CHAPTER 2

MATERIALS AND METHODS

2.1 Location of Study Area

The study area is located within the Matang Mangrove Forest Reserve (MMFR) in Perak, on the West Coast of Peninsular Malaysia (4° 50'N, 100° 35'E). The MMFR is hailed as one of the best managed mangrove forests in the world with a total forest area of 40,151 hectares. The main types of mangrove trees are *Rhizophora apiculata* (*'bakau minyak'*), forming pure stands with occasional understorey of *Bruguiera parviflora* (*'lenggadai'*), followed by *Rhizophora mucronata* (*'bakau kurap'*) along river banks and in more deeply flooded areas (Gan, 1995).

The MMFR is located within three river basins, namely Sungai (Sg.) Sepetang, Sg. Larut and Sg. Terong. It comprises of 19 independently gazetted forest reserves, and has been under the direct jurisdiction of Perak State Forest Department since 1902. The mangrove reserves are located in a large crescent-shaped bay of 52 km long and 13 km wide in the middle. Major parts of the mangrove reserves include 7 deltaic islands, namely, Pulau Gula, Pulau Kelumpang, Pulau Selinsing, Pulau Sangga Kecil, Pulau Sangga Besar, Pulau Terong and Pulau Pasir Hitam.

The mangrove trees in MMFR have been traditionally harvested on a sustainable basis for fuel (as firewood or converted to charcoal) and polewood (for piling in construction work). The District Forest Office of Larut and Matang is responsible

for the implementation and monitoring of the Working Plan of silvicultural operation which currently runs on a 30-year rotation basis (Gan, 1995).

The waterways of MMFR serve as important nursery and feeding grounds for fishery resources and support a thriving aquaculture industry. The total production of brackishwater cage cultured fish in the Larut-Matang area was reported to be 286.64 tonnes with a total farm size of 4.2 ha in 1999 (Perak State Department of Fisheries, 2000). A total of 3,596 cage units was reported to be operated by 61 fish farmers (Natin, 2001). Each fish farm is composed of a series of interconnected floating net cages with the size of each cage unit of 2.5 m X 2.5 m X 2.5 m. Farms vary in sizes, ranging from 100 to 150 units. The three main species of fish cultured in the Matang rivers in the order of abundance were the giant sea perch *(Lates calcarifer)*, golden snapper (*Lutjanus johni*) and red snapper (*Lutjanus argentimaculatus*).

The cultured fish were fed with ground trash fish from trawler-by-catch which consists predominantly of small anchovy (*Stolephorus commersoni*), clupeids *(Sardinela fimbriata, Illisha melastoma*) and a wide range of other species. The number of feeding depends on the tide. During spring tide, feeding is normally done once a day while on neap tide, twice a day. On the other hand, feeding also depends on availability of trash fish. The amount of trash fish given per cage unit was approximately 8-15 kg/day for adult fish and 2-4 kg/day for juveniles. The weight of trash feed given per farm varied from 300 to 800 kg/day.

The specific study area (see Figure 2.1.1) consisted of the mangrove estuaries of three rivers, namely, Sg. Sangga Besar (SSB), Sg. Jaha (SJ) and Sg. Sangga Kecil (SSK).

SSB and SJ were rivers with fish cage culture. SSB had a higher density (more than 15 fish farms) of cage culture and SJ a lower density (3 fish farms). SSB (also known as a distributary) branches off from the larger Sg. Sepetang. SSB receives runoff from Sg. Sepetang as well as human settlements from the upstream and downstream. The nearest populated areas of SSB are Kuala Sepetang town on the upstream and a small fishing village downstream known as Bagan Kuala Sangga Besar. Apart from the few cages, no human settlement exists at SJ. On the other hand, SSK did not have any fish cages and served as a control river (the non-aquaculture river) in this study. SSK is also a distributary of Sg. Sepetang.

2.2 Meteorology

2.2.1 Rainfall

Rainfall data of Taiping obtained from the Malaysian Meteorological Services (MMS) for three years (1998, 1999 and 2000) were compiled and shown in Figure 2.2.1. Taiping town, located approximately 11 km from the study area, is known to be one of the wettest areas in Malaysia. In the year 2000, Taiping received 3,930.6 mm of rain and the average monthly rainfall was 327.6 mm. The rainfall pattern of Taiping is influenced by the Northeast Monsoon wind (November – March), the Southwest Monsoon wind (May – September) and the variable Intermonsoonal winds (April and October). Generally, two rainfall peaks during the months of

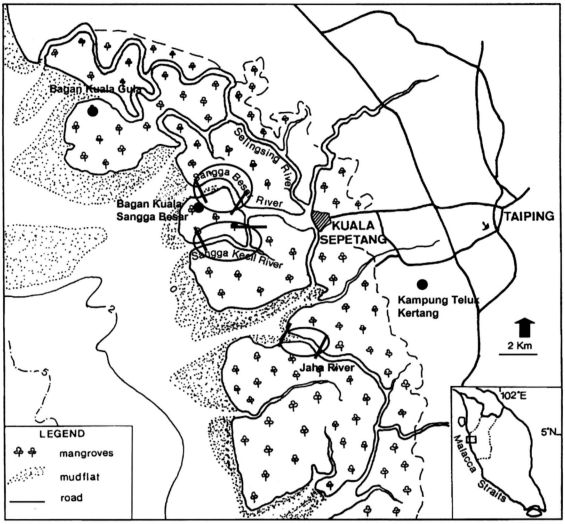

Figure 2.1.1 - The Matang Mangrove Forest Reserve in the State of Perak, Peninsular Malaysia (adapted from Chong *et al.*, 2001). Open circles indicate approximate position of study sites. The blue lines indicate the first and last transect at each estuary. See Figures 2.3.1 to 2.3.3 for the layout of the fish cages.

March/April and November coinciding with the intermonsoons were observed. A dry period with low rainfall (less than 200 mm per month) was observed in June/July.

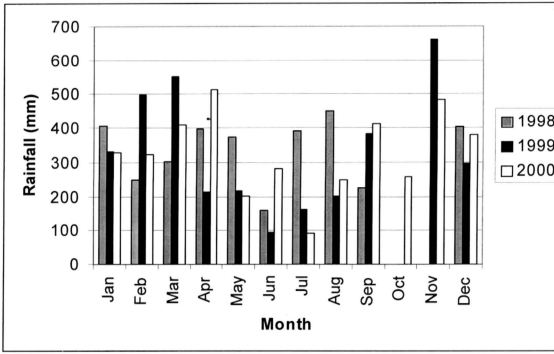

Figure 2.2.1 – Monthly Rainfall Data at Taiping Hospital in the Years 1998, 1999 and 2000.

For the year 2000, April and May were considered as wet months due to the high rainfall received in April. On the other hand, August was regarded as a dry period due to the lowest rainfall experienced in July, although in this particular year, August received slightly higher rainfall than May.

2.2.2 Tides

The tides at Kuala Sepetang (4°50' N, 100°37' E) are semidiurnal. The slack water times at Kuala Sepetang were estimated from the tide table for Lumut (4°14' N, 100°37' E) (Royal Malaysian Navy, 1999). The slack times at Kuala Sepetang were set back 1 hour 41 minutes before that in Lumut. The tidal heights of high and low

water for Kuala Sepetang were then calculated by interpolation based on the tidal height differences between Lumut and Kuala Sepetang. The calculated mean spring and neap tidal amplitudes at Kuala Sepetang during the sampling exercise are summarized in Table 2.2.1.

Table 2.2.1 – Tide Schedule and Computed Tidal Amplitudes at Kuala Sepetang.

Date	Time	Tidal Height (m)	Tidal Amplitude (m)	Average Tidal Amplitude (m)
20 April 2000	0338	1.9		
(1 day after full	0939	0.2	1.7	
moon)	1549	2.2	2.0	
	2211	0.2	2.0	1.7
21 April 2000	0409	1.8	1.6	
(2 days after full	1007	0.4	1.4	
moon)	1613	2.1	1.7	
	2238	0.2	1.9	
13 May 2000	1023	0.6		
(6 days before full	1132	1.9	1.3	
moon)	1804	0.7	1.2	
	2345	1.6	0.9	1.3
14 May 2000	0551	0.5	1.1	
(5 days before full	1226	2.1	1.6	
moon)	1856	0.6	1.5	
19 August 2000	0507	2.2		
(4 days after full	1109	0.2	2.0	
moon)	1654	2.1	1.9	
	2317	0.3	1.8	1.7
20 August 2000	0537	2.1	1.8	
(5 days after full	1145	0.6	1.5	
moon)	1728	1.9	1.3	
	2350	0.3	1.6	

2.3 Sampling Design

2.3.1 Grid Sampling

Grid sampling was carried out to map the concentrations of nutrients and chlorophyll *a* within an estuary. This type of sampling served two purposes:

a) To examine if there was any difference in the concentrations of nutrients and cholorophyll *a* inside and outside cages.

b) To detect whether nutrient plumes are generated from the fish cages or otherwise, by constructing iso-concentration contours.

Grid sampling was designed for the three rivers, by creating cross-transects and establishing water sampling stations on each transect. Grid samplings were conducted for the months of May (wet season) and August (dry season) in 2000. Locations of the sampled stations were achieved by hand-held GPS (Garmin GPS 75 and Ensign GPS). The layout for grid sampling is shown in Table 2.3.1 and the station locations are shown in Figures 2.3.1, 2.3.2 and 2.3.3 for SSB, SJ and SSK respectively. The widths of the estuaries are approximately 300 – 600 m for SSB, 100 – 300 m for both SSK and SJ.

Table 2.3.1 - Layout for Grid Sampling at Sg. Sangga Besar, Sg. Sangga Kecil and Sg. Jaha (Year 2000).

Month	Tide	SSB		SSK		SJ	
		Transect	No. of Station	Transect	No. of Station	Transect	No. of Station
19-20 August 2000	Flood	B, C, D, E, F, G, H, I, J, K, L, M	56	P, Q, R, S, T	15	U, V, W, X, Y, Z	25
	Ebb	A, B, C, D, E, F, G, H, I, J, K, L, M	60	P, Q, R, S, T	15	U, V, W, X, Y, Z	24
13-14 May 2000	Flood	C, D, E, F, G, H, I, K, L, M	47	-	-	V, W, X, Y	19
	Ebb	C, D, E, F, G, H, K, L, M	32	-	-	V, W, X, Y	19

Table 2.3.2 shows the codes of each station as used in Figures 2.3.1, 2.3.2 and 2.3.3 respectively. The location of each station is identified as 'N' (outside cage and on the same side of river bank), 'I' (inside cage), 'M' (outside cage and at mid river section) and 'O' (outside cage and on opposite bank). For transects without fish

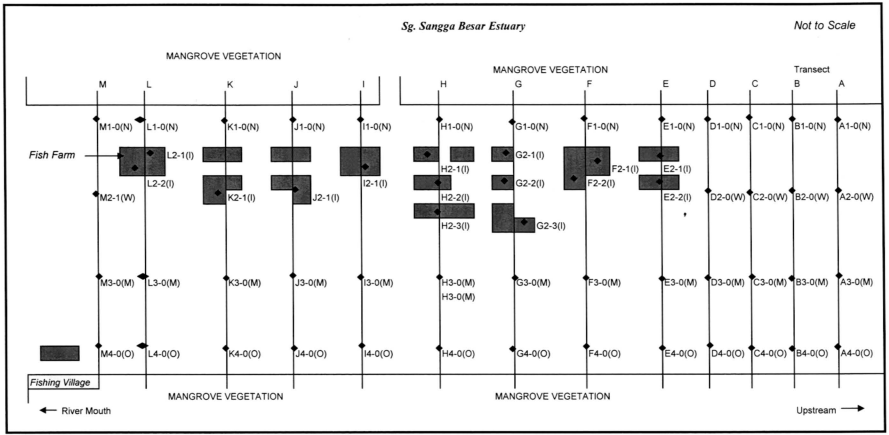

Figure 2.3.1 - Schematic Layout of the Fish Cage Farms and Water Quality Sampling Stations at Sg. Sangga Besar Estuary. Station locations (see Figure 2.1.1) are marked within parentheses as outside cage and at same side of river bank (N), inside cage (I), outside cage and at mid riversection (M), outside cage and on opposite side of river bank (O) and without cage (W) respectively. Distance between Transect A and Transect M is approximately 1500m. River width ranges from 300m to 400m.

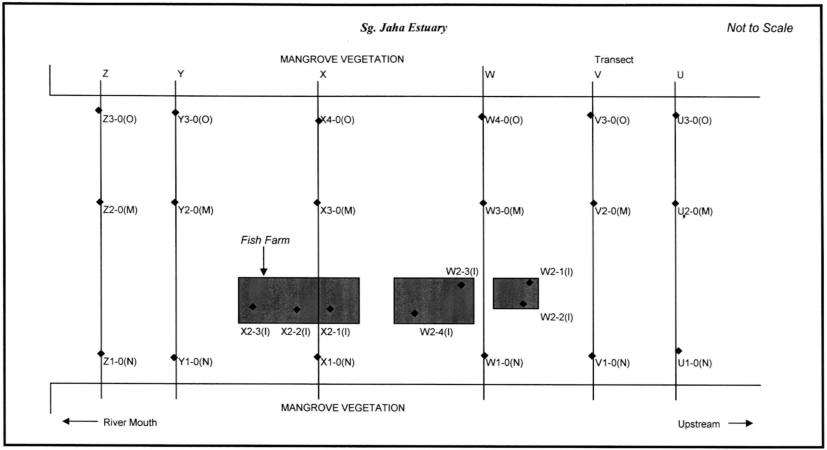

Figure 2.3.2 - Schematic Layout of the Fish Cage Farms and Water Quality Sampling Stations at Sg. Jaha Estuary (see Figure 2.1.1).
Station locations are marked within parentheses as outside cage and at same side of river bank (N), inside cage (I),
outside cage and at mid river section (M) and outside cage and on opposite side of river bank (O) respectively.
Distance between Transect U and Transect Z is approximately 700m. River width ranges from 200 to 300m.

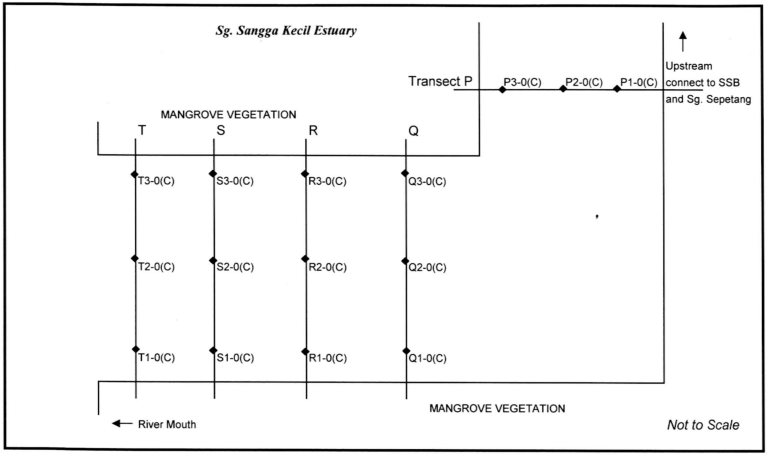

Figure 2.3.3 - Schematic Layout of the Water Quality Sampling Stations at Sg. Sangga Kecil Estuary (see Figure 2.1.1). Station locations are marked within parentheses as cage free (C). Distance between Transect P and Transect T is approximately 1000m. River width ranges from 250 to 350m.

cages (Transect A, B, C and M), stations along the same longitudinal axis as the 'I' station were identified as 'W' (without cages) instead.

Table 2.3.2 – Codes of Water Sampling Stations.

River	Transect	Station					
SSB	A	A1-0(N)	A2-0(W)	-	-	A3-0(M)	A4-0(O)
	B	B1-0(N)	B2-0(W)	-	-	B3-0(M)	B4-0(O)
	C	C1-0(N)	C2-0(W)	-	-	C3-0(M)	C4-0(O)
	D	D1-0(N)	D2-0(I)	-	-	D3-0(M)	D4-0(O)
	E	E1-0(N)	E2-1(I)	E2-2(I)	-	E3-0(M)	E4-0(O)
	F	F1-0(N)	F2-1(I)	F2-2(I)	-	F3-0(M)	F4-0(O)
	G	G1-0(N)	G2-1(I)	G2-2(I)	G2-3(I)	G3-0(M)	G4-0(O)
	H	H1-0(N)	H2-1(I)	H2-2(I)	H2-3(I)	H3-0(M)	H4-0(O)
	I	I1-0(N)	I2-1(I)	-	-	I3-0(M)	I4-0(O)
	J	J1-0(N)	J2-1(I)	J2-2(I)	-	J3-0(M)	J4-0(O)
	K	K1-0(N)	K2-1(I)	K2-2(I)	-	K3-0(M)	K4-0(O)
	L	L1-0(N)	L2-1(I)	L2-2(I)	-	L3-0(M)	L4-0(O)
	M	M1-0(N)	M2-0(W)	-	-	M3-0(M)	M4-0(O)
SSK	P	P1-0(C)	P2-0(C)	-	-	P3-0(C)	-
	Q	Q1-0(C)	Q2-0(C)	-	-	Q3-0(C)	-
	R	R1-0(C)	R2-0(C)	-	-	R3-0(C)	-
	S	S1-0(C)	S2-0(C)	-	-	S3-0(C)	-
	T	T1-0(C)	T2-0(C)	-	-	T3-0(C)	-
SJ	U	U1-0(N)	-	-	-	U2-0(M)	U3-0(O)
	V	V1-0(N)	-	-	-	V2-0(M)	V3-0(O)
	W	W1-0(N)	W2-1(I)	W2-2(I)	W2-3(I) W2-4(I)	W3-0(M)	W4-0(O)
	X	X1-0(N)	X2-1(I)	X2-2(I)	X2-3(I)	X3-0(M)	W4-0(O)
	Y	Y1-0(N)	-	-	-	Y2-0(M)	Y3-0(O)
	Z	Z1-0(N)	-	-	-	Z2-0(M)	Z3-0(O)

Note: (N) denotes outside cage and on the same side of river bank
(I) denotes inside cage (station in bold), (W) denotes outside cage along the same longitudinal axis
(M) denotes outside cage and at mid of river section
(O) denotes outside cage and on opposite side of river bank
(C) denotes without cages (control)

2.3.2 12-hour Sampling

The purpose of 12-hour sampling was to observe the effects of tide (flood versus ebb) and light (day versus night) on the concentrations of nutrient and chlorophyll *a* in the mangrove estuaries.

This study was conducted at SSB and SSK, during spring tide in April, 2000. At SSB, samples were taken from 'I' (inside the cage) and 'O' (outside cage and on opposite side of river bank) stations. One control station was established at SSK,

which had no aquaculture activity. Details of the sampling layout are shown in Table 2.3.3.

Table 2.3.3 – Tidal and Diel Information and Sampling Layout for 12-hour Study.

River	Station	Location	Sample	Time	Tide	Diel
SSB (20 April 2000)	E2-2	IN (Inside Cage)	1	1010	Flood	Day
			2	1130	Flood	Day
			3	1330	Flood	Day
			4	1600	Slack High	Day
			5	1720	Ebb	Day
			6	1930	Ebb	Night
			7	2100	Ebb	Night
	E4-0	OUT (Outside cage & on opposite side of river bank)	1	0900	Slack Low	Day
			2	1215	Flood	Day
			3	1410	Flood	Day
			4	1530	Slack High	Day
			5	1800	Ebb	Day
			6	2005	Ebb	Night
			7	2140	Ebb	Night
SSK (21 April 2000)	R2-0	CTRL (Cage-free as control)	1	1035	Slack Low	Day
			2	1235	Flood	Day
			3	1440	Flood	Day
			4	1640	Slack High	Day
			5	1845	Ebb	Day
			6	2115	Ebb	Night
			7	2315	Slack Low	Night

2.3.3 Short-interval Serial Sampling (Nutrient Leaching Experiment)

The purpose of this sampling was to follow the concentrations of nutrients and chlorophyll *a* in the water column over time after fish feed was introduced into the fish cages. Dry pellet and ground trash fish feed were given separately to two sets of fish cages located in Sg. Jaha. Water samples were collected from the fish cages as soon as the feed was given to the fish. Samples were collected at half-hourly intervals over 2 hours (see Table 2.3.4).

Table 2.3.4 - Sampling Layout for the Nutrient Leaching Experiment.

Station		Site A	Site B	Site C (Control)
Feed Type		Dry pellet	Trash fish	No feed
13 October 2001 (Flood tide)	Time	0956	1000	1005
		1027	1030	1035
		1054	1056	1100
		1122	1125	1130
		1144	1148	1154
13 October 2001 (Ebb tide)	Time	1300	1303	1308
		1325	1327	1333
		1354	1357	1400
		1425	1427	1430
		1450	1453	1500
14 October 2001 (Flood tide)	Time	1015	1017	1021
		1045	1047	1051
		1115	1118	1122
		1146	1150	1155
		1215	1217	1221

2.4 Analytical Procedures

2.4.1 Sampling and Sample Pre-treatment

Sampling of water was carried out from within the fish cages and a few distances away from the cages. Duplicates of water samples were taken at each station using acid washed polyethylene bottles just below the water surface. The sample bottles were screw-capped, labeled and stored in an ice-chest.

Upon reaching shore, the water samples were filtered (GF/C Whatman glass microfibre filter paper) into acid-washed polyethylene bottles. Filtered samples were stored in the freezer immediately. The sample bottles were kept frozen until laboratory analysis. The pH value and salinity of remaining unfiltered samples were measured by the YSI 3800 Water Quality Logging System (pre-calibrated in the laboratory). A precalibrated Ecoscan portable pH meter (for pH meter) and Reichert

refractometer (for salinity) were used in the May and August 2000 sampling due to malfunction of the YSI 3800 instrument.

A few drops of 1% $MgCO_3$ solution were dropped onto the filter paper (with filtered phytoplankton cells) which was then folded and kept inside an opaque disc container. The container was screw-capped, labeled and stored in the freezer until laboratory analysis.

2.4.2 Laboratory Analyses

The frozen samples were thawed to room temperature before laboratory analysis commenced.

a) Analysis of Nutrients

Concentrations of NH_3-N, NO_3-N, NO_2-N and PO_4^{3-} were measured by using the HACH DR/2010 spectrophotometer.

The sample cell was filled with the water sample. Pre-packed reagent in powder pillow was added and allowed to react. The blank, which consisted of the same water sample without reagent was used to set the spectrophotometer to zero. Then the reagent-treated sample was measured at the respective wavelength. The step-by-step analysis procedures were based on the HACH Water Analysis Handbook. Table 2.4.1 summarises the methods of analysis for the nutrient parameters.

Table 2.4.1 - Method of Analysis for Nutrient Parameters.

Para-meter	Method	Detection Range	Wave-length	Reagent	Summary of Method*
NH_3-N	Salicylate method	0.00 to 0.50 mg/L NH_3-N	655 nm	Ammonia salicylate reagent powder pillow Ammonia cyanurate reagent powder pillow	Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidised in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution.
NO_3-N	Cadmium reduction method	0.00 to 0.40 mg/L NO_3-N	507 nm	NitraVer 6 nitrate reagent powder pillow NitriVer 3 nitrite reagent powder pillow	Cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt which couples to chromotropic acid to form a pink-colored product.
NO_2-N	Diazotiza-tion method	0.00 to 0.300 mg/L NO_2-N	507 nm	NitriVer 3 nitrite reagent powder pillow	Nitrite in the sample reacts with sulfanilamide to form an intermediate diazonium salt. This couples with N-(1-napthyl)-ethylenediamine dihydrochloride to produce a red-colored complex directly proportional to the amount of nitrite present.
PO_4^{3-}	PhosVer 3 (ascorbic acid) method	0.00 to 2.50 mg/L PO_4^{3-}	890 nm	PhosVer 3 phosphate reagent powder pillow	Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

* HACH Water Analysis Handbook, 1997

Results in the unit of mg/L were converted to μmol/L. For example, the readings of NH_3-N, NO_3-N and NO_2-N were divided by the molecular weight of nitrogen (N = 14), while readings of PO_4^{3-} were divided by the molecular weight of PO_4^{3-} ion (PO_4^{3-} = 95).

b) Analysis of Chlorophyll *a*

Chlorophyll *a* concentration was determined based on a fluorometric method (Parsons et al., 1984). The filter paper with its phytoplankton content was torn into small pieces and put into a polypropylene test tube. 10 ml of 90% acetone (with a few drops of 1% $MgCO_3$) was added into the tube with a pipette. The phytoplankton cells and pieces of filter paper were repetitively crushed with a glass rod. The tube was screw-capped and stored without light in a refrigerator at 4°C for 24 hours to allow for extraction of chlorophyll *a*.

After extraction, the tubes were taken out and spun in a centrifuge at 3,000 rpm for 10 minutes. The concentration of chlorophyll *a* in the supernatant was measured by a Turner Quantech fluorometer based on a pre-set standard curve. Blank (90% acetone) was measured and all readings were re-adjusted with the blank reading.

The standard curve of chlorophyll *a* was established based on a high but known concentration of chlorophyll extract. *Chlorella* algae were cultured in the laboratory to obtain a bloom, from which a chlorophyll sample of high concentration was extracted following the procedure described above. The concentration of the extracted chlorophyll solution was measured with a Shimadzu UV-VIS Spectrophotometer. Three absorbance readings corresponding to three wavelengths (665, 645 and 630 nm) were obtained. The concentration of chlorophyll *a* in the solution was calculated using the following equation (Strickland & Parsons, 1968):

$$C = 11.6 \times OD_{665} - 1.31 \times OD_{645} - 0.14 \times OD_{630}$$

Where OD = the absorbance at different wavelengths

C = concentration of chlorophyll *a* in mg/ml.

The concentration of chlorophyll *a* in mg/L was calculated based on the following equation:

$$\text{Chlorophyll } a \text{ (mg/L)} = \frac{\text{C x 10 ml (volume of extract)}}{\text{0.1L (volume of sample water filtered) x 1000}}$$

The solution with known chlorophyll *a* concentration was then serially diluted to give five (known) different concentrations. The known chlorophyll *a* concentrations were used to set the standard curve in the fluorometer following the Turner Quantech Fluorometer operation manual.

2.4.3 Constraints and Limitations

Several constraints and limitations were experienced in the course of sample collection and laboratory analysis, which might have influenced the accuracy of the results obtained. However, care was taken at each step of the analyses to ensure consistent results.

The constraints and limitations encountered were as follows:

a) Salinity was measured in the April samples using the YSI 3800 multi-parameter probe and logger. However, the equipment failed to function properly for

several occasions, resulting in inconsistent reading and loss of data. Salinity of May and August samples was measured using a refractometer.

b) Nitrate-nitrogen (NH_3-N) analysis method. After adding the NitraVer 6 powder into the water sample, the mixture was shaken for 3 minutes. The shaking time and method are known to influence the color development (HACH, 1997). Hence a consistent shaking technique was used.

c) Reactive phosphorus (PO_4^{3-}) analysis method. The PhosVer 3 phosphate powder reagent was very sensitive to the swirling method and time. Therefore, a consistent swirling method had to be adopted.

d) Chlorophyll *a* analysis method. In the preliminary trials, several extraction times (duration) from 15 to 48 hours were tested. Most of the samples did not undergo complete extraction after 15 hours. In the final analysis, a second cell crushing was done 2 hours after the first and a standard 24-hour extraction time was adopted.

2.5 Statistical Analysis

2.5.1 Univariate Analysis – ANOVA

Analysis of Variance (ANOVA) was performed using the STATISTICA Version 5.5 Software Package. The purpose of ANOVA is to test for significant differences between means of more than two groups (Statsoft, 1994). ANOVA was used to detect significant differences in the water quality of the three estuaries. The raw

data were logarithmically transformed [$\log_{10}$ (x + 1)] so that the transformed data were normally distributed and homoscedastic (Barnes, 1952). The Student Newman-Keuls test was used when ANOVA showed significant difference and pairs of means were further tested for significant differences. The null hypothesis of no difference was rejected if $p < 0.05$.

All the water samples in SSK were used in the ANOVA. For SSB and SJ, only water sample results from transects with fish cages (SSB and SJ) were accounted in the test. ANOVA was conducted for the following cases:

a) Comparison of background nutrient and chlorophyll *a* concentrations among estuaries

Two-factor ANOVA of nutrient and chlorophyll *a* concentrations in relation to estuaries (SSB*SSK*SJ) and tides (flood*ebb) at 'O' stations (outside cage and on opposite side of river bank) for the August 2000 data.

The purpose of this ANOVA was to compare the 'background' water quality among the three estuaries which had no or varying degree of cage culture activity.

b) Comparison of Wet (May) and Dry (August) Seasons

Four-factor ANOVA of nutrient and chlorophyll *a* concentrations in relation to seasons (Wet*Dry), estuaries (SSB*SJ), tides (flood*ebb) and stations (N*I*M*O).

The purpose of this ANOVA was to compare the water quality in the dry and wet seasons between two estuaries with different intensity of cage culture, and whether water quality was affected by sample locations (inside or outside fish cages) and tides.

c) 12-hour Study

Two-factor ANOVA of nutrient and chlorophyll *a* concentrations in relation to stations (IN*OUT*CTRL) and tides (flood*ebb). Station IN was located at SSB, inside cage; station OUT was located at SSB, outside cage and on the opposite side of river bank and station CTRL was located at SSK (cage-free estuary).

Two-factor ANOVA of nutrient and chlorophyll *a* concentrations in relation to stations (IN*OUT*CTRL) and diel effect (day*night).

d) Nutrient-leaching Study

Two-factor ANOVA of nutrient and chlorophyll *a* concentrations in relation to feed types (pellet feed*trash fish feed*control) and time intervals (0.0*0.5*1.0*1.5*2.0 hour).

2.5.2 Multivariate Analysis – Principal Components Analysis (PCA)

Multivariate (multidimensional random variable) statistics are designed to tackle the analysis of complex data sets. In general, the purposes of multivariate analysis

include summarizing community data, relating community variation to environmental gradients and understanding community structure so that increasingly effective multivariate techniques may be developed. This wider perspective includes ordination.

Ordination serves to summarize community data by producing a low-dimensional ordination space (of typically one to three dimensions) in which similar species or samples are close together and dissimilar entities are far apart, producing effective, low-dimensional summaries from field data by relatively convenient and objective means (Gauch, 1982).

Examples of the commonly used ordination techniques include principal component analysis (PCA), canonical correspondence analysis (CCA), multidimensional scaling (MDS) and principal coordinate analysis (PCoA).

PCA is an ordination technique for projecting a multidimensional cloud of points into a space of fewer dimensions, using rigid rotation to derive successive orthogonal axes, which maximise the variance accounted for (Gauch, 1982). A data set must meet several assumptions of the PCA model, primarily that the components have normal distributions and be uncorrelated (Gauch, 1982). Hence, the raw data of this study were logarithmically transformed [$\log_{10}$ (x + 1)] to achieve normality and homogeneity of variance.

PCA was used in the present study to determine which combinations of variables (nutrients and chlorophyll *a* concentrations) explain the largest amount of variation in the multivariate data set.

The data set comprised of six variables, namely, NH_3-N, NO_3-N, NO_2-N, PO_4^{3-}, chlorophyll *a* and pH value, measured from a total of 224 water samples from SSB, SSK and SJ (April, May and August 2000).

A labeling system was established for the water samples collected at different times. To indicate the specific sample taken from the respective month, a numeric was added in front of each station label. Samples taken from the April, May and August were indicated by the numbers 4, 5 and 8 respectively, preceding the station labels. For example, the sample from station E2-1 taken in May is labeled as 5E2-1, while the sample taken from the same station in August is labeled as 8E2-1.

PCA was carried out on transformed values of these variables using the STATISTICA Version 5.5 Software Package. A varimax rotation was carried out to facilitate the interpretation of the results. This is based on the assumption that the interpretability of a factor can be measured by the variance of the square of its factor loading. Varimax rotation therefore maximizes the sum of these loadings for all the factors (Manly, 1986).

2.5.3 Contour Plots of Nutrient and Chlorophyll *a* Concentrations

Contour plots of the nutrient and chlorophyll *a* concentrations at the three estuaries during the flood and ebb tides in the wet and dry seasons were performed using the 3-dimensional XYZ graph with least square fit of the STATISTICA Version 5.5 Software Package. Since the estuaries are winding with uneven widths, the positions of sampling stations at the bends were 'linearized' (but maintaining the

relative positions) to avoid analytical distortions and to facilitate the interpretation of the contours.