CHAPTER II

MATERIALS AND METHODS

1. General Field Observations

Early after the onset of bleaching reported in late May, observations on coral bleaching were conducted by means of snorkeling and scuba reconnaissance at 13 reef sites in Phuket, Phang-Nga and Krabi provinces (Fig. 1: site 1-13). Informations on the date, location, depth, seawater temperature, and species of bleached corals were collected. In addition, percentage cover of live coral at those sites were estimated visually.

Furthermore, the occurrence of bleached corals, i.e. number of species and colonies, was quantified at 8 selected sites, namely Ko Hae (site 2), Ko Loan (site 4), PMBC (site 5), Ko Racha Yai (site 6), Ko Dokmai (site 8), Ko Hong (site 10), Ko Damkwan (site 11), and Ko Phi-Phi Don (site 13). All were fixed sites for the reef monitoring program of the PMBC. At each particular site, the transect tape (100 meters in length) was laid on the reef slope (3-6 meters depth) parallel to shore. Coral taxa and number of colonies encountered on the line transects were counted individually. Bleaching responses of those corals were classified as completely bleached, partially bleached and unbleached.

The partially bleached responses included either paling: the entire coralla was uniformly lighter than normal in color but not stark white, or white patching: some portions of the coralla retained normal color, while other portion was contrastingly stark white. The completely bleached responses implied total loss of color of whole colony and brought about the becoming visible of the underlaying white calcareous skeleton

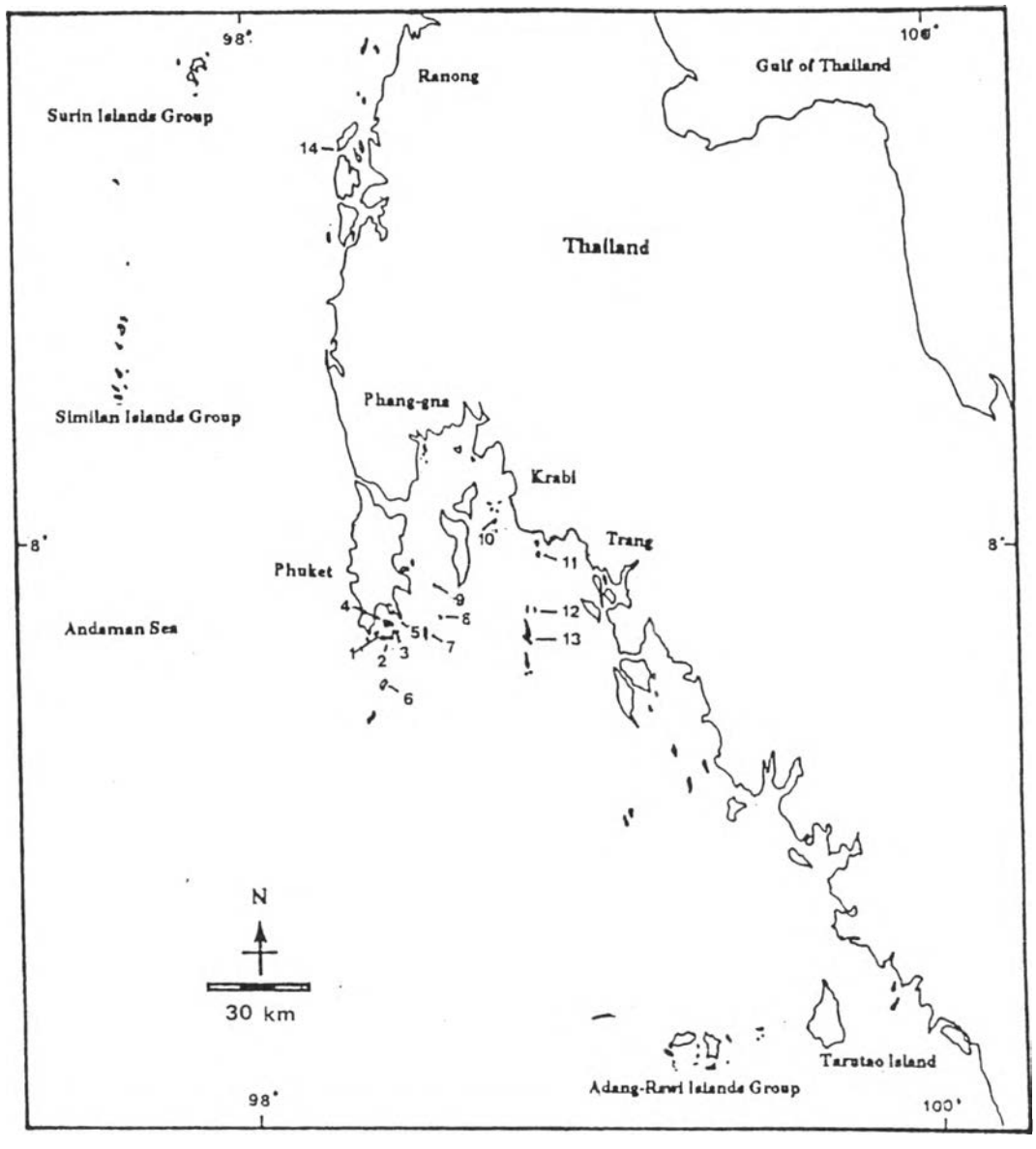


Fig. 1. Map of the Andaman Sea coast of Thailand showing areas of reef where bleaching were recorded.
1. Ko Keow and Ko Bon, 2, Ko Hae, 3. Ko Ell, 4. Ko Loan, 5. PMBC, 6. Ko Racha Yai, 7. Ko Maithon, 8. Ko Dokmai, 9. Ko Khai Nok, 10. Ko Hong, 11. Ko Damkwan, 12. Ko Phai, 13. Ko Phi-Phi Don, 14. Ko Ra.

through the translucent tissue. In some completely bleached specimens, however, the accessory pigments which specific to certain part of colony or species becomes visible and display faint shades of yellowish, pink, blue or some other color.

2. Field and Laboratory Studies

In the present study two levels of study, coral community and coral colony levels, were carried out at single selected areas.

2.1 Description of Study Site

The PMBC reef site, Cape Phanwa (Fig. 2), was chosen as a representative site for the study of the responses of corals, at both the colony and community levels, to the bleaching event. This reef is a simple fringing reef, firstly described in detail by Ditlev (1978). It is characterized by a sand flat, exposed reef flat, reef edge, and reef slope which drops to a depth of about 4 meters. The inner part of the reef flat is generally dominated by small microatoll forms of massive corals, *Porites lutea*, together with some other small heads of massive corals, including *Goniastrea pectinata*, *G. retiformis* and *Platygyra sinensis*. Some branching corals (i.e. *Acropora aspera*, *A. pulchra*, *Montipora digitata*, etc.) which are distributed in patches, are dominant in the outer part of this zone. Exposure to various physical factors during the tidal cycle governs the development of this reef zone. The reef edge is dominated by ramose growth-forms of *Acropora formosa, A. hyacinthus, A. digitifera* and *Porites nigrescens*. The reef slope is rather steep, 60-90 degree inclination, and narrow, 10-15 meters width. Several species of Acroporids, such as *Acropora formosa*, *A. nobilis*, *A. hyacinthus* as well as *Porites lutea*, *Pectinia alcicornis*, and faviids are the major component of corals in this zone.

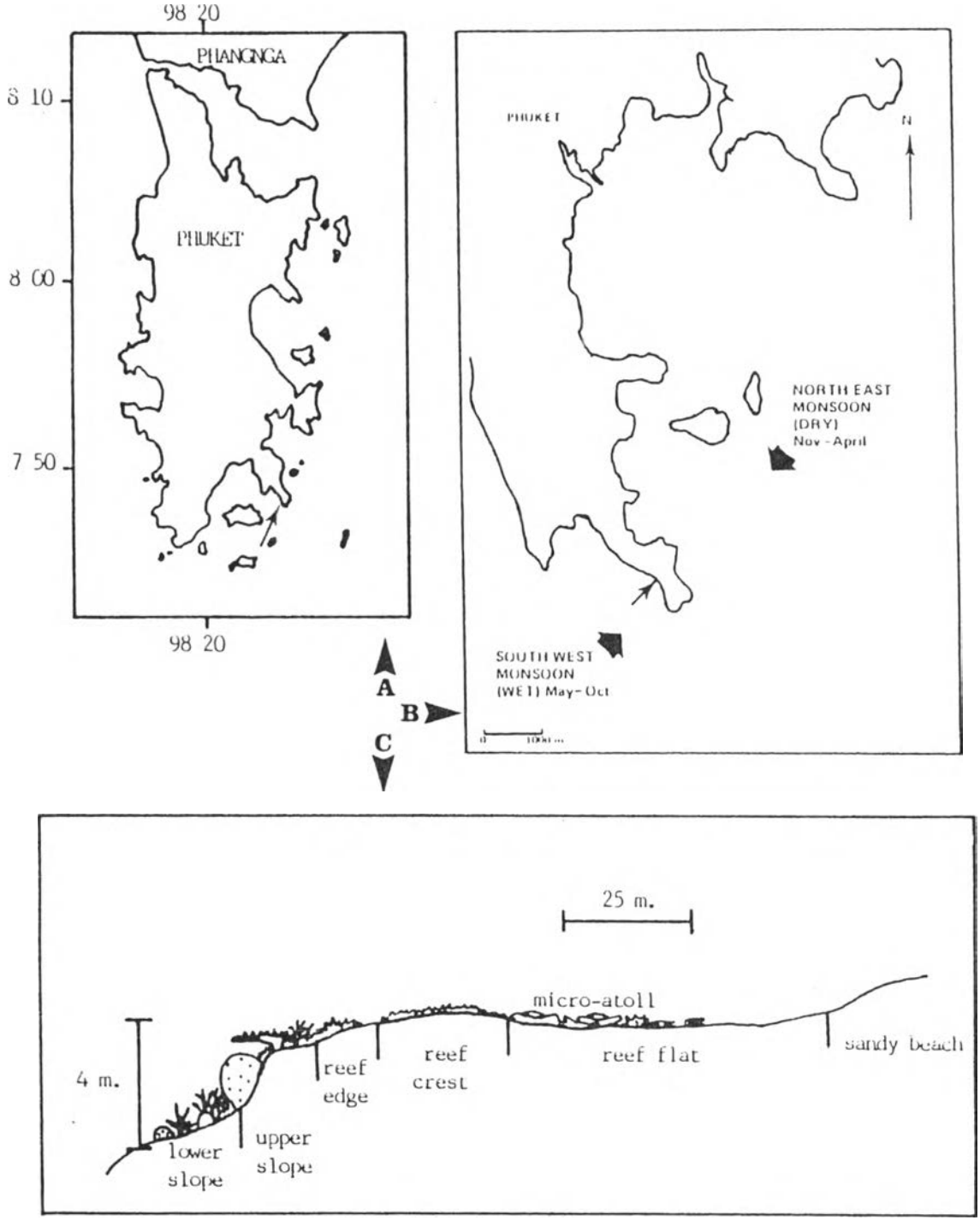


Fig. 2. Location of the PMBC reef site (A,B) and reef profile (C).

2.2 Coral Community Level Studies

2.2.1 Tagging

On 7 August 1991, bleached colonies of several coral species on the reef slope at PMBC site were initially tagged using rectangular pieces of plastic with a coded number. An exception was the soft coral, *Sarcophyton* sp., which was recorded by counting colonies within a fixed area. All tagged corals were the prolonged bleached colonies which started in late May. Tags were fixed onto colonies. For massive, columnar and encrusting coral growth forms, i.e. *Porites lutea*, *Lobophyllia* sp., *Symphyllia* sp., *Physogyra lichtensteini*, *Favia* sp., *Psammocora digitata*, *Lithophyllon edwardsi* and *Mycedium elephantotus*, tags were fixed onto corals with stainless steel nails. Corals of the others growth forms, including branching, ramose and solitary, i.e. *Acropora* spp., *Pocillopora damicornis*, *Hydnophora rigida*, *Pectinia alcicornis*, *Merulina ampliata*, *Fungia* spp., *Herpolitha limax* were tagged with plastic coated wire. Two later observations were conducted on 12 September and 15 December 1991.

The living conditions of corals through the recovery process were classified into 8 categories: partially bleached, completely bleached, bleached with partly dead, bleached with the process of recovering, bleached with dead and recovered part, normal, normal with partly dead, and dead.

2.2.2 Line transect assessment

A transect, one hundred meters long, was laid across the reef slope at the PMBC site, parallel to the shore, according to the method described in Dartnall and Jones (1986). Any coral species which was beneath the line was recorded and its projected length on the line was measured precisely at centimeter level. Practically, an individual was defined as any colony growing independently of its neighbours. In cases when the transect line intercepts an individual colony more than once, e.g. when a massive *Porites* colony with dead patches and living tissue is under the line, each intercept with living tissue was recorded

as belonging to the one colony (see also Fig. 3). For monitoring purpose, the transect line was located by marking with iron rods hammered into massive corals along the line.

Three successive assessments were conducted on 29 July 1991, 15 March 1992 and 22 April 1993. For the first assessment, centimeters cover of partially bleached corals, completely bleached corals, and recently dead corals due to bleaching were measured separately. As the recently dead and previously dead corals could be clearly distinguished; recent death was indicated by the presence of freshly-exposed, white skeletons without mucous, at most only slightly overgrown by microscopic algae. Therefore, it should be reasonable enough to back calculate the amount of coral coverage prior to bleaching by taking the later proportion into account.

Using this line technique, estimation of percentage live coral cover, colony size frequency, coral diversity and evenness could be obtained. The Shannon & Weaver's index of diversity was calculated both from the relative abundance using number of colonies (H'n) and from the relative living coverage (H'c) (after Loya, 1972).

$$H'n = -\sum p_i \log_e p_i \quad \text{----------(1)}$$

where $p_i = N_i/N$ is the proportion of the total number of individuals (N) belonging to the $_i$th species (N_i), and

$$H'c = -\sum p_i \log_e p_i \quad \text{----------(2)}$$

where p_i is the live coverage proportion of the $_i$th species in the sample. The evenness index was also computed basing on H'n or H'c (Pielou, 1977).

$$J'(n \text{ or } c) = H'(n \text{ or } c; \text{ observed})/H_{max} \quad \text{---(3)}$$

where $H_{max} = \log_e S$ is the equitability of all S species in a sample.

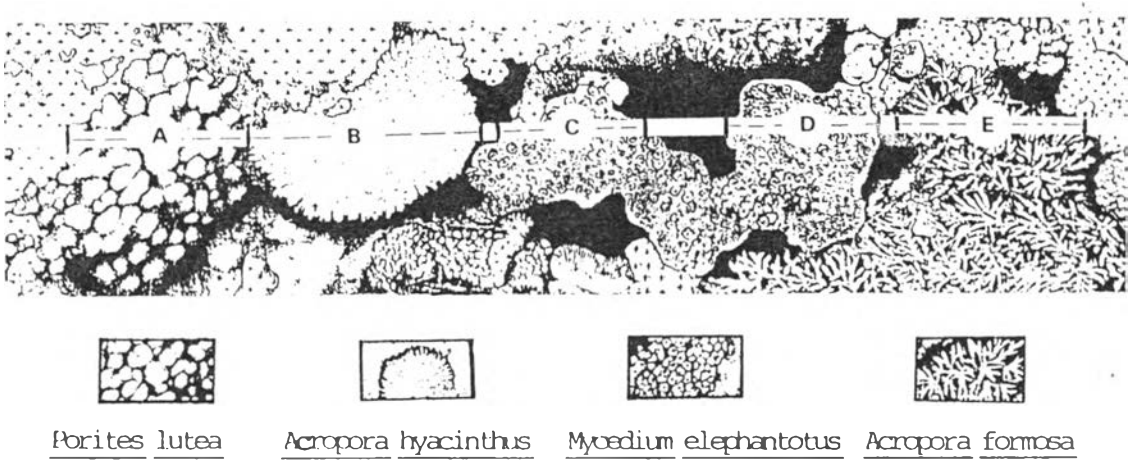
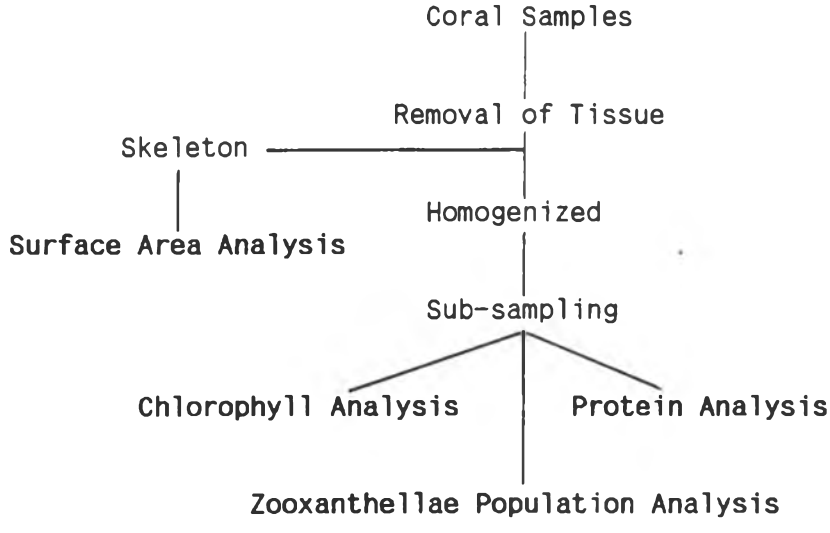


Fig. 3. Illustration of coral cover assessment using line-transect method. The projected lengths for each colony of: P. lutea = A, A. hyacinthus = B, M. elephantotus = C+D, and A. formosa = E.

Comparison among these parameters could lead to the calculations for the degree of damage and responses of coral community resulted from bleaching.

2.3 Coral-Colony Level Studies

The outline of this experimental design is shown below.



2.3.1 Sample Collection

Tagged colonies of bleached species which survived the early mortality and continued their recovery process, were chosen for time series collection. Three colonies of each of the prolonged bleaching species, *Psammocora digitata*, *Pectinia alcicornis*, *Lithophyllon edwardsi*, *Mycedium elephantotus* and *Merulina ampliata* were studied.. The first collection was carried out on 13 September 1991. Tagging number of each collected colonies was recorded and recognized for later sampling. Altogether nine collections were performed.

Tissue/skeleton samples were carefully taken from the colony with the aid of hammer and chisel. One fragment was collected from each colony during each sampling date. Each sample was placed in a labelled plastic bag, wrapped in aluminum foil and frozen at -20 ^{0}C until they could be processed.

2.3.2 Tissue Biomass Sampling and Analysis

The tissue covering the coral skeleton was removed using a dental Water-Pik with distilled water, and then placed in a clean plastic bag (Johannes and Wiebe, 1970). Skeletons were dried and kept for later determination of the surface area . The slurry was gently ground in a loose-fitting glass tissue grinder. The volume of homogenate was recorded and sub-samples were taken for zooxanthellae counts, chlorophyll analysis and tissue protein analysis.

2.3.2.1 Determination of Zooxanthellae Population size

Samples for zooxanthellae counts were initially preserved in Lugol's iodine solution (Appendix A). The preserved homogenate was tranferred onto the Neubauer chamber (hemocytometer). All fields with the total area of 25 mm^2 from each of six replicated chambers were counted using a compound microscope.

The zooxanthellae content of each coral sample was calculated from the resulting zooxanthellae density to the total volume of homogenate and standardized by converted to zooxanthellae cells per square centimeter of living coral surface.

2.3.2.2 Photosynthetic Pigment Analysis

Homogenate sample of known volume was filtered through Whatman glass fiber filter. As the sample was being filtered, few drops of suspension of $MgCO_3$ in distilled water were added to prevent acidity on the filter. Filter was drawn dry, folded in half, wrapped in aluminum foil, and stored in a freezer at -20 ^{0}C. until being extracted.

For extraction, the filter was then ground in a loose-fitting glass tissue grinder together with 4-5 ml of 90% acetone. The homogenate was then made up to volume of 10 ml with 90% acetone and then stored in centrifuge tube refrigerated in darkness for about 20 hours.

Chlorophyll extracts were then centrifuged and read against acetone blanks on a spectrophotometer at 750, 664, 647 and 630 nm and calculated the chlorophyll a concentration using the equation of Jeffrey and Humphrey, 1975 (as cited in Parson et al., 1984).

$$\text{Chlorophyll-a (mg)} = \{11.85E_{664}-1.54E_{647}-0.08E_{630}\}x\{V/v \times l\} \quad \ldots.(4)$$

where v = volume (ml) of filtered homogenate, V = total homogenate volume (ml), l = path length of spectrophotometer cell, and E = extinction values, at wavelengths indicated by subscripts, after correcting for a blank measuring at 750 nm.

2.3.2.3 Tissue Protein Analysis

Samples for protein analysis were taken from the remaining homogenate. Wholly homogenized again before sub-sampling for total protein (host tissue plus zooxanthellae) analysis is needed. The soluble protein concentration were determined by Lowry's method as described in Peterson (1977), using bovine serum albumin standard (see Appendix B).

2.3.2.4 Coral Surface Area Analysis

Coral surface area was determined by the aluminum foil method (Marsh, 1970). The coral skeletons were carefully overlaid with pieces of aluminum foil, so as to avoid overlapping and folding of the foil. The pieces of foil were weight and skeleton surface area (cm^2) were then calculated by comparison with weights of foil pieces of known surface area (see Appendix C).