CHAPTER 2

MATERIALS AND METHODS

2.1 Study area

Matang Mangrove Forest Reserve (MMFR) in Perak is located on the west coast of Peninsular Malaysia (4° 50'N, 100 °35'E). This forest reserve is about 40,711 ha of mainly *Rhizophora apiculata* and is considered to be the best managed mangrove forest in the world (Gan, 1995). It is the largest single tract of mangrove forest in Malaysia and has been under sustainable yield management since the early part of the 20th century.

Matang Mangrove Forest Reserve thrives and is built on deltaic sediments brought down from three river basins, that of the Sungai Sepetang, the Sg. Larut and Sg. Terong from north to south (Fig. 2.1). Major portions of the reserve essentially sit on seven deltaic islands. While about 85% of the forests are productive forests, the waterways amongst and separating the islands from the mainland have previously been shown to be important nursery areas for fish and prawns (Sasekumar et al., 1994a; Chong et al., 1994). The deltaic estuary of Matang is shallow, with well-mixed waters (Chong et. al., 1998). Climate is monsoonal with an average annual rainfall of 3500-4800 mm; rainfall peaks normally in May and November, coinciding with the start of the southwest and northeast monsoons, respectively. However, in 1999-2000, rainfall was sporadic with no clear seasonality. Tides are typically semidiurnal with Mean High Water Springs of 2.65 m (Sasekumar et al., 1994).

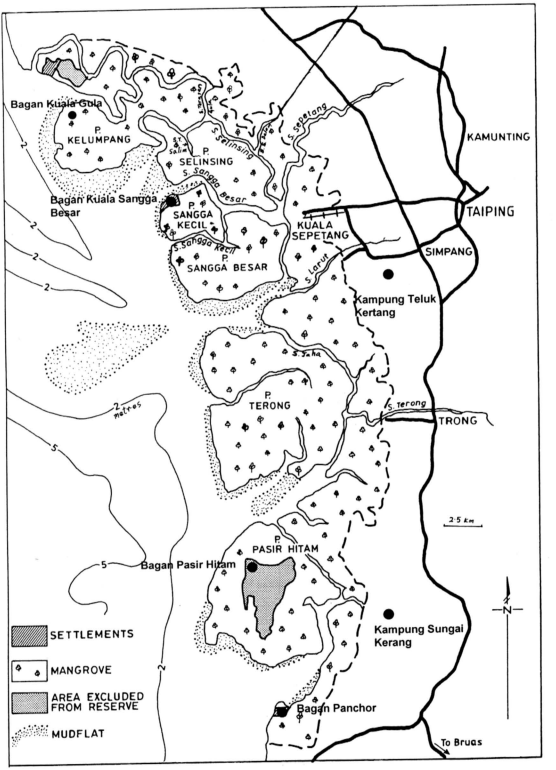

Figure 2.1 The Matang Mangrove Forest Reserve in the state of Perak, Peninsular Malaysia (adapted from Sasekumar et al. 1994). Dark filled circles indicate the main fishing villages.

There are a total of 28 fishing villages scattered within but mainly on the inner fringe of forest reserve. The major mainland fishing villages are Kuala Sepetang, Kampung Telok Kertang, Kampung Sungai Kerang and Bagan Panchor, while the island fishing villages are in Bagan Kuala Gula, Kuala Sangga Besar, Bagan Pasir Hitam and Bagan Panchor (Fig. 2.1). Sangga Besar river is one of the major waterways for fishing boats. The aquaculture operation is more prevalent in this river, occupying approximately the lower half of its length. Human influence on the river is further heightened by the fact that the river forms a major passage between the fishing village at Kuala Sepetang and the sea. The average depth at Sangga Besar river is 2 m and is 8.5 km in length (Yap, 1995). The depth in Sangga Kecil river is deeper with an average depth of 7 m.

The total number of cages operating in Sangga Besar river is 6,564 which are owned by 91 operators. The cages cover a total area of 5.29 ha (Perak Fisheries Department, 2000). Each fish farm in Sangga Besar river is composed of a series of interconnected floating net cages, each approximately 2.5 m x 2.5 m in dimension and 1.5 – 2.5 m in depth. The size of each farm varies between 100-150 cage units. The three main species of fish cultured in Matang in order of abundance are the giant sea perch (*Lates calcarifer*), golden snapper (*Lutjanus johnii*) and red snapper (*Lutjanus argentimaculatus*). The fish cultured are fed with trash fish and feeding depends on the tide. During spring tide, feeding is normally once a day while at neap tide, it is twice a day. The weight of trash fish fed per day varies between 300-800 kg per farm. The main feed provided to the cultured fish in Sangga Besar river is trash fish which comprises mainly of young slender shad (*Illisha 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*) (Natin, 2001). Cage culture fish are harvested after 7-8 months when the average harvest size is 600g, or after 10-11 months (at 800g) depending on the market demand.

2.2 Sampling design

Zooplankton sampling and water parameter measurements were carried out in Sangga Besar (SSB) and Sangga Kecil (SSK) rivers within MMFR from December 1999 to April 2000. In SSB, zooplankton were sampled inside floating net cages from four fish farms on Transects 1, 2, 3 and 4, respectively in the offshore direction. The length of the river in SSB from Transect 1 to downstream is 3.5km. Three farm sites in transects 1, 2 and 3 were located on the right bank of SSB, while the fourth on Transect 4 was located on the left bank, near to the island village of Bagan Kuala Sangga Besar (Fig. 2.2). Similar samplings were carried out at non-cage sites (or control sites) on the same transect but located on the opposite bank, i.e. away from the fish farms. Similar samplings were also carried out in Sangga Kecil river, representing the control river (without aquaculture), where three sampling transects were established downstream (Transect 5), mid-stream (Transect 6) and upstream (Transect 7). The length of the river from Transect 7 to downstream is 4.1km. The study area and sampling station are shown in Figure 2.2. The GPS readings for each sampling station are given in Table 2.1. The field sampling time-table for the overall studies is shown in Table 2.2.

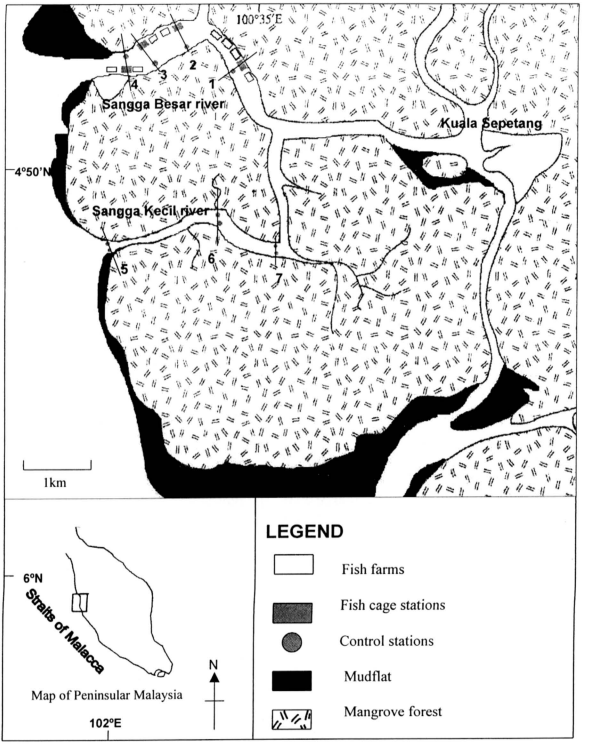

Figure 2.2 Sampling location in Sangga Besar and Sangga Kecil rivers in Matang mangrove swamp, Perak. Cross-river transects (numbered 1-7) are shown.

Table 2.1 GPS reading for each sampling station in Sangga Besar (SSB) and Sangga Kecil (SSK) river. station "IN" : inside cage culture area ; station "AWAY" : away from cage culture area; station "RIGHT" : right bank of the river ; station "LEFT" : left bank of the river

River	Transect	IN	AWAY
SSB	1	N 4° 51.512' E 100° 34.710'	N 4° 51.430' E 100° 34.596'
SSB	2	N 4° 51.748' E 100° 34.387'	N 4° 51.638' E 100° 34.273'
SSB	3	N 4° 51.708' E 100° 34.125'	N 4° 51.520' E 100° 34.008'
SSB	4	N 4° 51.348' E 100° 33.564'	N 4° 51.513' E 100° 33.540'
River	**Transect**	**RIGHT**	**LEFT**
SSK	5	N 4° 49.456' E 100° 33.774'	N 4° 51.512' E 100° 34.710'
SSK	6	N 4° 49.513' E 100° 34.690'	N 4° 51.512' E 100° 34.710'
SSK	7	N 4° 50.057' E 100° 35.257'	N 4° 51.512' E 100° 34.710'

Table 2.2 Field sampling time-table for study

Study	Month	Date	River	Transect
4-month study	December	20/12/1999	SSB	1,2
	January	23/01/2000	SSB	1,2
	Early March	07/03/2000	SSB	1,2
		08/03/2000	SSB	3,4
	Late March	28/03/2000	SSB	3,4
		29/03/2000	SSK	5,6,7
	April	22/04/2000	SSB	2,3,4
12-hour study	April	20/04/2000	SSB	1
		21/04/2000	SSK	6

4- month Study

The purpose of this study was to compare the composition and abundance of zooplankton inside fish cages and non-caged areas over a period of 4 months, in order to see whether there are temporal effects.

In SSB, along each transect 1, 2, 3 and 4, three zooplankton samples were routinely taken from 2 sampling stations. The first station was located directly inside the fish cage and was referred as inside station (IN). The second station, referred to as away station (AWAY), was located about 180-210 meters away from the first station, on the opposite bank (Fig. 2.3).

In SSK, three replicates were taken from each of two sampling stations established along transects 5, 6 and 7. The first station was located at the left bank of the river (LEFT). The second station on the same transect was located at the right bank of the river (RIGHT) (Fig. 2.4). Samples were taken randomly at each station. Thus, the 'RIGHT' and 'LEFT' stations in SSK had equivalent positions to the 'IN' and 'AWAY' stations in SSB respectively and this facilitated the ANOVA test between rivers.

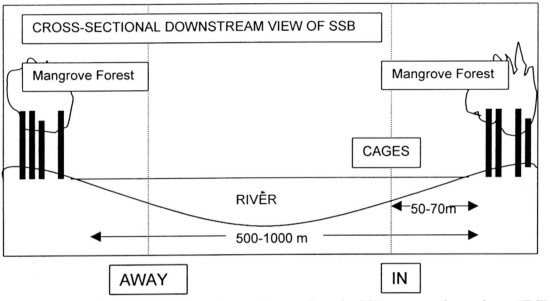

Figure 2.3 Designated location of sampling stations in SSB, cage culture river. "IN" : inside fish cage culture station, and "AWAY" : away from fish cage culture station on each transect.

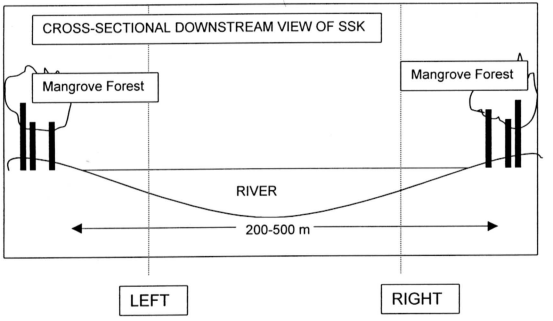

Figure 2.4 Designated location of sampling stations in SSK, control river. "LEFT": left bank of the river station and "RIGHT" : right bank of the river station on each transect.

12-hour Study

The purpose of this study was to determine any difference in zooplankton biomass and density (including water parameters) due to tidal and diel effects. This study was carried out in both, SSB and SSK on Transect 1 and Transect 6 respectively from 0852 hr to 2250 hr (see Appendix 2.1).

2.3 Sampling of zooplankton

For fish cage areas, it is impossible to sample zooplankton using boat-towed nets because of the limited space within the fish cage areas. It was also not practical to use a vertical-sampling net because the depths in between cages ranged from 2 – 4 m. Because of this, a pump was used to sample zooplankton for this study. Pumping systems sample intermediate volumes of waters (tens of liters to tens of cubic meters). Sampling of zooplankton with pumps in the open sea has several advantages over towed nets such as reliable measurements of the filtered volume, depth control, and control of the filtering process with the possible use of several mesh sizes (Miller and Judkins, 1981). For example, the EZY-ZOOP, a self-contained pump sampler was calibrated against net samples and provided comparable data on taxonomic composition, total densities of zooplankton and the densities of the major taxa (copepods, nauplii and brachyuran zoea) encountered (Dixon and Robertson, 1986).

Zooplankton samples were collected using a hose-on-suction side system (pump on deck) between December 1999 to April 2000. The pump used was attached to a HONDA 'Forced Air Cooling 4-Cycle Gasoline Engine'. [model SEF-50X (G200)]. The

maximum suction capacity was 580 liter/min. To deploy the pump-system, an intake hose was placed 0.5m below the water surface randomly inside the fish cage (and also in controls). The amount of water filtered ranged from 2 m^3 to 7 m^3 (see Appendix 2.1) and was measured using a factory-calibrated flowmeter, Helix 3000. The Helix 3000 is a high-capacity in-line helical rotary (Woltmann) type water meter with a precision injection moulded mechanism eminently suitable for high and sustained flows associated with bulk metering. The discharge hose was placed directly above the mouth (50 cm dia.) of a suspended 153 μm-mesh plankton net. The zooplankton was then washed down into the net bucket. The sample was kept in a 500ml plastic container with air-tight lids. The samples were immediately preserved in 10% buffered formaldehyde. Three replicates were taken at each station. Each replicate of zooplankton sample was from filtering the intake water for 10-20 min.

2.4 Laboratory analysis

2.4.1 Determination of wet weight

Mangrove zooplankton samples that were collected contained large amounts of sediment and plant detritus. Zooplankton had to be separated from the sediment and detritus before the determination of wet weight were made.

Each zooplankton sample was seived through a 125 μm Endecott seive using running tap water to remove fine clay, silt and sand sediment. The retained zooplankton (including plant detritus) was washed onto a pre-weighed steel gauze (w_g) of similar

mesh size, which was then placed onto a blotting paper to absorb moisture. The zooplankton sample on the gauze was then weighed accurately to 2 decimal points (w_z). The zooplankton wet weight was determined by wet weight difference (w_z-w_g). Estimation of the amount of plant detritus in terms of percentage volumetric composition was made for each sample. This was done by placing subsamples of the 'zooplankton' in a Sedgewick-Rafter cell and estimating the percentage volume of detritus under a microscope, with the aid of a 10 x 10 graticule micrometer (eye estimation method, see Chong and Sasekumar, 1981). The estimated detritus and hence weight was then subtracted from the total wet weight to obtain the actual zooplankton wet weight. The actual zooplankton biomass was then expressed as gram per cubic meter of water filtered (gm^{-3}). The zooplankton were resuspended in buffered 5% formalin and kept in a 100 ml storage bottle for subsequent examination.

2.4.2 Enumeration

Large samples were split using a Folsom plankton splitter, until the split sample obtained was not too large. Usually, 2 splits were sufficient, but the maximum number of splits was 4 (n). A fixed volume of 1 ml was then subsampled onto a Sedgewick-Rafter cell using a Stempel pipette. Enumeration was done on the entire subsample (N_s). The volume of split sample (V_s) was measured. The total number of individuals in the field sample was estimated by $N_s \times V_s \times 2^n$. Density was expressed as number of individuals per cubic meter (ind. m^{-3}), after taking into consideration the total volume of water filtered.

2.4.3 Identification

Zooplankton were identified using the following keys and reference: Newell and Newell (1977), Sirota (1966), Arvin (1977), Gosner (1971), Broad (1957), Bookhout and Costlow Jr. (1974), Ferrari (1977), Hiromi (1981), Ueda and Hiromi (1987), Lawson and Grice (1973), Walter (1987), Walter (1984), Oka, Saisho and Hirota (1991), Nishida and Ferrari (1983) and Todd, Laverack and Boxshall (1996).

2.5 Measurement of environmental parameters

Environmental parameters were taken monthly during the study period at all the sampling locations. Surface water temperature (°C), dissolved oxygen (mg/l), pH and salinity (‰) were measured at each sampling location using the YSI 3800 water logging system (multi–parameter probe). For 12-hour study in April, dissolved oxygen and turbidity readings recorded by a Hydrolab DataSonde 3 datalogger were obtained from Dr. D. M. Alongi from Australian Institute of Marine Science (AIMS). Dissolved inorganic nutrients (NH_3-N, NO_2-N, NO_3-N and PO_4) were determined using a Hach Kit (DR2010) following the Manual for DR/2010 Spectrophotometer Procedures. Surface chlorophyll-*a* concentrations were determined by fluorometry method (Parsons, 1984). Nutrients and chlorophyll-*a* results were obtained from Ms. Wong S. C. (thesis in preparation). Records of average monthly rainfall data for Taiping (located 10km east of Kuala Sepetang) were obtained from the Malaysian Meteorological Service. Tide levels were determined based on Tide Tables of Peninsular Malaysia for the year 1999 and 2000.

2.6 Statistical analysis

2.6.1 Univariate analysis

Analysis of variance (ANOVA) followed by multiple range test (Newman Keul test) was used to compare differences in total biomass and density of zooplankton among the months, transects and stations (3-factor ANOVA). For these analyses, raw data were logarithmically transformed [$\log_{10}$ (x+1)] to achieve normality and homogeneity of variance before analysis (Zar, 1998). The logarithmic transformation was applied because it has the effect of compressing the upper end of the measurement scale and thus reduce the importance of large values relative to smaller values in the data matrix (Digby & Kempton, 1996). All statistical analyses were performed using the Statistica Version 5.0 Software Package. Levels of significance were accepted at $p < 0.05$.

ANOVA was carried out for the following

a) A three-factor ANOVA applied to logarithmically-transformed biomass (wet weight) and density data, with months (December*January*Early March), transects (1*2) and stations (IN*AWAY) as the possible influencing factors; for Sangga Besar river only.

b) A three-factor ANOVA applied to logarithmically-transformed biomass and density data with months (early March*April), transects (2*3*4) and stations (IN*AWAY) as influencing factors; for Sangga Besar river only.

c) A one-factor ANOVA applied to logarithmically-transformed biomass and density data with river (SSB*SSK) as influencing factor for late March data .

d) A two-factor ANOVA applied to logarithmically-transformed biomass and density data with tide (ebb*flood) and stations (IN*AWAY) for SSB; (LEFT*RIGHT) for SSK as influencing factors for April data during the 12-hour study for SSB and SSK.

e) A two-factor ANOVA applied to logarithmically-transformed biomass and density data with diel (DAY*NIGHT) and stations (LEFT*RIGHT) for SSK as influencing factors for April data during the 12-hour study.

2.6.2 Multivariate Analysis

There are numerous multivariate methods that may be used to reduce complex community data. Examples include hierarchical clustering, multidimensional scaling and principal component analysis. All require generation of a similarity or dissimilarity (distance) matrix for input into a cluster or ordination (Legendre and Gallagher, 2000). Statistical techniques based on simple distribution as the unidimensional normal distribution are not really appropriate for analyzing complex ecological data sets. Multivariate or multidimensional statistics consists of methods that are able to analyze complex ecological data sets comprising many variables which, may and often do 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 enables 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 data (species abundance or measured environmental variables) or binary data (species presence-absence).

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), multidimensional scaling (MDS) and canonical correspondence analysis (CCA). The latter is a class of ordination methods that permits the simultaneous analysis and comparison of two data matrices. In ecology, these are most often the ones containing the species abundance data and environmental variables (Ledgendre and Legendre, 1998).

2.6.2.1 Multidimensional scaling (MDS)

The method of non-metric Multidimensional Scaling (MDS) attempt to place samples on a map, usually in two dimensions, so that the rank order of the distances between samples on the map exactly agrees with the rank order of the matching similarities taken from the triangular similarity matrix (Clarke and Warwick, 1994).

A successful MDS ordination is measured by a stress coefficient which reflects the extent to which the two sets of ranks do not agree (high stress = high disagreement). Stress increases with reducing dimensionality and with increasing quantity of data. For 2-dimensional (2-D) ordinations, stress < 0.05 gives an excellent representation, while stress < 0.1 corresponds to a good ordination, stress < 0.2 gives a potentially useful 2-D picture and stress < 0.3 indicates that the points are close to being arbitrarily placed on the 2-D map.

The MDS procedure was used as an ordination technique for analyzing the zooplankton abundance data. Multidimensional scaling (MDS) was performed on the species abundance data to investigate similarities between each station.

The MDS procedure is available in the Statistica 5.0 software, where it requires a distance matrix as input. This distance matrix has to be derived from species abundance data in the Cluster procedure. However, the cluster procedure in Statistica computes the distance matrix using Euclidean distances. Euclidean distances based on species abundances data however suffer from "distortion" and to circumvent this paradox, other distance measures are recommended (Legendre and Legendre, 1998). 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 procedure (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 saving the computed (now chord) distances among sites in matrix form. This matrix was then submitted to the MDS procedure.

2.6.2.2 Principal Component Analysis (PCA)

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. PCA was performed after "chord transformation" to all the species abundance data (see above). The PCA was used to group the sampling stations according to the presence of different types of zooplankton. PCA is performed using the CANOCO software (ter Braak and Smilauer, 1998). The species abundance data from left and right banks of SSK were pooled together in the analysis, as also in the MDS analysis above, because there were no significant difference between species groups.