CHAPTER TWO

MATERIALS AND METHODS

2.1 FIELD SAMPLES

2.1.1 Study area and sampling stations

The study area in the Klang Strait consists of several habitats on the coast of Selangor (Fig.1). Along the eastern part of Klang Strait are mangroves and mudflats, with three major rivers namely, River Selangor, River Klang and River Langat, that are responsible for the dilution of the Strait's waters. The Klang and Langat rivers are also responsible for the downstream transport of large quantities of sediment loads from the Klang hinterland. Angsa Bank, a large tongue-like sandy mud shoal, bears testimony to the significant hydrodynamic and sedimentation processes in the Klang Strait. The shoal extends in the north-west direction from the Klang-Langat delta, reflecting the dominant ebb flow in the same direction (but flood flow in the opposite direction). The shoal with water depths of less than 5 m. is partially exposed during low spring tides. At the lowest tides, the coastal mud flats are uncovered for seaward distances of 1-3 km.

Strong tidal currents scour the main channel of the Klang Strait, keeping it deep although the greater part of the strait does not exceed 10m depth (Fig.1). The coast of Selangor experiences strong semi-diurnal tides, with a maximum range of 5 meters and above during extreme spring tides. The coast may be classified as a high mesotidal environment. The waters are well mixed vertically with negligible stratification of water temperature and salinity (Chong *et al.*, 1996). Tides are semidiurnal with diurnal tides occurring only twice monthly. During the 24-hour cycle, there is one high and one low water succeeded by a second high and low water of lower magnitude at night. During the study period the maximum tidal height was 5.1 meters above Chart Datum and the lowest was -0.8 meters.

The geographical location of the study area was confined within an area defined by Long. 3° 05.29' to Long. 3° 21.30' N and Lat. 101° 08.00 to Lat.101° 20.60'E (Fig.1). The whole sampling area covered approximately 540,000 ha. The study area was divided into a sampling grid comprising fifteen 6 x 6 km squares (except coastal grids). Each square was referenced by its south-west corner and was given an unique grid station number (Fig.1).

2.1.2 Rainfall and hydrology

Land precipitation data were obtained from the Drainage and Irrigation Department (D.I.D.), Kuala Lumpur, which maintains a number of meteorological stations in the state of Selangor. Data from five stations that were distributed near the study area were utilized. These stations are located in Kuala Selangor (Gauge no. 045), Braunston Estate (Gauge no. 057, about 1.6 km. South-east of Sungai Buloh), Athlone Estate (Gauge no. 059, about 3.2 km. North-east of Sungai Janggut), Harpenden Estate (Gauge no. 087, about 3.2 km. north-east of Sungai Sementa Besar) and in the D.I.D. office in Klang (Gauge no. 084, about 8 km. from Sungai Sementa Besar). The rainfall data were collected over a two-year period, from November 1996 to April 1998.

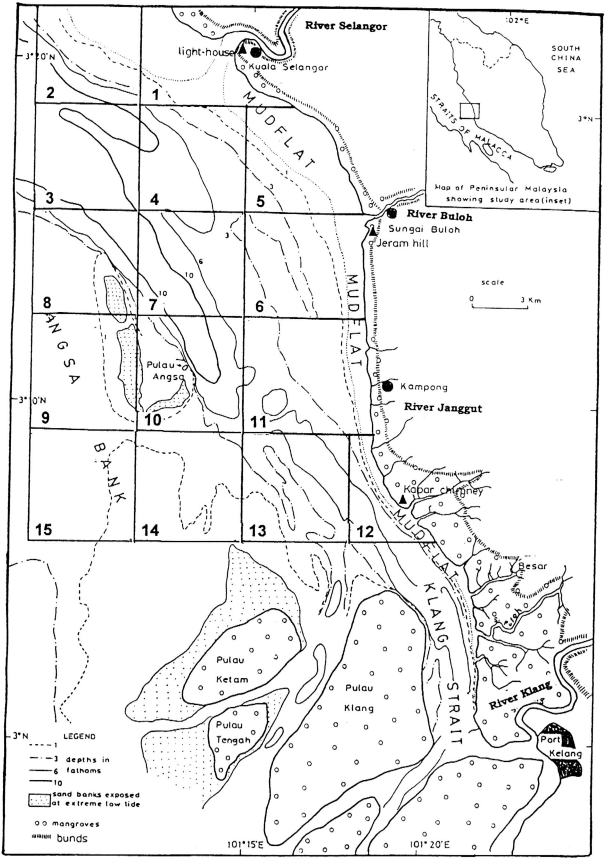

Fig. 1. MAP OF STUDY AREA

DEPTHS ADAPTED FROM MAP OF KLANG STRAIT AND APPROACHES, ADMIRALTY, 1952, LONDON.

Map adapted from (Chong, 1984).

2.1.3 Collection of fish and macroinvertebrate.

Monthly otter trawl samples of fish and macroinvertebrates were taken from 4 or 5 random stations during each cruise. The same square grid was revisited after every two months. During the period of this study most of the grid squares were covered five times each, that is, five samples were collected within each grid square.

Sixty-five samples were collected during a total of fifteen trawl cruises. The details of the sampling stations during the fifteen cruises are given in Appendix 1. Some stations were not trawled over because of unsuitable conditions such as the depth, presence of stationery fishing gears, boat traffic and presence of cockle beds. Only one trawl was taken from station 3, and station 7 was completely left out due to deep water and unsuitable bottom conditions (Appendix 2).

The start of the trawl haul corresponded to the moment when the winch brakes were put on after the two ropes were let out, and the end to the moment when the winch was clutched in and heaving began. The duration of the tow varied from 15 to 20 minutes each. The trawl distance was computed by calculating the ground speed of the towed net which was estimated using the chipboard method. A chipboard was thrown at the bow and the time taken for the stern to reach the chipboard (or for a known distance) was taken using a stop-watch. This was repeated three times and the average ground speed was estimated. Trawl information were recorded on field data forms as shown in Appendix 3.

After the catch was hauled on deck the fish and macroinvertebrates were sampled according to standard procedures (Per Sparre and Venama, 1989) with sub-samples taken when large quantities were encountered. Samples were immediately kept on ice in an icebox, but were kept in a freezer in the laboratory until examination.

During the study period the otter trawl was used as a sampling gear. However, two nets of different dimensions had to be used during the surveys due to unavoidable circumstances. The first net used during the first three months had a head rope length of 30 m and cod-end mesh size of 1 cm., whereas, the second trawl net used in subsequent trawls had a head rope length of 48.87 m. and a cod-end mesh size of 1.91 cm.

2.1.4 Water parameters

All readings of the water parameters were taken at the water surface and just above the sea bottom before the start of each trawl tow. The depth (m), turbidity (Ntu), pH, O_2 (mg/l), salinity (ppt) and temperature (°C) were measured using a YSI 3800 water quality logger. The water parameters were monitored five times at each station during the study period, from November 1996 to April 1998.

2.1.5 Sediment Collection

Sediment samples were collected before the start of trawling and after hauling in the trawl net. An Ekman box grab of dimensions 20 cm x 20 cm., was used for sampling. The grab jaws closed on impact by a messenger sent down from the boat deck. The sample was hauled on deck and placed on a plastic tray before temperature, pH and redox potential of the sediment were immediately measured by a field pH meter (Hanna, HI 8314 membrane pH meter). Sediment information at each station was recorded on a field data form as shown in Appendix 4. A portion of the collected sediment was then taken to assess the organic matter content and particle size of the sediment. All samples collected were kept on ice in an icebox and taken back to the laboratory where they were kept frozen in a freezer until analysis.

2.2 LABORATORY ANALYSIS

2.2.1.Sediment analysis

All sediment samples were kept in a deep freezer in the laboratory until further analysis. A portion of the sample was air dried for particle size analysis (PSA). Pretreatment of samples to enhance separation of aggregates is a key step in PSA and is generally recommended, since sediment contains aggregates that are not readily dispersed. Sediment generally contains organic matter and other chemicals. The air dried sediment was then treated with hydrogen peroxide to remove organic matter; this method has been recommended as the standard oxidising method for most soils (Day, 1965). After all the organic matter had been digested the treated samples were washed with distilled water to remove soluble salts before being analysed using a Coulter counter L230 Particle Size Analyzer. The results obtained by the Particle Size Analyser method were verified by the hydrometer method (Bouyoucos, 1951), which showed almost similar results (see next chapter, Table 2). Particle grain sizes were categorised according to the Wenworth grade scale.

Organic content was determined by loss in weight of a known weight of air-dried soil, ashed for five hours at 550 °C (Buchanan, 1984). Mean values were calculated for each station.

Sediment contour maps for Klang Strait were drawn based on the sediment type and percentage composition. These contours were drawn separately for each type of sediment (clay, silt or sand), by first plotting the percentage composition of the sediment type on the map based on the samples' GPS readings (positions). The desired contour lines, based on the observed range in composition of the sediment type, were then drawn by interpolation. Based on their observed ranges, the selected contour lines for clay were 3%, 5%, 7% and 10%; for silt 25% and 50%; and for sand 25%, 50% and 75%. Similar contour lines for sediment organic matter were also drawn based on 3%, 5%, 7% and 9% organic matter content.

2.2.2 Sorting and Identification of Fish and Macroinvertebrates

Sorting of the trawl samples were carried out in the laboratory. By using published taxonomic keys most of the catch were identified up to the species level while unidentified specimens specially crabs, gobiid fish and echinoderms were sent overseas for expert identification. The fish, prawn and other macroinvertebrates were identified using the following references: Smith (1950), Tan (1955), Scott (1959), Mohsin, A., and Ambak, M. A. L. (1996), Fishscher and Whitehead (1974), Trewavas (1977), Rau and Rau (1980), Donald (1981), Tan and Ng (1988), Arnold and Birtles (1989), Miyake (1991), AIMS (1992), George et al.(1994), Addyhanis (1995), Gerry (1997), and Lim and Low (1998). The species names used and accepted by FAO for used and accepted by FAO for fish (Fishscher and Whitehead, 1974) and prawn (Holthuis, 1980-1993) were adopted. If a species name was not available, the name as given by any of the above authors was adopted consistently.

Contour maps showing the density and biomass of species of fish and macroinvertebrates were drawn base on their average values for the whole study period. These density or biomass contours were estimated and drawn using the "Least Square Fit 3 –D Contour Plot" in the computer software package, Statistica Version 5.

2.3 DATA AND STATISTICAL ANALYSIS

2.3.1 Multivariate analysis

Statistical techniques based on simple distribution as the unidimensional normal distribution are not really appropriate for analysing complex ecological data sets. Multivariate or multidimensional statistics consist of methods that are able to analyse complex ecological data sets comprising many variables which, may and often do co-vary (Legendre and Legendre, 1998).

These techniques also permit the description of the variability of species composition data as a whole, rather than the analysis of each species independently (Legendre and Legendre, 1998). In contrast, conventional univariate statistical analysis like correlation and regression only enable the investigation of how pairs of variables are related.

The approach taken in multivariate techniques is to compare sites or groups of sites to find out how similar they are based on their species composition or environmental conditions. Similarity between pairs of sites are most often measured using association coefficients (Legendre and Legendre, 1998). These coefficients may be based on either quantitative (species abundance or measured environmental variables) data or binary (species presence-absence) data.

Cluster analysis is used to classify various sites based on their similarity, and typically results in a dendrogram (or tree-diagram) which provides a visual display of the hierachical structure of similarities between sites. Clustering can also be used to detect associations of species based on their occurrence together (similarities). There exists a variety of clustering methods (Legendre and Legendre, 1998).

Ordination enables the representation of the multidimensional aggregated data in two or three dimensions. By isolating the environmental variables that contribute to the greatest variation in the aggregated data, ordination techniques are able to identify possible causes for observed associations between sites. Among the commonly used ordination techniques are principal component analysis (PCA), and canonical analysis, which includes canonical correspondence analysis (CCA). The later is a class of ordination methods that permits the simultaneous analysis and comparison of two data matrices. In ecology, these are most often the one containing the species abundance data and the one containing the environmental variables (Legendre and Legendre, 1998).

2.3.1.1 Sediments and Water parameters

Principal component analysis (PCA) of various water and sediment characteristics, namely, depth, turbidity, salinity, dissolved oxygen, pH, temperature, composition of clay, silt, very fine sand, fine sand, medium sand, and coarse sand, sediment pH and organic matter, were carried out. Basically the technique reduces the number of variables in the data set by finding linear combinations of those variables that best explain most of the observed variability. Prior to analysis by using the computer software Statistica 5, the data sets of percentages were arcsine-transformed to approximate multivariate normality and homoscedascity (Zar, 1984, Sokal and Rohlf, 1974). The PCA was used to group the sampling station according to their water and sediment characteristic similarities.

2.3.1.2. Fish and macroinvertebrate distribution and abundance

Standard procedure for estimating demersal fish density and biomass according to the "swept area method" (Per Sparre and Venema, 1989) was adopted. Species abundance and biomass were measured in term of number of individuals ha^{-1} and kg wet weight ha^{-1}, respectively. The mean catch per unit of area is an index of the stock abundance. This index may be converted into an absolute measure of biomass. An escapement factor of 0.5 was used (Pauly, 1979; Per Sparre and Venema, 1989). [See below].

The trawl sweeps a well defined path, the area of which is the length of the path times the width of the trawl. Called the swept area or the "effective path swept", the swept area (a) can be estimated from:-

$$a = D*h*x_2$$

Where

D = Distance of tow path (= $v * t$)

v = The velocity of the trawl over the ground when trawling.

h = The length of the head-rope.

t = The time spent trawling.

x_2= The fraction of the head-rope which is equal to the width of the path swept by the trawl

Pauly and Navaluna, (1983) suggested a value of x_2 equals to 0.5 as the best estimate (see also Per Sparre *et.al*, 1989).

Density is the total number of fish or macroinvertebrate stock, or of some defined portion of it in a given area. The density (Di) of each species (i^{th} species) was estimated by the swept area method as

$$Di = \frac{\text{Total number of individuals of } i^{th} \text{ species}}{\text{Area swept (a)} * x_1}$$

Where x_1 = Portion of individuals or fish actually retained by net (assumed to be 0.5, see Per Sparre *et al.*, 1989).

Biomass is the weight of fish or macroinvertebrate stock, or of some defined portion of it in a given area. The biomass of the i^{th} species (Bi) was estimated by the swept area as follows:

$$Bi = \frac{\text{Total wet weight of } i^{th} \text{ species}}{\text{Area swept (a)} * x_1}$$

2.3.1.3 Cluster Analysis

Cluster analyses of stations (cases) by abundance (dissimilarity matrix) of fish and macroinvertebrates (variable) were performed to see whether there were any relationships between station clusters based on species similarity/dissimilarity and station clusters based on similarity/dissimilarity of sediment and water characteristics. The unweighted pair-group average clustering procedure was used. The cluster analysis was done using the computer software Statistica version 5.

The Bray-Curtis index (Bray and Curtis, 1957) quantifies the community relationships between pairs of samples, and has been used widely in benthic ecology (Boesch, 1974). This index was computed to form a dissimilarity matrix of species-standardised abundance, after $\log_{10}(x + 1)$ transformation, for the cluster analysis. The Bray-Curtis index D (X1, X2) has the following relationship:

$$D(X_1, X_2) = 1 - \frac{2W}{A + B}$$

Where D = Percentage difference between Site or Sample X_1 and X_2,

W = Is the sum of the minimum abundance of the various species, this minimum being defined as the abundance at the site where the species is the rarest.

A and B are the sums of the abundances of all species at each of the two sites (X_1 and X_2) or, in other words, the total number of specimens observed or captured at each site, respectively.

2.3.1.4 Community structure

The following indices were used to describe the community structure of fish and macroinvertebrates in each specific station in the Klang Strait:

1. Shannon-Wiener's index (H`) for heterogeneity or diversity (Shannon and Weaver, 1963):

$$H` = \ln (\Sigma n_i) - [(\Sigma n_i \ln n_i)/ \Sigma n_i]$$

Where n_i = number of individuals of each i^{th} species in specific station.

Σn_i = total number of individuals of all species in specific station.

2. Maximum species diversity (H`max), i.e species diversity under condition of maximum equitability:

$$H`max = \ln (\text{total number of species in the station}).$$

3. Pielou's index (J) for evenness (Pielou 1969):

$$J = H` / H`max$$

4. Margalef's index (D) for species richness (Margalef 1968):

$$D = [(\text{total number of species in specific station} - 1)/ \ln (\Sigma n_i)$$

2.3.1.5 Fish and macroinvertebrate species abundance in relation to abiotic variables

Canonical correspondence analysis (CCA) was performed for each common species or taxon using the abiotic factors (water and sediment parameters) and abundance matrices of the fish and macroinvertebrate species simultaneously. This procedure allowed for the ordination of both sampling stations and species along the same axes which were derived from the abiotic variables (water and sediment variables) (Legendre and Legendre, 1998).

The procedure for CCA was obtained from the computer software package CANOCO version 2.2 (Ter Braak, 1988).