CHAPTER 2

MATERIALS AND METHODS

A. Scope of Research Activities

The scope of this research is outlined into 4 steps. Each step would provide useful information in order to meet the objectives:

1. Landuse Analysis: Changes on land-use particular to shrimp farm area around the Kung Krabaen Bay was investigated. Changes in shrimp farm area was analyzed from the available publications and related reports distributed by the Kung Krabaen Bay Royal Development Study Center (KKBRDSC) and other agency. The change in land-use in particular the area of shrimp farms were focused on the period before the establishment of KKBRDSC project as Phase I. Phase II was the period from 1981 to 1998 before the construction of seawater irrigation system and Phase III was the period after the seawater irrigation system construction and commenced its operation.

2. Development of Budget Models: This step would develop the budget models of water, alt and nutrient budget in the Kung Krabaen Bay system. This analysis would determine the eutrophic condition of the system during the three above phases. The environment factors concerned were the physical, hydrological and chemical characteristics of the bay system, i.e. area of the system, water depth, the existing shrimp farm area, amount of precipitation and evaporation, salinity nitrogen and phosphorus compounds including inorganic and organic forms. Data used in these models based on the secondary data .

3. Analysis of Benthic Community Structure: This step would classify and analyze the structure of benthic community, particular to polychaete assemblage, in order to determine the abundance, species diversity, during the period when the seawater irrigation was in operation. This included the field sampling survey and the laboratory works. The dominant species would be identified in relation to several environmental factors, namely, salinity, dissolved oxygen, pH, hydrogensulfide content, organic matter and grain size. Focus would be placed on the polychaete assemblage and taxonomy.

4. Analysis of Data: This step would conclude and discuss the impacts of organic enriched effluents from shrimp farms on the benthic communities and the bay itself. Changes in the benthic communities would be identified. Indicator species in particular certain polychaetes would also be identified. The outcome of this research would be recommended for the implementations for environmental management in the Kung Krabaen Bay.

B. Landuse Analysis

This part would evaluate the development of land-use particular in the use of land for shrimp farms and analyze their activities in order to determine the degree of effluents released into nearby environment. The analysis showed the correlation between the shrimp farming expansion operated within the three above phases with the nutrient concentration released. The statistic correlation was used to analyze the relationship between nutrients N and P and landuse. The secondary data of landuse for the year before 1981 to 2001 were reported by the Department of Land Development (1981), the Kung Krabaen Bay Royal Development Study Center and personal communication with the KKBRDSC's staffs. Landuse in 1999 was conducted in the present study by classifying the image of LANDSAT5 TM data (path 130 Row 53, January 1999) Bands 4, 3, 2 RGB. The procedures of rectification and classification were proceeded by using the computer program ArcView version 3.1.

C. Development of Budget Models

The method used in calculation of water, salt and nutrient budget models is applied following the LOICZ biogeochemical modeling guidelines (Gordon, et al., 1996). Fluxes of water, salt, nitrogen, phosphosrus, and carbon will be compared for dry (November-April) and wet (May-October) season. The temporal change between the period be (1994-1998) and after (1999-2000) the seawater irrigation installation. Meteorological data on the annual of evaporation, precipitation and number of rain day during 1994-2000 (Climatology Division, Meteorological Department) was used to calculate daily average evaporation and precipitation for dry and wet season. Nutrient data as well as physico-chemical data in the bay and the canals were obtained from the Kung Krabaen Bay Royal Development Study Center.

The physico-chemical parameters: salinity, temperature, dissolved oxygen, total nitrogen, nitrite, nitrate, ammonia, total phosphorus, phosphate in the drainage canals, the bay water body and the open sea (outside the bay) from routine monitorings during 1994 – 2000 were obtained from the Kung Krabaen Bay Royal Development Study Center. The average values of dissolved inorganic and organic nutrients during different water irrigation schemes and seasons were calculated

The water budget of Kung Krabaen Bay was determined mainly by the average precipitation (V_P), the average evaporation (V_E) rather than the average water from drainage canals (V_Q). Calculate and build up the water model using the procedure of LOICZ. Water budgets was calculation from equation.

$$dV_1/dV_t = V_Q + V_P + V_G + V_O + V_{IN} - V_E - V_{OUT}$$

$$V_R = V_{IN} - V_{OUT} = \text{Residual flow}$$

Where water and salt circulation parameters estimated from the water and salt budgets for Kung Krabaen Bay during the period before and after seawater irrigation system. V_Q = water flow from shrimp ponds, V_R = residual flow, V_P and V_E = volume of precipitation and evaporation, V_X = water exchange rate, $V_X(S_{sea} - S_{syst})$ = exchanged salt flux and τ = water exchange time in day.

Salt budget exchange calculated from the following equation

$$V_X (S_2-S_1) = V_1(dS_1/dt) + V_R S_R$$

The exchange time (τ) of water calculated from the equation of system volume divided by the volume of water exchange plus the volume of residual flow.

$$\tau = Vsyst / (V_X + V_R)$$

Nutrients budget of N and P was calculated from the equation

$$\Delta Y = VdY/dt + Y\,dV/dt - \Sigma V_{IN}Y_{IN} + \Sigma V_{OUT}Y_{OUT}$$

ΔY = Net nutrients, sink or source

Net ecosystem metabolism (p–r) and stoichometric analysis (nfix-denit) for Kung Krabaen Bay would be calculated.

D. Analysis of Samples

Sampling Design

Based on literature reviewed, the physical morphology of the Kung Krabaen Bay was semi-circular in shape. Fringes of mangroves and small canals lined on the north, east and south part of

forests. Organic gradients occur that the high concentration recorded in the drainage canals and the shoreline and slightly decreased in area far from the shoreline 500, 1000 and 1500 m respectively (Sangrungruang, 1997). The transect line method was chosen to cover the gradient characteristics of the study site. Holme and McIntyre (1984) suggested that a transect normal to the shoreline running from shallow into deeper water is commonly used for in situ studies. The profile of a transect is usually determined trigonometrically from differential depth and increment transect length between adjacent points.

Drew (1971) has pointed out that most ecological investigations of benthic macrophytes have based on transect and quadrate methods, while similar techniques have been applied to sessile animals. As we knew the point source giving gradient contamination, the transect approach has the advantage that, since it depends on demonstration change along an gradient, seasonal and other such factors are not relevant (Holme and McIntyre, 1984). For this study, the transect approach should serve to observe gradients from the location of the shrimp farms where effluents drained through canals as the point source. It also provided the interval sampling stations across the organic content gradients followed Sangrungruang (1997).

From the pilot field study, I have attempted to use the grab sampler due to the previous benthic studies in the area failed to collect appropriate sample to best represent the area. However it was difficult to use the grab sampler on the hard and packed sandy floor. The core sampler made from PVC with 20 cm in diameter with mesh-sieve cap put on top in order to prevent benthos from escaping were used instead. They were found to be more effective. SCUBA diving were necessary because of the hard and packed sediment. The diver put the core sampler in position and used the hammer to drive the core into sediment at approximate 20 cm deep. Three core samplers were collected at each station. A total of 5 transect lined were conducted as in Figure 2.1. There were 6 sampling stations at intervals along each transect. The stations were designed to represent the point source area of drainage canals, the area inside the bay and the control area on the coastal area inside and outside the bay.

Samplings were conducted at 3 month interval in January, April, August and November 2000 along the stations as shown in Table 2.1

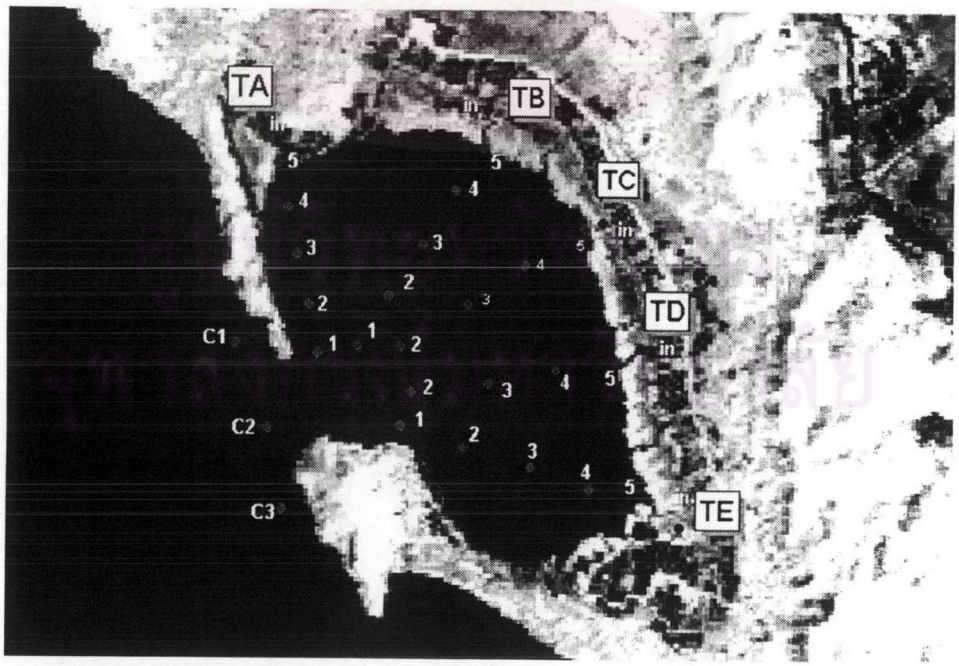

Figure 2.1 Study site the Kung Krabaen Bay with sampling line transects: TA, TB, TC, TD, TE with numbers representing sampling stations along each transect and the coastal stations (C1, C2 and C3).

Field Sampling

Four trips by long-tailed fishing boat were carried out in every 3 months interval, namely, 24-29 January, 23-28 April, 24-29 August and 1-5 November in 2000. Sample of 0.03 m^2 were taken with a core sampler. Thirty four stations were sampled. Three core samples of bottom sediment were collected at each station, resulting in a total of 408 core samples during the present study.

Table 2. Position of sampling stations in Kung Krabaen Bay, Chanthaburi Province by UTM grids during January – November, 2000.

Station	North	East
TAin	813,368.88	1,394,679.13
TA5	813,556.51	1,394,403.71
TA4	813,635.70	1,394,062.88
TA3	813,702.83	1,393,706.11
TA2	813,788.90	1,393,319.24
TA1	813,861.20	1,392,949.14
TBin	815,171.17	1,394,809.96
TB5	815,136.74	1,394,515.60
TB4	814,919.85	1,394,197.15
TB3	814,671.97	1,393,785.74
TB2	814,418.93	1,393,381.21
TB1	814,171.05	1,393,002.51
TCin	816,080.06	1,393,952.71
TC5	815,938.90	1,393,909.68
TC4	815,463.80	1,393,630.81
TC3	815,017.97	1,393,315.80
TC2	814,504.99	1,393,004.23
TDin	816,377.86	1,392,993.18
TD5	816,190.23	1,392,882.01
TD4	815,708.24	1,392,826.93
TD3	815,172.89	1,392,725.37
TD2	814,584.18	1,392,659.95
TEin	816,672.21	1,391,613.63
TE5	816,443.27	1,391,833.69
TE4	815,988.82	1,391,916.32
TE3	815,506.84	1,392,083.29
TE2	814,985.26	1,392,224.44
TE1	814,506.72	1,392,393.14
C1	813,234.62	1,393,026.61
C2	813,479.05	1,392,369.04
C3	813,592.66	1,391,756.23

The measurement of physical and chemical factors, salinity and dissolved oxygen, were conducted *in situ* at bottom water with portable salinity-oxygen meter. Temperature was measured with alcohol thermometer.

Sediment Analysis

Sediment samples for pH, H_2S, grain size and organic content analysis were subdivided from sediment surface layer about 5 cm depth and kept in ice box.

The pH measurement would be immediately conducted after field sampling had been done each day using Yokoyama's (1984) procedure and measured with the INDEX pH-meter.

Measurement of H_2S in term of acid-volatile sulfide (AVS) content (H_2S+ FeS) of the surface sediment was determined by acidifying sediment samples with sulfuric acid and collecting the discharged H_2S in a dosimeter tube with an H_2S-absorbent column (Gastec, Model 201H) (Chareonpanich, et al., 1993; Tsutsumi, 1995). The analysis was carried out immediately after the field sampling had finished.

For the determination of grain size composition, a sample of approximately 50 g sediment collected from the surface of each core sample was dried for 24 hrs at 105°c. Each dried sample was sieved for 10 minutes in an automatic sieving machine provided with sieves of 2.0, 1.0, 0.5, 0.25, 0.125, and 0.063 mm mesh size. Each grain size sample was weighed to the nearest 0.1 g. The mean value of each class of grain size were calculated for each station and expressed as a percentage of the total dried weight. During sieving, small particles often clogged the 0.063 mm mesh and the amount of particles smaller than 0.063 mm therefore represents minimum values.

The median grain size were determined graphically in mm units from the cumulative percentage curve at 50%. These values were recalculated in phi (ϕ) units where 1 mm is defined as zero, and phi calculated as the negative log 2 to the particle diameter in mm. It should be note that a small particle size is a larger positive number on the phi scale. Besides median grain size, the sorting coefficient σ (sigma) was calculated by reading the cumulative weight at 95%, 84%, 16% and 5% and using the formula:

$$\sigma = \frac{\phi 84 - \phi 16}{4} + \frac{\phi 95 - \phi 5}{6.6} \qquad \text{(Gray, 1981)}$$

For determination of organic content, the procedure was followed Walkly and Black (1934).

Benthos Sorting and Identification

The sediment was washed through a set of sieves, the smallest one with a mesh size of 1 mm. Visible animals retained on the sieve were picked out by hand. The sieving residue was then washed three times in sea water and the supernatant of each washing, containing small suspended animals, was poured through 0.3 mm mesh plankton net. Organisms were fixed in 4% formaldehyde. The numbers of individuals of seven taxonomic groups (Polychaete, Oligochaete, Crustacea, Mollusca, Echinodermata, Chordata, and other groups) were recorded. Biomass was determined as wet weight (including hard parts), to the nearest 1 mg. Excess water was blotted off before weighing. The identification of benthic organisms would be focused on polychaetes which were the dominant groups. Polychaete specimens were specific identification to species level by using various available documents, in particular Fauvel (1953), Day (1967), Blake (1975) and Fauchald (1977). Descriptions and drawings were made after the identification under the camera lucida.

E. **Analysis of Data**

Statistical Analysis

The paired t-test was used to test the difference of mean at 0.05 significant for a null hypothesis Ho: $\mu 1 = \mu 2$ at 0.05 significance. This test was applied to determine a change in mean abundance between the difference period of dry and wet season.

Correlation coefficient would be calculated in using to estimate the functional relationship between polychaete species and the environment parameters from the equation

$$r = \frac{\Sigma XY}{\sqrt{\Sigma X^2 . \Sigma Y^2}} \qquad \text{(Zar, 1996)}$$

Three indices, species diversity index, evenness index and richness index, were computed by using the PRIMER package program of the Plymouth Marine Laboratory (Carr, 1997).

The Shannon-Wiener Diversity index was calculated from the equation
$H' = - \Sigma$ pi (log pi), where pi was counting species at i^{th}.

Species richness is computed from equation , d = (s-1)/log N, where s = total number of each species and N = total number of individual.

The equitability or evenness index was computed with Pielou's evenness index equation, J' = H' (observed) / H' (max).

The cluster analysis in multivariate technique was applied for analyzing the polychaete community structure by using the PRIMER computer package program of the Plymouth Marine Laboratory (Carr, 1997).

The evaluation of organic enriched environment was applied from Pearson and Rosenberg (1978) by creating the number of species (S), abundance (ind/core) (A) and biomass (wet weight-g/core) or SAB curves relationship along the organic enrichment in term of distance from the organic material source point. The ecotone point will be the point dividing the polychaete assemblage into two different assemblages.

For the determination of organic induced perturbed levels for the Kung Krabaen Bay will base on the analysis from Warwick (1986). The species abundance (ind/core) and biomass (wet weight-g/core) curves or ABC curves relationship were ranked by log plotted with cumulative dominance in percentage. The calculation was used the package computer program, PRIMER, developed by the Plymouth Marine Laboratory (Carr, 1997).