CHAPTER II

MATERIALS AND METHODS

STUDY AREA

The sampling area located at the end of the PMBC (Phuket Marine Biological Center) pier (Fig. 4), south-eastern coast of Phuket Island in the Andaman Sea, about 100 meters from the shore, where the water depth is about 7.5-9.5 meters with muddy-sand bottom next to the reef flat area. Two distinct monsoon seasons characterize this area, the north-east, November to April, dry and hot and the rest of the year is wet and cooler due to the south-west monsoon.

MATERIALS AND METHODS

Environmental Factors

1. Primary Production

Data of primary production could be obtained by the ^{14}C method with four-hour in situ incubation around noon as recommended by Sundström et al. (1987). Experiments were conducted weekly at the PMBC pier for a period of one year. Incubation bottles were placed at five depths, surface, 1, 2, 4 and 6 meters. Dark bottles were incubated at two levels, surface and 6 meters. Approximately four microcuries of ^{14}C were added in each bottle. After incubation, the samples were filtered onto Sartorius membrane filters (0.2 micrometer pore size) and exposed to fumes of hydrochloric acid and formalin before being dried. Activity

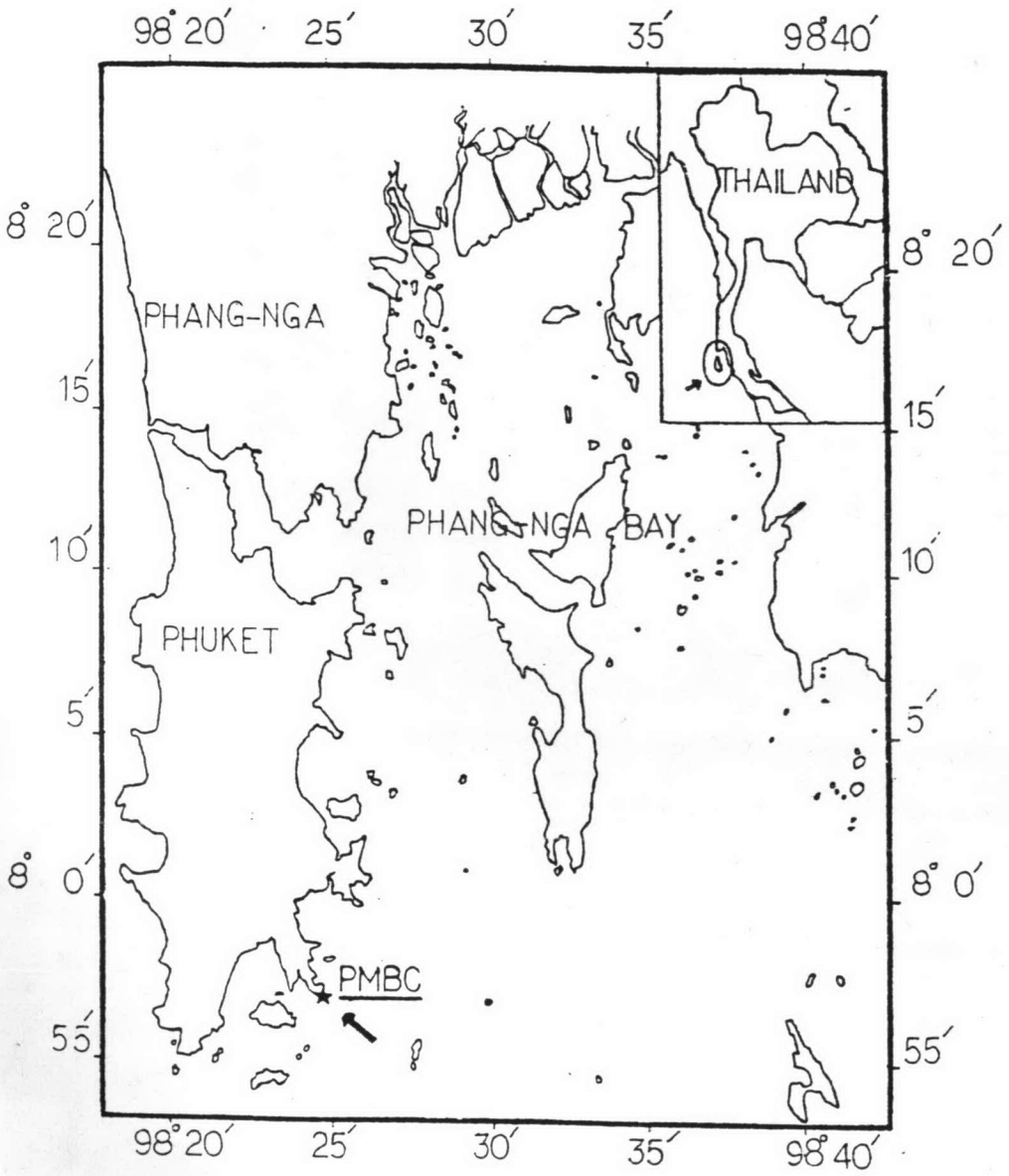

Fig. 4 Location of the study site at the PMBC, Phuket Island.

counts were made by a Geiger-Muller counter. Selected filters were also counted in a liquid scintillation at the ^{14}C-Center, Hørsholm, Denmark, for calibration. Primary production throughout the water column was calculated by integrating data from different depths.

2. Chlorophyll Concentration

For chlorophyll determination, sea water was sampled at the same depth by a 3-liter fiberglass and stainless steel water sampler. As it had been reported that production of copepods was effected by phytoplankton cell size, so, two fractions of chlorophyll concentration were determined. The two selected size fractions were ; the fraction of size larger than 1 micrometer, as representative for total chlorophyll concentration and the other fraction was larger than 8 micrometer, as representative for the size which is able to be consumed by copepods. In order to get two fractions of chlorophyll concentration, one liter of water sample was filtered onto Whatman GF/C filter paper and another one liter was filtered onto 8-micrometer membrane filter. The filters were homogenized an extracted in 90 per cent acetone. Chlorophyll-a was determined by spectrophotometer and value determined by substitution in the equation used by Jeffrey and Humphrey (1975).

3. Temperature

Temperature and salinity were recorded at all sampling depths. Temperature was recorded from a thermometer, until the last half of the study period, it was recorded by a STD (salinity-temperature-depth) sensor.

EXPERIMENT IN COPEPOD CULTURE

Cultivation of any copepod is an important thing to be done as to obtain the information of development stages, juvenile growth rate, size-weight relationship and also to use the stages of the animals, nauplius 1-6 and copepodites 1-6, in various aspects of laboratory experiment. Since the preliminary study in culturing of *Acrocalanus gibber* in the laboratory was succeeded as adult females were developed in the culture, the experiment in cultivation of *A. gibber* was decided to be carried out. The experiment was expressed as follow :

1. Collection

Copepods were collected during daytime at high tide by a 500 micrometer mesh size plankton net with 0.75 meter opening diameter. Oblique towing from about 1 meter above the bottom up to surface was applied with the towing distance of about 30 meters. The content of the cod-end was transferred carefully into a plastic bucket with about 15 liters of seawater from the same are. The accumulated sample collected from 8-10 tows was immediately taken to the laboratory. The collected sample was filtered onto a 180 micrometer sieve which was kept submerged all the time. Healthy, mature female of the calanoid copepod, *A. gibber*, were sorted out under dissecting microscope by a small pipette.

2. Culture

The sorted *A. gibber* females were transferred to a 10-liter glass jar with filtered seawater (through 1 micrometer sieve) and bubbled gently with air. After 4-5 hours at room temperature, all females were filtered out with a 180 micrometers sieve and only eggs were left in a jar for hatching.

The animals were fed daily with unicellular algae, *Tetraselmis* sp. in excess amount. Subsampling of about 30 animals were done every 12 hours to check the development stages.

Carbon Content of the Animals

In order to measure weight in term of carbon content of the animals, copepodid stages of *A. gibber* were sampled from PMBC pier by using vertical tow of 200 micrometer net and eggs were obtained from incubation mature females of *A. gibber* in laboratory for about four hours.

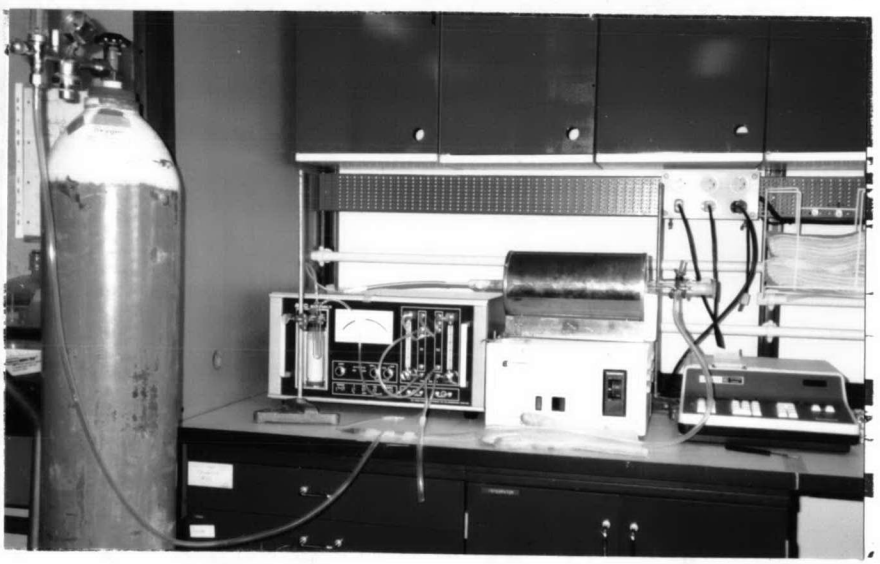

Fig. 5 Infra Red Gas Analyzers (IRGA) type 225 Mk3.

Carbon content of *A. gibber* was measured by an Infra Red Gas Analyzers (IRGA). The animals were prepared as follows : body length of the animals, and diameter of eggs, were measured in the unit of micrometer and the exact number of animals were counted. They were then put into a small foil bowl, 4 millimeters in diameter, which had been pre-combusted in a muffle furnace (550 ^{o}C) for 2 hours. In the bowl, the animals were rinsed many times with artificial seawater and finally the water was removed as much as possible by a mouth pipette. Samples were dried at 60 ^{o}C for 3 hours and kept in a freezer until analyzed.

For carbon analysis, the IRGA (Fig. 5) was calibrated with different known concentrations of oxalic acid. The output data and weight in microgram carbon of oxalic acid were plotted into a standard curve. The dry copepod samples in foil bowls were then introduced one by one into a small chamber which was heated to 600 ^{o}C. The instrument would measure the integrated carbon dioxide development in gas flow. The result would appear immediately on an integrating machine connected to the instrument. By comparing the figures recorded to the standard curve, weight in microgram carbon of the animals in different size classes could be obtained.

Calculation of Body Weight

The average carbon contents of the animals derived from the IRGA were plotted against the length of each stage. This relationship between the weight and body length would result in the form of the equation :

$$W = a L^b$$

where, W = weight of the animals in microgram carbon

L = body length of the animals in micrometer

a = intercept

b = exponent

Estimation of Abundance, Biomass and Production

To describe the seasonal variation in abundance, biomass and production of *A. gibber*, a field sampling program was conducted at PMBC pier. Copepod egg production was estimated by the egg production method (Berggreen et al., 1988). Copepod egg production measurements were conducted weekly between July 1990 and July 1991. Samples for abundance and biomass estimates were collected simultaneously every week between October 1990 and September 1991.

1. Abundance

A 200 micrometers mesh size plankton net with an opening diameter of 0.50 meter (WP-2 type) was slowly towed vertically from bottom to surface. The samples collected were preserved in 10 per cent formalin. Three replicate samples were taken in the same area every week from October 1990 to September 1991.

One fourth of each samples was subsampled to sort out *A. gibber* both in copepodid and adult stages. The number of all *A. gibber* was counted and the prosome length was measured in micrometer under dissecting microscope. The weekly abundance of *A. gibber* was calculated

to the same standard of number of individuals per cubic meter of water filtered from the average of the three replicates.

2. Biomass

From the length-weight relationship equation obtained from the carbon analysis, the body length at each size class of *A. gibber* was converted to weight in microgram carbon. The weekly biomass of the animals were then calculated as weight in microgram carbon per cubic meter of water.

3. Egg Production Rate

For egg production experiments, once a week, a 500 micrometer mesh size plankton net, 0.75 meter in diameter, was used for oblique towing in the same way as towing for culture. After 3-5 tows, the sample was immediately taken to the laboratory. Approximately five mature females of *A. gibber* were transferred to each of fifteen 200-ml screw cap bottle (filled with 40 micrometers filtered seawater).

After about 4 hours, the females and eggs were filtered out through 180 and 40 micrometers sieves, respectively. Number of living females and eggs produced were counted and their sizes were measured. Incubation time was recorded.

Specific egg production rate (G_f) was calculated by :

$$G_f = \text{egg.female}^{-1}.\text{day}^{-1} \times (W_{egg}/W_{female}) \times (24/T)$$

(Peterson et al., 1991)

where, W_{egg} = averaged weight in microgram carbon of egg

W_f = averaged weight in microgram carbon of incubated females (from length-weight regression)

T = incubation time (hours)

4. Production

Copepod secondary production (PR) could be estimated from measurements of stage specific instantaneous growth rates and biomass (Kimmerer, 1987) :

$$PR = \sum g_i B_i$$

where, B_i = biomass of copepod stage i (microgram carbon per square meter)

g_i = specific growth rate of stage i

Berggreen et al. (1988) showed that, for *Acartia tonsa* and several other species of copepods, juvenile specific growth rate is exponential and equal to the specific egg production rate (specific growth of female copepods). Therefore,

$$g_1 = g_1 = g_3 = \ldots = G_f$$

It is assumed that this was the same for *A. gibber*, thus,

$$PR = G_f \sum B_i$$

where, $\sum B_i$ = the total *A. gibber* biomass (microgram carbon per cubic meter)

G_f = specific growth rate of female copepods (per day)

Production was estimated each week during one year period.

Role of *Acrocalanus gibber* in the Coastal Pelagic Food Web

1. Ingestion Rate

The algae, *Tetraselmis* sp. in exponential growth, as food for copepods was used in six levels of cell concentration, 50, 100, 200, 400, 800 and 1500 microgram carbon per liter. Prior to each experiment, size of about 100 cells of the algae were measured. Average cell size was 16 micrometers of the first three experiments and 14 micrometers for the rest. Carbon content of the cell could be estimated from the equation developed by Strathmann (1967) by measurements of cell size and assuming spherical cell shape. His equation showed that specific carbon content of the algae is equal to 0.11×10^{-6} microgram carbon per cubic micrometer.

In order to estimate the ingestion rate of *A. gibber*, the experimental procedure was conducted as follows :

1) Different concentrations of cells were prepared by dilution from the stock culture into 20 micrometers filtered seawater. Number of cells to represent each level of carbon content per liter were calculated based on the Strathmann equation. Concentrations corresponding to 50, 100, 200, 400, 800 and 1500 microgram carbon per liter were prepared for about 2 liters of each concentration. Concentrated growth medium was added to the suspensions to prevent nutrient limitation during experiments.

2) From the stock made for each concentration, it was then transferred to five 200-millimeter screw cap bottles and 10-12 mature females of *A. gibber* were added into three of the bottles. These female copepods were taken from the sea and left them with excess phytoplankton overnight. The remaining two bottles without animals were left as control.

3) The bottles were put on a shaker for 24 hours at a speed of 60 rounds per minute to keep phytoplankton suspended. The light and dark period were in the ratio of 10:14 and they were left at room temperature.

4) After 24 hours, the animals were filtered out onto 180 micrometers sieve and the alive individuals were counted under the compound microscope.

The ingestion rate could be calculated by :

$$I = F \times C \qquad \text{(Kiørboe et al., 1982)}$$

where, I = ingestion rate

F = filtering rate (clearance rate)

$= (V/Nt) \times \ln (C_1^* . C_2 / C_1 . C_2^*)$

V = volume of incubation bottle (millimeter)

N = number of experimented female

t = incubation time (hours)

C = average cell concentration

$= (C_2^* - C_1^* / \ln(C_2^* / C_1^*)$

C_1 & C_2 = number of cells in the control bottles at the beginning and the end of the experiment.

C_1^* & C_2^* = number of cells in the experimental bottles at the beginning and the end of the experiment

2. Biomass and Production of Other Zooplankton Groups

From zooplankton samples collected each month, one fourth was used for the identification of other zooplankton groups. Number of all zooplankton groups were recorded and calculated to the amount of individuals per cubic meter of water.

The leftover three-fourth of the samples was filtered onto 100 micrometer plankton screen, dried at 110 ^{o}C overnight and then heated to 550 ^{o}C for 3 hours in a muffle furnace. The sample was weighed after each step and biomass of the total zooplankton would then be calculated as milligram ash free dry weight per cubic meter of water.

Zooplankton production was crudely estimated by assuming that the specific growth rate of *A. gibber* could represent other zooplankton specific growth rate at the sampling site as well. Thus, zooplankton production could be estimated from equation ;

$$PR = \sum G_f . B_i$$

which give $\sum B_i$ = total zooplankton biomass.

3. Relationship between *Acrocalanus gibber* and Other Groups of Zooplankton

Correlation between density of *A. gibber* and other zooplankton groups was shown by using Spearman Rank Correlation. Cluster analysis was also employed for determining of similarity within groups of zooplankton, with emphasis on *A. gibber*.

4. Stomach Content of Pelagic Fishes Living Around the Study Area

Fishes were collected for stomach analysis at the PMBC pier by 1 centimeter mesh size gillnet and by spearing at the PMBC pier. Samples were taken at four occasions within three months, October to December 1991.

From 36 species of fish collected, gut content from 157 fishes were examined. The species and number of fishes which contain *A. gibber*, other species of copepods and also other zooplankton in their guts were recorded.