CHAPTER TWO

MATERIALS AND METHODS

2.1 FIELD SAMPLES

2.1.1 Study Area and Sampling Stations

2.1.1.1 Study Area [Matang Mangroves Forest Reserve (MMFR)]

The study was carried out in the Matang Mangrove Forest Reserve (MMFR) which is reknown as the best managed in the world. MMFR is the largest of the mangrove forests in Peninsular Malaysia which occur mainly along its western coast in the state of Kedah, Perak, Selangor and Johor. The mangroves of MMFR, which is in Perak (Figure 1), form a continuous belt facing the Straits of Malacca, from 4^0 15'N 100^0 2'E to 5^0 1'N 100^0 45'.

The MMFR comprises of 19 independent gazetted forest reserves, collectively known as the Matang mangroves, which are located on seven deltaic islands of Pulau Gula, Pulau Kelumpang, Pulau Selinsing, Pulau Sangga Kecil, Pulau Sangga Besar, Pulau Terong and Pulau Pasir Hitam. These islands are separated by numerous waterways (estuaries, channels and inlets). The Matang mangroves are located in a large crescent-shaped bay which is 52 km long and 13 km wide at the middle.

The mangrove area encompasses a total of 40, 711 ha (Gan, 1995) of mainly silvicultured *Rhizophora apiculata* mangroves. MMFR has been under sustainable yield

management by the State Forestry Department of Perak since its reservation in 1902. The District Forest Office of Larut / Matang is responsible for the implementation and monitoring of the Working Plan which provides the policy guidelines in the management, conservation and preservation of the reserve (Gan, 2000).

Although several changes were made to the silvicultural system, it was Noakes (1952) who devised the first comprehensive ten-year working plan for the period 1950-59. Since then the management regime has been modified only slightly and currently runs on a 30-year rotation basis.

Apart from the forest being cropped on a rotational basis for producing charcoal, fuelwood and polewood, the waterways are also nursery and feeding grounds for fisheries resources and support thriving cockle and cage aquaculture industry. Among the waterways, the Sungai (River) Sangga Besar (SSB) and Sungai Sangga Kecil (SSK) serve as the main access routes for fishers, while the former is also utilized for cockle and finfish cage cultures. Thus, the Matang mangroves support a forestry as well as a very important fishing industry.

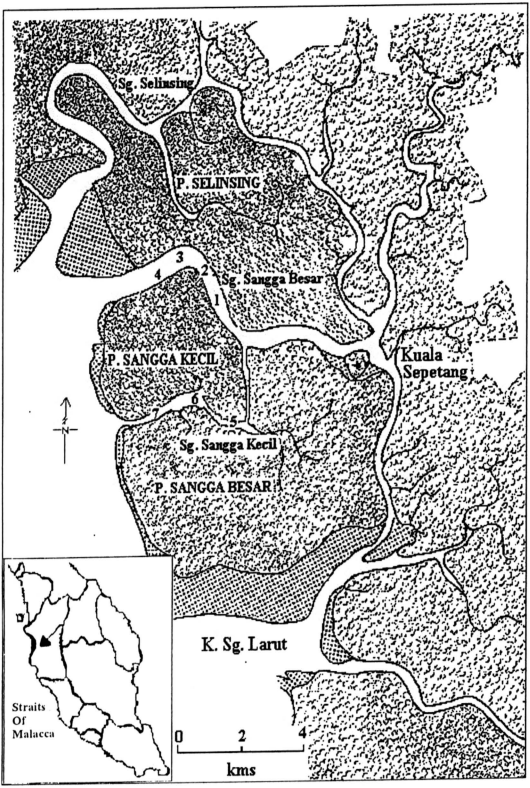

Figure 1: Map of Study Area in MMFR, Perak, Peninsular Malaysia, showing sampling transects in SSB (Transects 1, 2, 3, 4) and SSK (Transects 5, 6, 7). Inset shows location of MMFR. (Map adapted from Ahmad Husin, A. 1997).

The mean monthly temperature for 1997 at Lubok Merbau (closest principal meteorological station at, 4^0 48'N 100^0 54'E) was 26.6 ^{0}C and the mean annual humidity was 83%. The mean number of sunshine hours was 6.1 per day with a minimum of 3.1 for September. Annual rainfall in the MMFR ranges from 2000-3000 mm (Gan, 1995). Taiping, the nearest town to the MMFR (located 10 km east inland of Kuala Sepetang) is reknown for receiving the highest rainfall in Peninsular Malaysia. Freshwater flow into Matang is through numerous streams and ground run-off. The tides at Kuala Sepetang are semi-diurnal with a Mean High Water Spring of 2.65 m (Sasekumar *et al.*, 1994). The mean tidal range is 3.3 m.

There are two main industrial parks in the Sepetang River Basin, the Kamunting Industrial Park and the Tupai Industrial Park. The major sources of pollution of the rivers are effluent discharges from the factories in the Kamunting Industrial Park and silt from soil erosion from development and mining. Polluting sources are from mainly rubber textile, leather tanning and alcohol manufacturing industries. Chong *et al.* (1999) indicated that Sungai Sangga Besar is slightly polluted, with other sources of organic pollution, from the food industry, edible oil industry, rubber mills, palm oil plantations and pig farms.

Fish Cages in Sungai Sangga Besar (SSB)

Based on Fisheries Statistics from the Perak (state) Fisheries Department for the Larut-Matang district (year 2000), the number of fish farmers in the whole Matang swamp was estimated at 61, operating 3,596 cage units with a total surface culture area of approximately 27,207.2 m^2. In 1999, the total farm size of 4.2 ha in Larut-Matang had an annual production of 287 tonnes of cultured fish.

Each fish farm consists of a series of interconnected floating net cages. Each cage unit is approximately 2.5 m x 2.5 m in area and 2.5 m in depth. The three main species of fish cultured in the order of importance are the giant sea perch (*Lates calcarifer*), golden snapper (*Lutjanus johnii*) and red snapper (*Lutjanus argentimaculatus*).

The main feed given to the cage fish in SSB are trashfish, comprising mainly young slender shad (*Ilisha elongata*), gizzard shad (*Anadontostoma chacunda*), thyrssa anchovy (*Thyrssa kammalensis*), spined anchovy (*Stolephorus baganensis*), scaly hairfin anchovy (*Setipinna tata*), squid (*Loligo edulis*), djeddaba crevalle (*Alepes djedaba*) and white herring (*Escualosa thoracata*). The amount of feeding with trash fish is dependent on the tide. During spring tide, feeding is normally once a day, whereas at neap tide, it is twice a day. But feeding depends on trash fish availability. Shortage of supply could limit feeding to three times a week only. The weight of trash fish given per cage unit varies between 8-15 kg/day for adult fish and 2-4 kg/day for juvenile fish. The weight of trash fish feed given per day varies between 300 – 800 kg per farm. Cage culture fish are harvested after 7-8 months of culture when the average harvest size is at 600g, or after 10-11 months of culture (at 800g) depending on market demand.

2.1.1.2 Sampling Stations

The study areas covered two estuaries in the MMFR, namely SSB (cage culture area) and SSK (non-aquaculture area) (Figure 1). In SSB, four sampling transects (Transects 1 – 4)) were set across the river, cutting through four cage farms (Figure 1). The size of each farm varied between 100-150 cage units.

In SSK, three cross-transects were set along the river as follows: upstream (Transect 5), mid-river (Transect 6) and downstream (Transect 7) (Figure 1). In SSB, away stations (A) (see below) were without fish cages and serve as controls in the river, while SSK was chosen to serve as a control river, i.e. a river entirely without aquaculture.

The investigation consists of three parts: (1) 4-month study, (2) 12-hour study and (3) 1-day "grid" sampling study. The field sampling timetable for these studies is summarized in Table 1 below. During the 4-month study period, a total of 90 and 18 samples were taken from SSB and SSK, respectively, for four successive samplings in December 1999, January 2000, early March 2000 and late March 2000. In the 12-hour study, 45 samples were taken from SSB, while 20 samples were taken from SSK. In the "grid" sampling, a total of 40 samples were taken from SSB only.

Table 1: Field Sampling Timetable for Overall Studies

Study	Month	Date	River	Transect
4-Month Study	December	20/12/99	SSB	1, 2
	January	23/01/00	SSB	1, 2
	Early March	07/03/00	SSB	1, 2
		08/03/00	SSB	3, 4
	Late March	28/03/00	SSB	3, 4
			SSK	5, 6, 7
12-Hour Study	April	20/04/00	SSB	1
			SSK	6
1-Day "Grid" Sampling Study	May	14/05/00	SSB	2

2.1.2 Sampling Design

4-Month Study

The purpose of this study was to compare macrobenthos diversity and abundance in cage and non-cage areas, as well as to see whether there were any temporal differences in animal abundance.

In SSB, along each transect three replicate samples were routinely taken from three sampling stations. The first station was located directly under the cage, which was indicated as inside station (IN). The second station, referred to as the middle station (MID), was located about 100 – 150 meters away from the cages. The third station, referred to as the away station (AW), was located about 180 – 210 meters away from cages. The second and third stations serve as control stations (Figure 2).

In SSK, two replicate samples were taken from each of three sampling stations established along the transect. The first station was located at the left side of the river bank (L). The second station was located in the middle of the river (M), while the third station was located at the right side of the river bank (R) (Figure 3). The locations of these three stations (per transect) in SSK thus correspond to equivalent positions of the stations established in SSB, which will facilitate comparative analysis.

12-Hour Study

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during spring tide on two consecutive days (20th –21st April, 2000), on transect 1 (SSB) and transect 6 (SSK), respectively. Night set in at 1930 hr. For transect 1 in SSB, three replicate samples were taken from three sampling stations; inside station (IN), middle station (MID) and away station (AW). For transect 6 in SSK, two replicate samples were also taken from three sampling stations; left side of the river bank (L), middle (M) and right side of the river bank (R). The sampling regime with regards to time of sampling, tidal phase and light condition (diel) are shown in Table 2.

Table 2: Sampling Information for 12-Hour Study in SSB and SSK (20th – 21st April, 2000).

River	Transect	Stations	Sample	Time	Phase	Diel
SSB	1	IN	1a-I	1200	Ebb	Day
		MID	1a-M	0940	Ebb	Day
		AW	1a-A	0855	Ebb	Day
		IN	1b-I	1330	Flood	Day
		MID	1b-M	1300	Flood	Day
		AW	1b-A	1355	Flood	Day
		IN	1c-I	1620	Flood	Day
		MID	1c-M	1550	Flood	Day
		AW	1c-A	1530	Flood	Day
		IN	1d-I	1920	Flood	Day
		MID	1d-M	1845	Flood	Day
		AW	1d-A	1800	Flood	Day
		IN	1e-I	2100	Ebb	Night
		MID	1e-M	2130	Ebb	Night
		AW	1e-A	2145	Ebb	Night
SSK	6	L	6a-L	1045	Ebb	Day
		R	6a-R	1020	Ebb	Day
		L	6b-L	1410	Flood	Day
		R	6b-R	1440	Flood	Day
		L	6c-L	1705	Flood	Day
		R	6c-R	1630	Flood	Day
		L	6d-L	1845	Flood	Day
		R	6d-R	1940	Flood	Night
		L	6e-L	2250	Ebb	Night
		R	6e-R	2225	Ebb	Night

1-Day "Grid" Sampling Study

The aims of grid sampling over the cage culture area were to map the sediment texture and to examine the spatial distribution and abundance of the macrobenthos, over shorter distances. Faunal and sediment samplings covered areas directly under the fish cages as well as distances of 5 metres away from the perimeters of the farm which was located on the right bank of SSB at transect 2. This particular farm has approximately 150 net cages with an area cover of approximately 3,750 m^2.

Figure 5 gives the ground plan of the 'grid' stations that were sampled. Although a sampling grid of equidistant stations was originally planned, this was difficult to achieved due to boat drift and cage obstruction. Locations of the sampled stations were determined by a hand-held GPS (Garmin, GPS 75 or Ensign, GPS). Two replicate samples were taken from each station.

2.1.3 Macrobenthos and Sediment Collection

Sediment and macrobenthos were sampled by using a Petersen grab, which sampled an area of 0.1 m^2, and to a depth of approximately 20 cm. The grab was dropped from the boat deck and closed on impact. The grab was hauled onto the boat and the sample was placed inside a hopper. *In-situ* parameters, such as sediment pH, temperature and redox potential were measured using a field pH meter (Hanna Instruments, HI 8314 membrane pH meter and later Eutech, Ecoscan pH 6 / 01 portable pH meter). A portion of the collected sediment was scooped up and kept inside a labeled plastic bag for subsequent assessment of the organic matter content and particle size of the sediment.

Sediment samples collected were kept in a container and taken back to the laboratory where they were kept frozen in a freezer until analysis.

The rest of the samples were then washed through two sieves with mesh sizes of 2 mm and 0.5 mm. Fauna were then removed during the washing process and placed directly into labeled plastic bags and preserved in 10 % formalin for sorting in the laboratory.

2.1.4 Water Parameters

Water parameters such as pH, temperature (^{0}C), dissolved oxygen (mg/l), conductivity (mS/cm^2), salinity (ppt) and depth (m) were measured *in*-situ by a Grant, YSI 3800 Water Quality Logging System and also a SCT Meter (YSI). The data logger was calibrated as per factory instructions before each sampling trip. All readings of the water parameters were taken at the water surface and bottom but for the purpose of this study, only the bottom reading was used for analysis.

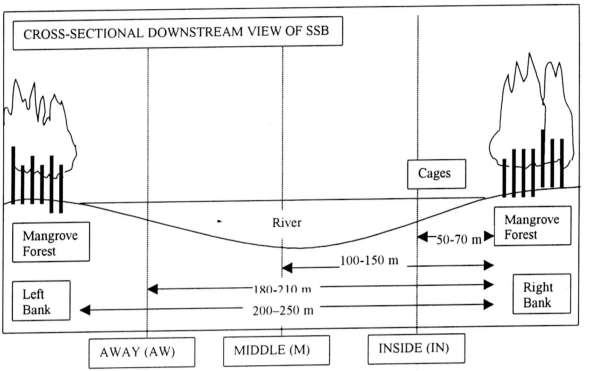

Figure 2: Designated Location of Sampling Stations in SSB

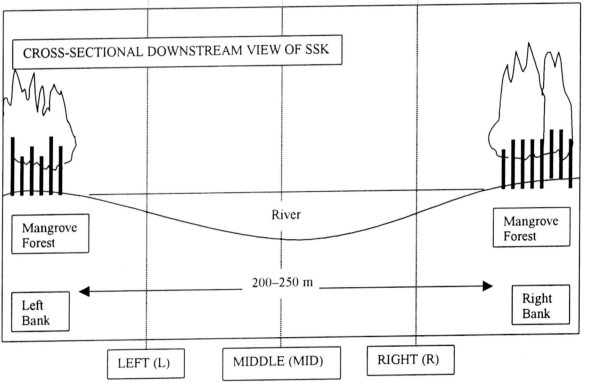

Figure 3: Designated Location of Sampling Stations in SSK

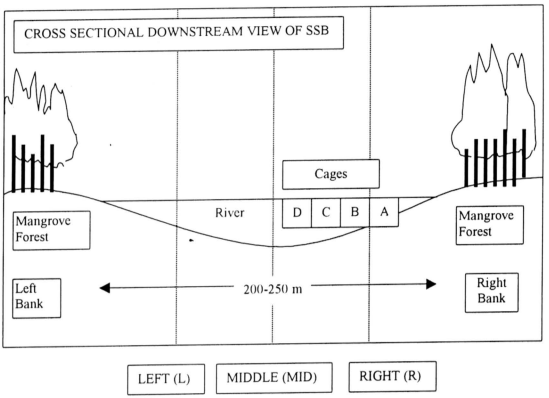

Figure 4: Location of Sampling Stations for 1-day Grid Sampling in SSB (in Transect 2). Alphabets A-D indicate Longitudinal Transect of Increasing Distance from the Right River Bank (Actual Sampling)

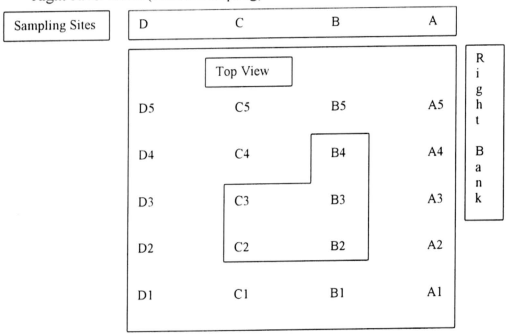

Figure 5: Ground Plan of Approximate Positions of Stations for Grid Sampling in SSB (in Transect 1). B2, B3, B4, C2 and C3 are Located Directly under Fish Cages.

2.2 LABORATORY ANALYSIS

2.2.1 Sorting and Identification of Macrobenthos

Sorting of samples were carried out in the laboratory. Samples were rinsed with freshwater and sorted for animals by using sieves with mesh sizes of 2.0 mm and 0.5 mm. By using published taxanomic keys, most of the samples were identified up to the species level whereas unidentified specimens especially gastropods, crabs and polychaetes were brought to The Raffles Museum of Biodiversity Research, Department of Biological Sciences, National University of Singapore (NUS) for identification. The macrobenthos were identified by using the following references: Day (1967a; 1967b), Barnes (1968), Fauchald (1977), Lovett (1981), Tan and Ng (1988), Arnold and Birtles (1989), Tan and Ng (1994), Todd *et al.* (1996), Carpenter and Niem (1998a; 1998b), Lim and Low (1998) and Ng and Sivasothi (1999). Dr. Peter K.L. Ng of NUS assisted in identifying the crabs.

2.2.2 Sediment Analysis

2.2.2.1 Particle Size Analysis

Sediment samples were first thawed, dried in an oven at 80^0C for 7 days. Aggregates were broken up in a mortar with a pestle to obtain the fine sediments. Pretreatment of sediment samples to enhance separation of aggregates is a key step in the analysis of particle size and is generally recommended, because sediment contains aggregates that are not readily dispersed.

The oven-dried sediment was treated with 10 % hydrogen peroxide to digest the organic matter and then left overnight. 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 (Fraunhofer Optical Model). The soil particle groups were then categorised according to the Wentworth grade scale.

Sediment contour maps for 1-day "grid" sampling were drawn based on the sediment type and percentage composition. These contours were drawn separately for each category of the sediment, namely silt, clay and sand, by first plotting the percentage component of the sediment type on the map based on the samples' GPS (Global Positioning System) readings. The sediment contour were estimated and drawn using the "Least Square Fit 3-D Contour Plot" in the computer software package, Statistica Version 5.

2.2.2.2 Organic Matter Content

A portion of air-dried sediment was weighed before being combusted in a furnace at 550^0C for five hours, after which it was then weighed again (Buchanan, 1984). The percentage of organic matter was calculated based on the weight loss during combustion. Mean values based on three replicate samples were calculated for each station.

2.3 STATISTICAL ANALYSIS

2.3.1 Univariate Analysis

Total macrobenthos abundance was logarithmically transformed [log (x+1)] to homogenise the variance and normalise the distribution, as required for parametric analysis (Sokal and Rohlf, 1998). Analysis of variance (ANOVA) followed by a multiple range test (Newman-Keuls test) were conducted to compare the differences in total macrobenthos abundance. All statistical analyses were performed using the Statistica Version 5.0 Software Package. Levels of significance were accepted at $p < 0.05$. Data presented in the text and figures are means $\pm$ 1 s.e.

ANOVA were carried out for the following:

a) A three-factor ANOVA applied to logarithmically-transformed abundance data, with months (December*January*March1*March2), transects (1*2) and stations (IN*MID*AW) as the possible influencing factors; for SSB only.

b) A two-factor ANOVA applied to logarithmically-transformed abundance data with transects (1*2*3*4) and stations (IN*MID*AW) as influencing factors; for early March data and SSB only.

c) A two-factor ANOVA applied to logarithmically-transformed abundance data with transects (3*4*5*6*7) and stations (IN*MID*AW) as influencing factors; transects 3,4,5 in SSB and transects 6,7 in SSK; for late March data.

d) A four-factor ANOVA applied to logarithmically-transformed abundance data with river (SSB*SSK), station [1 (IN)*3(AW)], tidal phase [1 (ebb)*2(flood)] and diel

[(1 (day) * 2(night)] as the influencing factors; for April data, mainly for Transects 1 and 6.

2.3.2 Multivariate Analysis

Multivariate or multidimensional statistics consist of methods that are able to analyse complex ecological data sets comprising many variables which, may and often do covary. 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).

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 which may be based on either quantitative (species abundance or measured environmental variables) data or binary (species presence-absence) data.

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. Ordination is the collective term for multivariate techniques that arrange sites along axes on the basis of species composition data.

Canonical ordination techniques are designed to detect the patterns of variation in the species data that can be most parsimoniously explained by the observed environmental

variables. The resulting ordination diagram expresses not only a pattern of variation in species composition but also the main relations between the species and each of the environmental variables. Canonical ordination thus combines aspects of regular ordination with aspects of regression (Jongman *et al.*, 1995).

Among the commonly used ordination techniques are Principal Component Analysis (PCA), Nonmetric Multidimensional Scaling (MDS), Principle Coordinate Analysis (PCoA) and Correspondence Analysis (CA), whereas canonical analysis includes Canonical Correspondence Analysis (CCA), Redundancy Analysis (RDA) and Canonical Correlation Analysis (CcorA). CCA and RDA is a class of ordination methods that permits the simultaneous analysis and comparison of two data matrices – very often a species-abundance matrix and an environment matrix.

Redundancy Analysis (RDA) was performed for common species using the abiotic factors (water and sediment parameters) and species abundance data. 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). RDA was chosen over CCA because a linear response model was assumed for the cage culture effect, and there is no good reason to assume an unimodal response model given the short environmental gradient in the study site (see Ter Braak and Smilauer, 1998).

RDA was performed on the species abundance and environmental data collected from the 4-month study. Only 22 species (i.e. only those with at least two occurrences) and 39 sites (i.e. stations sampled over the 4-month period) were used in the analysis. All environmental data (altogether 16 variables) in terms of percentages were arcsine-

transformed to approximate multivariate normality and homoscedascity (Zar, 1984). The procedure for RDA was carried out using the computer software package CANOCO for Windows Version 4.02 (Ter Braak and Smilauer, 1998).

A distance matrix was derived from species abundance data in the Cluster procedure from Statistica 5.0 software. To obtain a distance matrix based on Orloci's chord distance (see Legendre and Legendre, 1998), the species abundance data were first log-transformed before they were subject to a transformation program (Legendre and Gallagher, in press) downloaded from http://www.tas.umontreal.ca/biol/casgrain/en/labo/transformations.html. The program converts a matrix of species abundance in such a way that the Euclidean distance among rows of the transformed matrix is equal to the "chord distance" among rows of the original data matrix.

The transformed data were then submitted to the Cluster procedure of Statistica using the "Euclidean distance" option, and then computed (now chord) distances among sites were then used to construct tree diagrams (dendograms).