# CHAPTER II

# MATERIALS AND METHODS

## Study Area

The scallop samples used in this study were obtained from trawl catch by the governmental fisheries research vessels as well as private trawlers operated in the study area around Chang Islands located in the most eastern part of the Thai territory in the Gulf of Thailand between latitudes $11^{o}$ 30' to $12^{o}$ 15' N, and longitude $102^{o}$ 5' to $102^{o}$ 50' E.

Chang Islands compose of two large islands, one is Koh Chang, the largest island in the Gulf of Thailand, and the other which locates south-eastern to Koh Chang is Koh Kood. Other than these two islands, there are many (about 50) small islands inbetween, i.e. Koh Mak, Koh Kradad, Koh Wai, etc. (Figure 2)

## Sampling and Handling of Samples

Collecting of samples and data can be categorized into 2 groups according to the needs in data analysis as :

### 1. Samples for Collecting Length Frequency Data

Studying of the growth performance, mortality and recruitment in the population was carried out through an analysis of length frequency data. Scallop samples were obtained from trawl catches of the governmental marine fisheries research vessels;

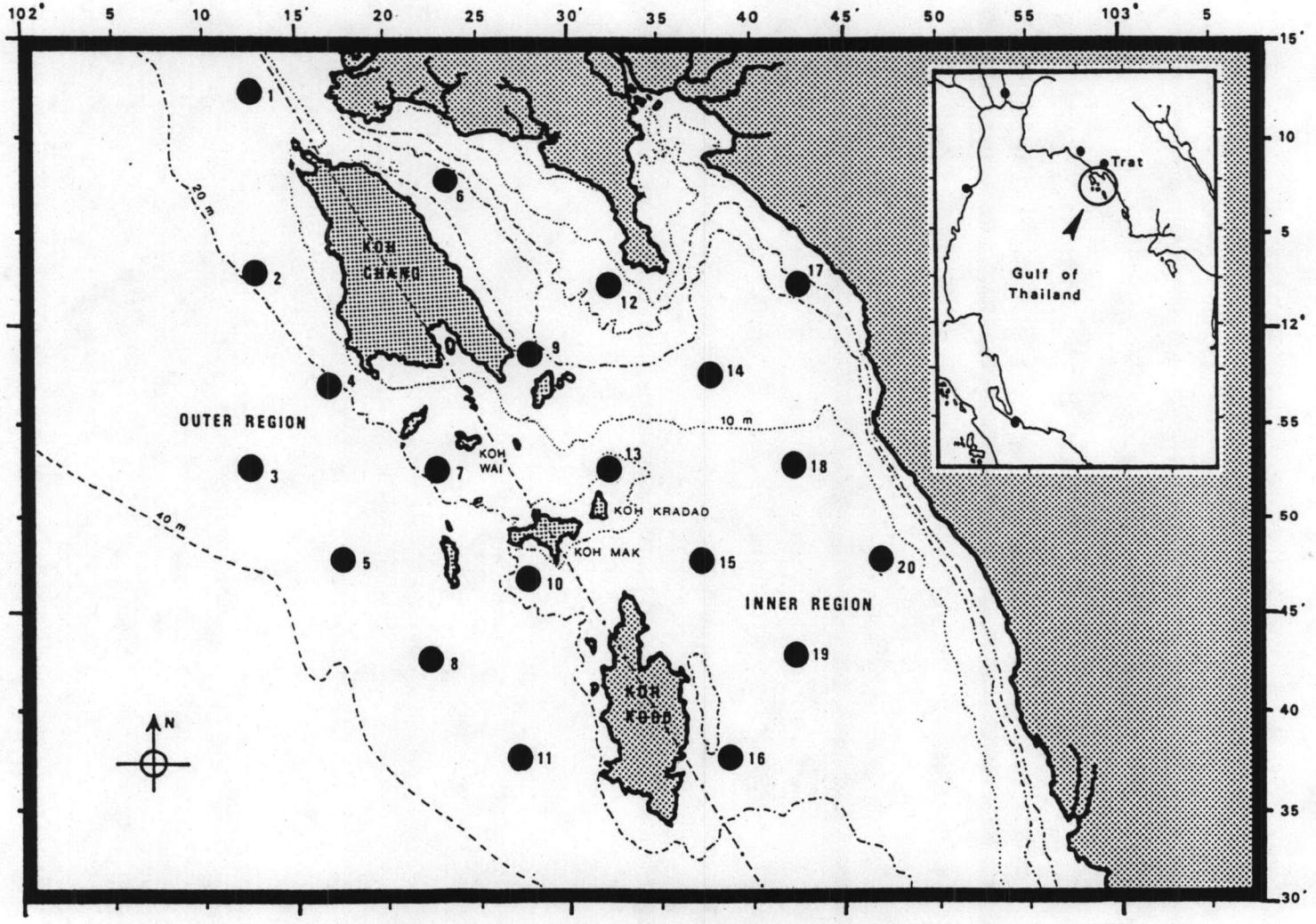


Figure 2 Study area and sampling stations; Chang Islands, Trat Province, Eastern Gulf of Thailand.

R/V Pramong Talay 12 and Pramong Talay 15 of the Eastern Marine Fisheries Development Center, Department of Fisheries. Sampling was carried out for one year, from January to December 1987.

Both the research vessels, Pramong Talay 12 (about 18 m in length), and Pramong Talay 15 (about 23 m in length) were equipped for bottom trawl nets, 39 m in upper-length and 51 m in lower-length size, with a 4 cm mesh cod-end.

Trawl samples were collected monthly from 20 stations as shown in Figure 2, with 1 hour trawl at each station. The total catch of scallops from each trawl was weighed and then individual shell height (dorso-ventral axis; i.e. the straight-line distance from auricles, or hinge, to the ventral edge of the shell) were recorded immediately on board the research vessel by punch-marking on an L-shaped measuring board (Figures 3 and 4). In this study, data of shell height frequency were used instead of shell length. The height frequencies with 0.5 cm class interval were compiled for analysis.

2. Samples for Laboratory Studies

For the determination of relationships between different shell dimensions and weight of various parts of the body as well as for gonad studies, a number of scallop samples were collected from both the governmental fisheries research vessels and private trawlers operating in the study area. The samples were chilled in ice-box while being transported to the laboratory for measuring, weighing and inspection afterwards. This part of work also covered a period of one year, June 1987 to June 1988.

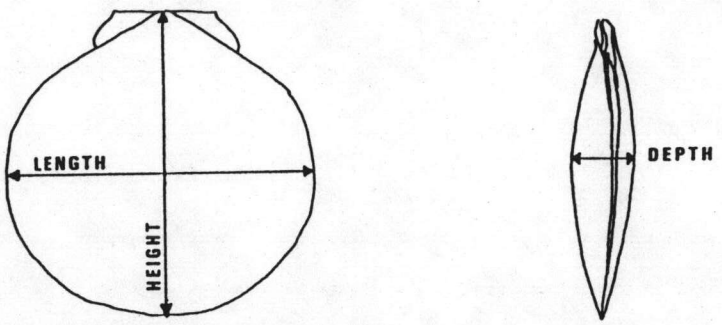


Figure 3 Shell dimensions for size measurement of the scallop *Amusium pleuronectes*.

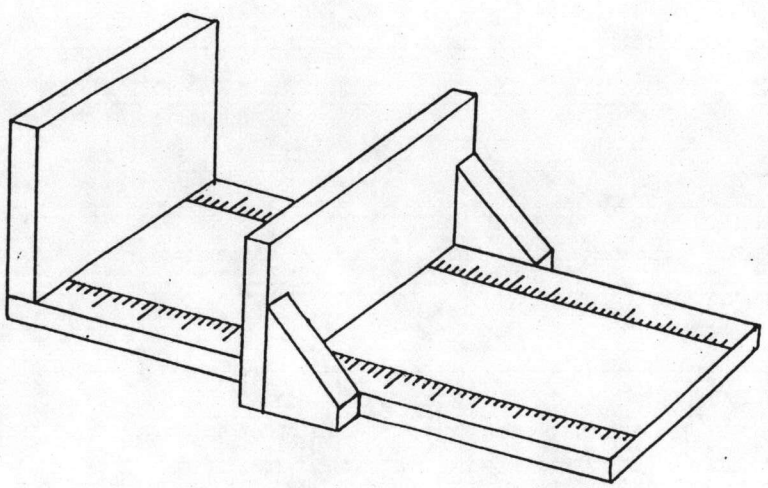

Figure 4 L-shaped measuring board.

**Length Frequency Data Analysis**

Compiled data of shell height frequency distribution obtained from punch-marking record were used to determine the growth, mortality and recruitment through the Compleat ELEFAN microcomputer program. The term "length", symbolized by "L" appeared anywhere when processing through the program in this study, actually refers to the shell height, instead of shell length.

To determine whether the growth would vary depending on location of sampling area, the study area was divided into two regions; the inner stations (station 6, 9, 12, 13, 14, 15, 16, 17, 18, 19 and 20) and the outer stations (station 1, 2, 3, 4, 5, 7, 8, 10 and 11) (Figure 2). Catch data were lumped together into each group of stations, then processed for growth parameters determination through the Compleat ELEFAN. Growth parameters derived from each region were compared, in term of 0' (Pauly and Munro, 1984) as expressed by the equation :

$$\emptyset' = \log K + 2 \log L_{\infty} \qquad \text{.....(10)}$$

When the results from both regions showed similarity of 0' value, which meant that the growth performance of the scallop populations in both regions were similar, all catch data were pooled together and further analyzed as one.

Original data with 0.5 cm class interval was entered through ELEFAN 0, a data entry and file managements routine. Then those data were used for further analysis through ELEFAN I and ELEFAN II.

1. **Estimation of growth parameters**

Computation of growth parameters, $L_\infty$ and K, from the length frequency data entered through ELEFAN 0, was obtained by using ELEFAN I. This yielded estimation of $L_\infty$ and K with corresponding Rn, a goodness of fit value.

Besides $L_\infty$ and K, estimation of the parameters C (amplitude) and WP (winter point) of the seasonalized VBGF, according to equation (4), was also available in the growth analysis by ELEFAN. Varying the C and WP values were tested to obtain the appropriate parameters set, corresponding with the highest Rn value and suitable growth curve. When variation of C and WP showed no better applicability, as for tropical climate where the seasonal difference was not evident, parameters C and WP were assumed to be zero.

The class interval of length frequency data was adjusted, from originally 0.5 cm, to 1.0 cm interval by ELEFAN 0. Then all parameters were reestimated when appropriate. Growth curve was then developed from selected parameters.

Values of $L_\infty$ and Z/K (ratio of the coefficients of mortality and growth) were also estimated by using the modified Wetherall's method (Wetherall, 1986; Pauly, 1986) through the ELEFAN II program.

Comparison of the growth of scallop *A. pleuronectes* from this study with other studies of same or different species from other areas was also performed, considering the growth performance index, O' values.

2. **Estimation of Mortality**

The data file with suitable class interval, including the values of $L_\infty$ and K obtained from ELEFAN I, were used for further analysis of mortality through ELEFAN II. The total mortality (Z) was estimated from the slope of length-converted catch curve, between the selected points at which scallop were fully recruited and vulnerable to the gear employed.

Natural mortality (M) was estimated by using the mean M/K value of 1.4 (Table 2) and the K value obtained from ELEFAN I.

The fishing mortality (F) was then computed as :

$$F = Z-M \quad \text{.....(11)}$$

3. **Determination of Recruitment**

Based on the $L_\infty$ and K values obtained from ELEFAN I, the recruitment pattern was determined by projecting shell height frequency data onto a time axis using ELEFAN II (Pauly, 1987; Gayanilo, et. al., 1988).

**Laboratory Study**

Scallop samples transferred to the laboratory were randomly sampling for approximately 50-60 individuals per month. Each individual was then carefully numbered, measured, weighted, dissected and examined for the followings :

1. **Size-Weight Relationships**

Measurement of each shell dimensions was carried out by means of a measuring board to the nearest 0.1 cm as shell height (H; dorso-ventral axis), shell length (L; anterio-posterior axis) and shell depth (D; thickness from one valve to another) (Figures 3 and 4).

After total weight (whole body weight with shell; TW) was recorded, each scallop was opened and dissected for weighing of various each body parts, i.e. the shell (shell weight; SW), whole flesh weight (FW), adductor muscle weight (AW) and gonad weight (GW), to the nearest 0.01 g. The diameter of each adductor muscle (ADM) was also measured.

Determination of the relationships between different shell dimensions, i.e. shell height *vs.* shell length, shell height *vs.* shell depth and shell length *vs.* shell depth, was performed by linear regression analysis where the relationships is expressed in the form :

$$Y = a + bX \qquad \text{.....(7)}$$

where Y and X represent shell dimensions pair to be determined, a is the intercept and b is the slope of the regression line.

The relationships of shell height and weight of various parts, such as total weight, shell weight, flesh weight, adductor muscle weight and gonad weight, were determined from the linearized equation:

$$\log W = \log a + b \log H \qquad \text{.....(9)}$$

where W is the weight of any parts, in grams and H is the shell height in centimeters.

To test whether the computed value of *b* is significantly different from 3, the t-test statistic is used (Vakily, et. al., 1988) with the t* value calculated from :

$$t^* = | b-3 | S_b^{-1} \quad \text{.....(12)}$$

where $S_b$ is the standard error of the regression coefficient. The value of *b* is different from 3 if the calculated t* is greater than the tabulated value of t for the degree of freedom of n-2.

## 2. Reproduction

### 2.1 Reproductive cycle

#### 2.1.1 Gonadal development

After measurement and weighing the random sampling specimens, gross observations of the gonads, particularly the color and condition, were made to determine the sexuality and stages of gonadal development based on the explanation by Llana and Aprieto (1980). The stages of development were roughly divided to 5 stages, immature, developing, mature or ripe, spawning, and spent. After a preliminary examination, each gonad was then preserved for histological study to confirm the stages of development.

On preparation of gonads for histological examination, each gonad was fixed with Bouin's fixative for 24-48 hours, then processed through paraffin embedding techniques for serial sactions and stained with Mayer's hematoxylin and Eosin Y (Humason, 1979).

Stages of gonadal development was then examined microscopically to confirm, as well as to compare with, the results obtained from macroscopic determination. Classification of the gonadal stages, in case that gamete development of an individual scallop might be found to vary from follicle to follicle, was based upon the predominant stage of development observed in the serial sections.

### 2.1.2 Gonad Indices

For each specimen, gonad indices was determined quantitatively and qualitatively to explain the condition of gonad.

#### 2.1.2.1 Quantitative gonad index (QT GI)

This index was based on the relative weight of the gonad with respect to the total weight of the soft body, or flesh. Expressed as a percent, the index is

$$QT\ GI = (GW/FW) \times 100 \quad \text{.....(13)}$$

(Mottet, 1979), as GW is gonad weight and FW is flesh or soft body weight.

#### 2.1.2.2 Qualitative Gonad Index (QL GI)

This index was expressed as the numerical weights, i.e. immature = 1; developing = 2; mature or ripe = 3; spawning and redeveloping = 2; and spent = 1 (del Norte, 1986), in order to describe the trend of events in the reproductive cycle. Similar to the QT GI, the QL GI values for the population increased

following the development and reached the maximum just before spawning. With spawning, the gonad index decreased, to remain subsequently at a sustained low level during the resting period, or started increasing again as redeveloping. Thus, the index numbers were corresponding to the level of gonad condition. The number was assigned to developmental stages of the ovary and testis in each individual. Therefore, this index was determined separately in the ovarian and testicular parts of each individual scallops.

Both indices were averaged for each monthly sample as a measure of the state of reproductive development in the population. Comparison of the monthly mean gonad indices was done by the Analysis of Variance.

### 2.2 Size at Sexual Maturity

Size of the smallest individual found with a ripe gonad, or with the stages following mature, i.e. spawning, redeveloping and spent, was considered as the minimum or threshold size at maturity.

## 3. Aspects on Association with Pea Crab

Occurrence of the commensal pea crab *Pinnotheres* sp. infestation in mantle cavity of *A. pleuronectes* samples in this study was checked. Data on size and gonad condition of infested and uninfested scallops were compared by the analysis of variance.