally. Then converted to weight to calculate the maximum bioerosion rate in gm $CaCO_3$ /m^2 /yr as follows :-

Maximum bioerosion rate = 225 x volume of bore holes

when 225 = D x T x A and D x T x V

D = Average density of dry coral

= 1.18 gm/cm^3

T (Time) = values converted to year

= 1.2

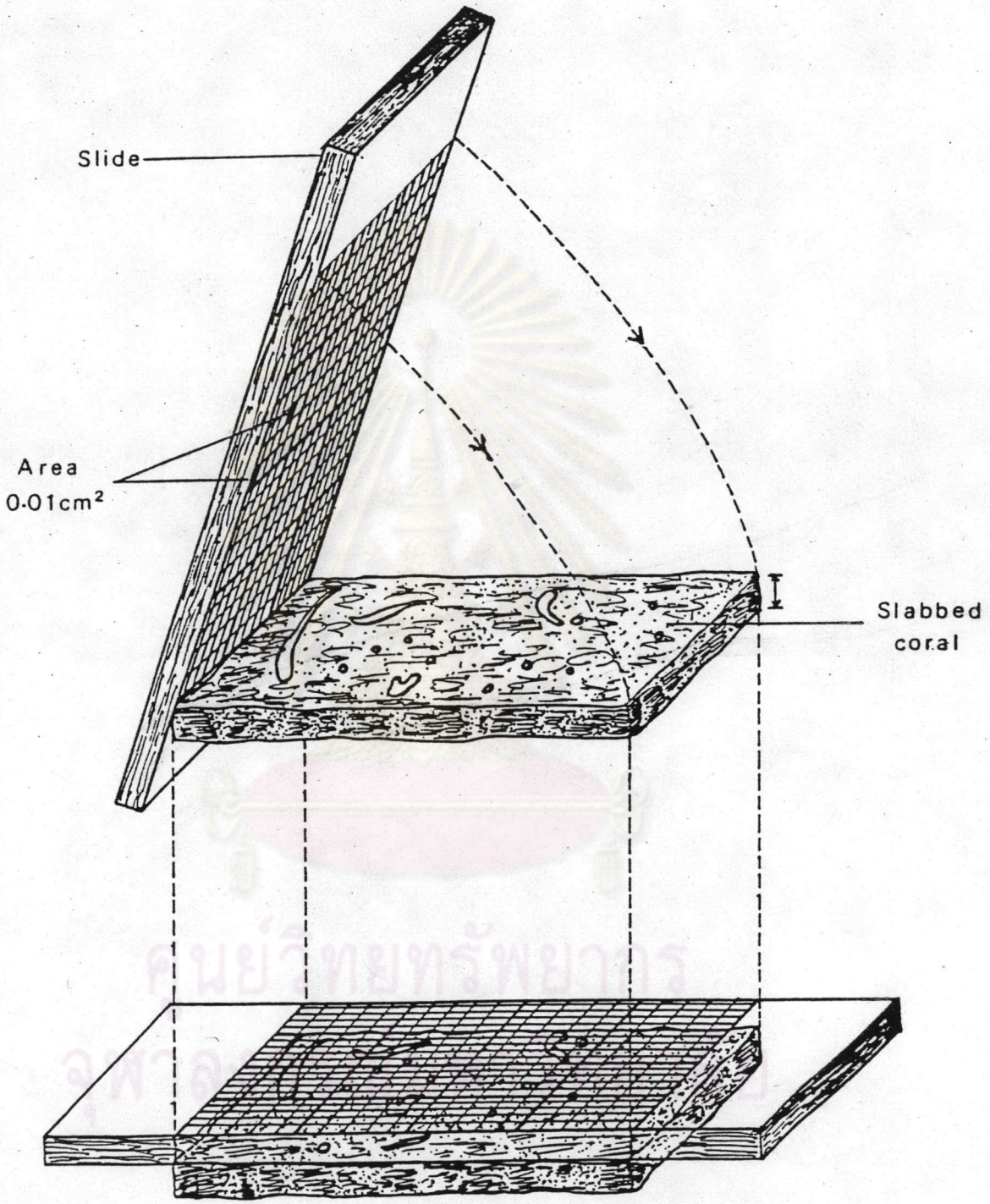

Figure 5 The maximum bioerosional estimation method and materials.

A (Area) = All of outer surface area values converted to square meters

= 104.167

V (Volume) = volues converted to m^3

= 10,416.7

7. Data analysis

7.1 Diversity Index and Evenness of borer species. Shanon index of general diversity and evenness (Odum, 1971) were used for borers tend in live coral and coral blocks in each zone.

$$\bar{H} = -\sum \frac{ni}{N} \cdot \log \frac{ni}{N}$$

or

$$\bar{H} = -\sum pi \cdot \log pi$$

and $$e = \bar{H} / \log N$$

when :-

$\bar{H}$ = Diversity index

e = Evenness values

ni = number of borers in each species

N = Total number of all borers

pi = $\frac{ni}{N}$

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7.2 Distribution pattern of borers (Poisson distribution)

The pattern of distribution of boring organisms, were investigated by using variance/Mean Ratio analysis i.e. random, uniform or clumped.

when :-

Mean $(\bar{X})$ = $\frac{\sum_i Xi}{n}$

Xi = Number of each borers in each coral head or coral blocks

n = Number of coral head or coral blocks

Variance$(S^2)n$ = $\frac{\sum_i (Xi-X)^2}{n-1}$

Variance/mean ratio = $\frac{S^2}{X}$

The interpretation of ration is as follows :-

Value of variance/mean ratio	Distribution pattern
1	random
> 1	clumped
< 1	uniform

Then, T-test was used to determine the significance level values of deviation of variance/mean ratio which differed from 1 by the calculation of standard error of the deviation for the variance/mean ratio

$$\text{Standard error of deviation} = \sqrt{\frac{2}{n - 1}}$$

$$t = \frac{\text{Observed ratio} - 1.0}{\text{Standard error of deviation}}$$

Observed ratio = Variance/Mean ratio

Degree of freedom = n - 1

7.3 Biological Index (McCloskey, 1970). In comparing dominant species of boring organisms between live corals and coral blocks, different zone, and different time.

The borer species are dominantly ranked by their biological index optained by giving 20 points for the first place ranking, 19 for the second, and so on. If one species ranked first in all twenty five samples, it would have the maximum index values of 500. However, the boring organisms are usually clumped togerther. Therefore, we have often found the alternation of ranking in all twenty five samples. So, the biological index value is normally less than 500.

7.4 Relationship of associated boring organisms which occured dominantly in all samples

From the above results the number of occurence of dominant boring organisms in each sample were filled up in 2 x 2 contingency table as following :-

		species B		
		Present	Absent	
species A	Present	a	b	a + b
	Absent	c	d	c + d
		a + c	b + d	T

when :-

a = number of coral heads found both species A and species B.

b = number of coral heads found only species A

c = number of coral heads found only species B

d = number of coral heads found neither species A nor species B

T = Total number of both species A and species B

And then, the determination of coefficient of association (c) is calculated by formula :-

Condition	Formula
1. when ad > bc	$c = \frac{ad - bc}{(a + b)(b + d)}$
2. when bc > ad and d > a	$c = \frac{ad - bc}{(a + b)(a + c)}$
3. when bc > ad and a > d	$c = \frac{ad - bc}{(b + d)(c + d)}$

Finally, To examine the statistical significance of the relationship chisquare (x^2) test is need.

$$\text{Chi-square } (x^2) = \frac{[(ad - bc) - 0.5\ T]^2\ (T)}{(a + b)\ (a + c)\ (b + d)\ (c + d)}$$

Degree of freedom = 1

8. Statistical analysis

Some numerical data were analysed by statistical formula which are packed in software statistical package (Lotus 1-2-3). The microcomputer IBM compatible and were used to keep record of some data, the standard statistical formula which are essential in some enumeration are :-

8.1 Average (Mean values, $\bar{X}$) :- It is applied to calculate most of data such as; the number of boring animals, sedimentation rate, meteorological and oceanographic data, bioerosion rate etc as follows :-

$$\bar{X} = \frac{\sum Xi}{N}$$

when :-

$\bar{X}$ = mean values

Xi = total number of data

N = Number of data

8.2 Linear correlation coefficient : This is useful for finding out the significance of relation of 2 variables

such as bioerosion rate and density of coral substrates; bioerosion rate and sedimentation rate; bioerosion rate and number of boring organisms etc.

The formula for calculation by electrical calculator is as follows :-

$$r_{xy} = \frac{\sum(XiYi) - (\sum Xi)(\sum Yi)/n}{[\sum Xi^2 - \frac{(\sum Xi)^2}{n}][\sum Yi^2 - \frac{(\sum Yi)^2}{n}]}$$

X, Y = From bivariate population and both of them relate each other

rxy = correlation coefficient, the value is range $-1 < r_{xy} < +1$

8.3 Linear regression :- It is useful for predicting the trends of relation of such as bioerosion rate and number of boring organisms, some relationship amongst boring organisms.

From : $Y = a + bX$

Y = dependent variables

X = independent variables

The calculator use the formula as follow

$$a = \frac{\sum y - b.\sum x}{n}$$

$$b = \frac{n.\sum xy - \sum x.\sum y}{n.\sum x^2 - (\sum x)^2}$$

when : A = constant

B = regression coefficient

8.4 Analysis of variances with two ways lay out

Use two ways for statistical significant. Then, calculate the Mean square by deviding sum of square with degree of freedom. After wards these results fill in the table to continue calculation as formula :-

Source of variance	df	Sum of square (SS)	Mean square (MS)	F	Ftable (df)
Between variable A	J-1	SSA	$\frac{SSA}{J-1}$	$\frac{MSA}{MSE}$	$\frac{J-1}{N-IJ}$
Between variable B	I-1	SSB	$\frac{SSB}{I-1}$	$\frac{MSB}{MSE}$	$\frac{I-1}{N-IJ}$
Interaction AB	(I-1)(J-1)	SSAB	$\frac{SSAB}{(I-1)(J-1)}$	$\frac{MSAB}{MSE}$	(I-1)(J-1)
Within group (Error)	N-IJ	SSE	$\frac{SSE}{N-IJ}$		
Total	N-1	SSTotal			

When :-

J = Number of zones or species

I = Number of periods or lines

N = Number of all total

The analysis of variance are useful to determine statistical significant the number of borers, in additional to compare the composition of dominant boring organisms.