3.0 MATERIALS AND METHODS

3.1 CAREY ISLAND AND SAMPLING SITE

Carey Island is 11, 667 ha in area and is located in the Straits of Malacca and closer to the western coast of Peninsular Malaysia. Carey Island is one of the largest islands that belong to that state of Selangor. Carey Island is not a real island due to its proximity to mainland and it is only separated from the mainland by a river known as Langat River. The island was named after an English officer who was granted several thousand acres of land by Sultan of Selangor for agricultural purposes in 1890s. Carey Island is also known as an island below the sea as a large part of it is submerged 2m deep during the high tides. Currently, 80% of the island area belongs to Sime Darby Plantation while the rest are declared as government reserves. Sime Darby Plantation has used up most of its area for oil palm cultivation and this was made possible by the construction of 120 kilometers bunds surrounding it that keep the area from seawater especially during the high tides. Human settlement on the island consists of several villages mostly belonged to the Orang Asli of the Mah-meri tribe.

Carey Island area has a humid tropical climate throughout the year with average rainfall of below 2,000mm^3. The driest month is in January (120mm^3) and the wettest month is in April (280mm^3) (Salleh & Tajuddin, 2006). Carey Island is also listed by the Malaysian Wetland Working Group as a mangrove island, since it is mostly surrounded by mangrove. Influx of water from Sungai Langat that flows into the mangrove ecosystem making the water becomes brackish and slightly acidic.

This study was carried out on three locations at Carey Island mangrove. An initial study was carried out at 5 stations in this island, but although result of the study showed significant different ($P < 0.05$) based on phytoplankton composition, the difference is due to present or absent of some species in those particular mangrove area. The composition showed more than 50% similarity regardless the distance between

stations. Consequently, the number of stations was reduced to 3 in distance less than 2 km for easy access and safety during sampling. Location of the stations were at ST1 (02°92’N, 101°35’E), ST2 (02°91’N, 101°36’E) and ST3 (02°90’N, 101°36’E). Location of Carey Island and the stations are shown in Figure 2.1. Photographs of each station are shown in Plate 3.1,3.2 and 3.3.

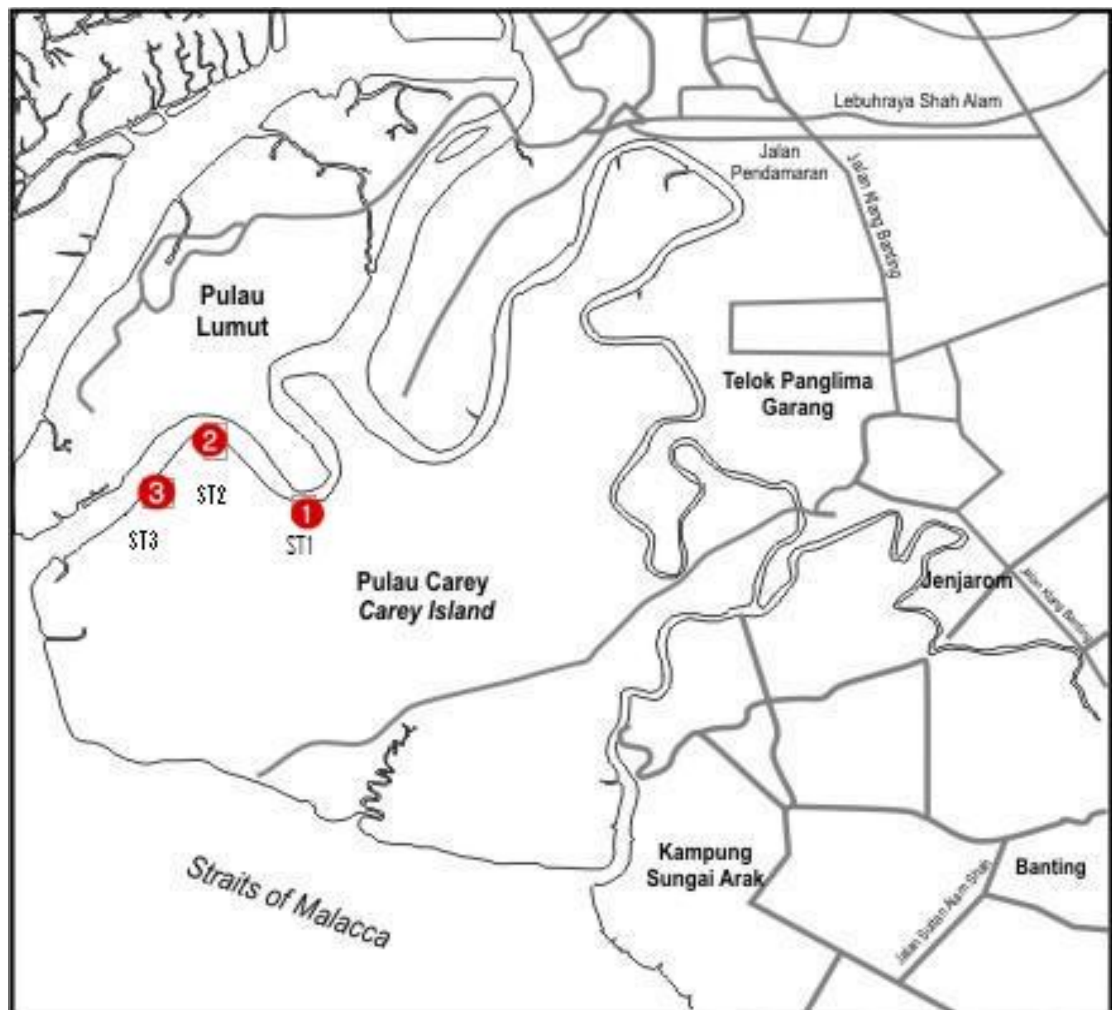

Figure 3.1: Map of Pulau Carey or Carey Island and study sites (ST1, ST2 and ST3).

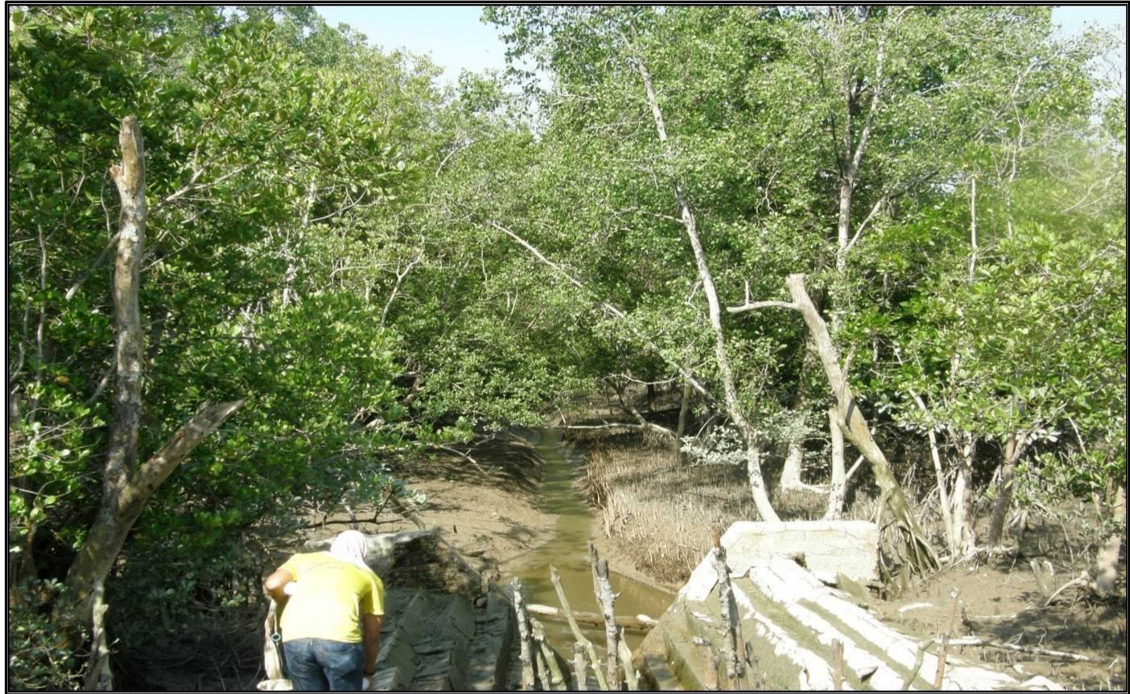

Plate 3.1: ST1, located upstream along Langat River, during low tide.

Plate 3.2: ST2 located midstream, during maximum low tide.

Plate 3.3: ST3, located downstream along Langat River, during maximum low tide.

3.2 PHYTOPLANKTON STUDY AND WATER QUALITY ANALYSES

Sampling for water quality and phytoplankton analyses were carried monthly from April 2009 to March 2010. The time interval between each sampling was between 29 to 31 days. Water samples and *in-situ* measurements were taken during both high and low tides in each sampling occasions.

3.2.1 Phytoplankton Study

3.2.1.1 Phytoplankton Sampling

Phytoplankton collected from mangrove ecosystem at Carey Island mangrove ecosystem were kept in 60ml vials and preserved by adding 2 to 3 drops of 5% formalin as preservation for identification and enumeration process. Both plankton net and water sampler were used in phytoplankton sampling. Water samples were collected using a 3 liter Ruttner sampler (Plate 3.4). The water sampled was transferred into 500ml samples bottles. 30µm wire mesh plankton net (Plate 3.5) was used in qualitative phytoplankton sampling for identification and micrographs purposes. In order to study the spatial diversity of phytoplankton at the research area; samplings were carried out at 3 different stations. Three replicates that were sampled horizontally were sampled from different parts of each station. While in determining the temporal diversity, phytoplankton samplings were done throughout a year for 12 months and in each of months, samplings were carried out in both high tide and low tide periods.

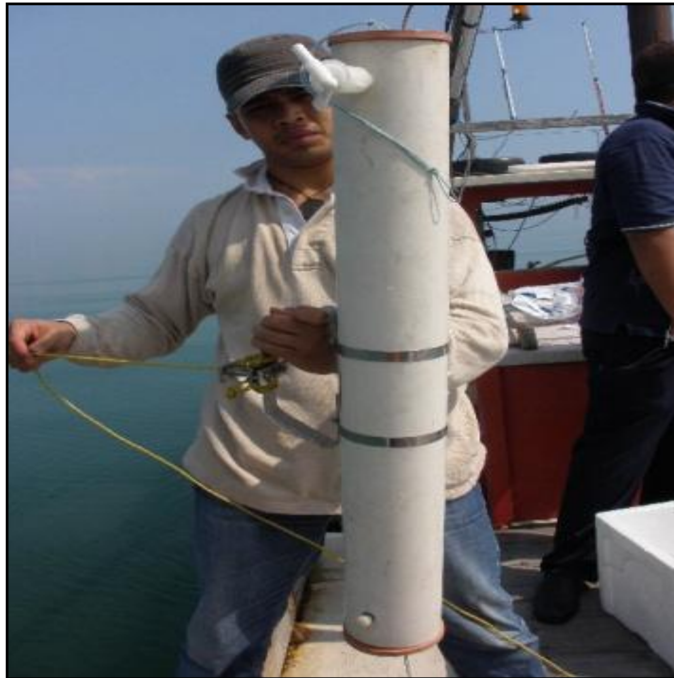

Plate 3.4: Ruttner sampler

Plate 3.5: 30µm wire mesh plankton net.

3.2.1.2 Phytoplankton Identification

Phytoplankton sampled from each station undergoes identification process until genus or species level. Identification of phytoplankton was based on the morphology. Identification and measurement of phytoplankton were carried out using light microscopy equipped with eyepiece graticules (Plate 3.6: a) and b). Aids from several taxonomic keys: Prescott (1962), Forest (1954), Aishah (1996), Lokman (1991) and Hasle & Syvertsen (1997) were important in identifying the phytoplankton up to genus or species levels. Phytoplankton species were identified by using the fresh specimen for division of Chlorophyta, Cyanobacteria and also some species of Bacillariophyta and Pyrrophyta. However, further process such as sedimentation was needed for some species of Bacillariophyta as well Pyrrophyta.

No acid cleaning was done on diatoms for identification, since the samples were found to have mild gathering of sediment and debris. This is due to the fact that only subsurface horizontal sampling was done in each sampling occasions and not the vertical samplings. Sterrenburg (2006) suggested that suitable procedure such as sedimentation is helpful in general microscopy to identify diatoms species. He also stated that the acid wash sometimes is time consuming, expensive and increase the possibility to break the diatoms structure if done by non professional. With the respect that the major aim was to study the spatial and temporal distribution of phytoplankton, less time was spent on identification process. Thus, the use of literature in key species and photographs mentioned above was vital to identify the diatoms and other divisions of phytoplankton in this study.

However, aid from SEM was used when there was limitation using light microscope to identify the phytoplankton. In preparing the samples for SEM examination, collected phytoplankton sample in 5% formalin were washed using

distilled water for 15 minutes twice. After overnight incubation, the specimen left in room temperature for 15 minutes.

The selected specimens are dehydrated in a series of ethanol dilutions, starting from 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, and finally 100% ethanol. For 100% ethanol, the ethanol is replaced twice, 15 minutes for each step. After that, the samples were immersed in 100% ethanol: 100 acetone solution with the ratio from 3: 1, 1: 1 and 1: 3 for 20 minutes.

The final step in preparing the specimen was to soak them in absolute acetone for 20 minutes and the same soaking process was repeated for three times.

Samples in the absolute acetone went through Critical Point Drying process by using Polaron E3000. After CPD steps completed, dried phytoplankton samples obtained were mounted on aluminum stub with diameter of 12.5 mm using conducting carbon cement (LEIT-C) and stored inside dryer apparatus. Gold sputtering process using Bio-Rad SEM Coating System was applied on the specimen thus plating it with thin gold layer (40-60mm). Later, the gold plated specimen was inspected under SEM model JOEL JSM6400 at 5 kV and recorded using photographic camera that connected to the SEM.

Plate 3.6: a) Light Microscopy Nikon Eclipse TS100 b) Light Microscopy Olympus BX5.

3.2.1.3 Phytoplankton Enumeration

In laboratory, slides were prepared to count the phytoplankton collected per ml. 1 ml of preserved algae sample was pipetted into sedimentation tube (Plate 3.7). 1ml of Lugol's iodine was added into the sedimentation tube. As the iodine weighted and fixed the cells, it would able to aid sedimentation which important in concentrating the cells (Bellinger & Sigee, 2010). Air tight condition was created by applying vaseline at the edge of the sedimentation tube.

The prepared sedimentation tubes were left for at least 2 hours before proceeding with enumeration process, which was carried out with aid from inverted light microscope. The specimen was enumerated by dividing the sedimentation tube into 4 divisions. The calculation was carried out from one division to another (Evans, 1972) and was expressed according to cells/L scale. The slides prepared were also used in identification process as mentioned in previous paragraph.

Graph of parameters over cell numbers/ml for each species was plotted and observations of factors that affect the growth of phytoplankton in the mangrove ecosystem using Pearson Correlation (*r*). The statistical analysis of phytoplankton data was done to obtain the Shannon-Weiner Index (H').

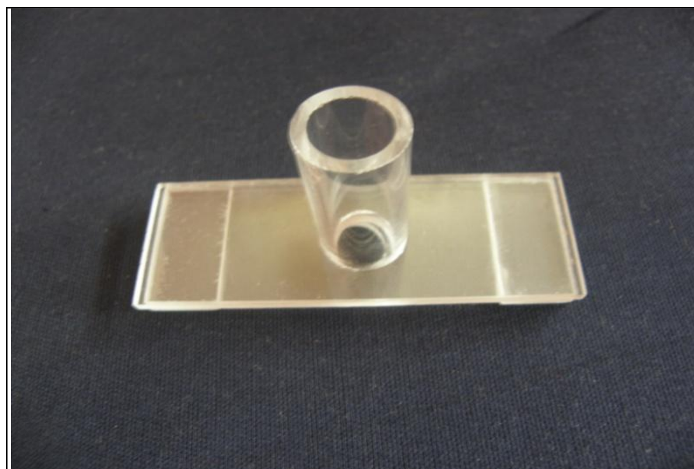

Plate 3.7: Sedimentation chamber for algae enumeration.

3.2.2 Water sampling

Water samples for chemical analyses were collected using Ruttner sampler (Plate 2.4) and were transferred into polythene bottles (500 ml) and preserved by adding 2 to 3 drops 4% formalin. Measurement of physical factors such as water temperature, pH, salinity, water conductivity, dissolved oxygen and total dissolved solid were recorded in situ at all sampling sites whereas, chemical analysis such as phosphate, nitrate, silicate and sulfate were carried out in the laboratory. Correlation coefficient was calculated to show the relation between phytoplankton with the physical and chemical parameters of the Carey Island mangrove water.

3.2.2.1 Oxygen, Temperature, Water Conductivity, Total Dissolved Solid, pH, Salinity

YSI Model 556 MPS (Plate 3.8) was used in order to measure oxygen concentration (mg/L), temperature (ºC), conductivity (µs/cm), TDS (mg/L) pH and salinity (mg/L) of water at study sites. All of the parameters were measured *in-situ*.

3.2.2.2 Chemical analyses

The collected water samples were filtered using Whatman-microfilter paper through glass microfiber filter. The filtered water was used to analyze chemical compound of the sampled water which were the nitrate ($NO3^{-2}$), phosphate ($PO4^{-3}$), silicate (SiO_3) and sulfate ($SO4^{-2}$). Powder Pillow HACH reagents were used to measure the chemical concentrations. The procedure to measure the nutrients were carried out using Spectrophotometer DR4000 HACH and the nutrient concentrations were expressed in mg/L.

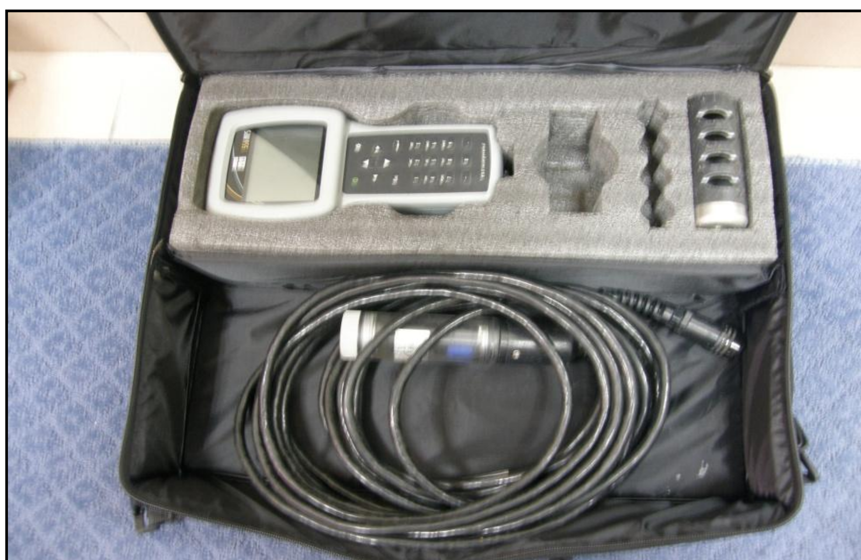

Plate 3.8: YSI Model 556 MPS

3.3 STATISTICAL ANALYSES

3.3.1 Physiochemical Parameters Analyses and Phytoplankton Study

Difference in chemical and physical parameters between stations, tides and months were done with aid from IBM SPSS Statistics 19 (2010). The parameters differences between stations and months were tested using *one-way* ANOVA while between tides were tested with aid from *2 tailed* t-test. *One-way* ANOVA also used to detect differences in phytoplankton cells' abundance and species richness between stations.

3.3.2 Relative Density (RD) and Relative Frequency (RF) of Phytoplankton

The values for both RF and RD were calculated for each species of phytoplankton and presented in the data for spatial phytoplankton distribution. Below are the formulas of both RD and RF.

Density = Number of individual/number of sampling area

Relative density (RD) = (Species density/Total densities of all species) X 100

Frequency = Plot/station in which species present/Total of plots

Relative frequency (RF) = (Species frequency/Total frequency of all species) X 100

3.3.3 Pearson's Correlation Test

Linear correlation between phytoplankton number and its pysio-chemical environmental factors were tested using Pearson Correlation at significant (*2-tailed*) level $p< 0.01$** and $p< 0.05$*. The test was done to study the factors those affecting distribution and phytoplankton's number. Scatter plot graphs were plotted when significant positive or negative correlations were showed between phytoplankton number and physical and chemical parameters. The r value strength was interpreted using Guilford's Correlation Interpretation (Guilford, 1956).

3.3.4 Diversity Index

Phytoplankton species diversity of each station during low and high tides of each month was investigated using evenness and Shannon Diversity Index. They were calculated with the aid of Multi Variate Statistical Package (MVSP) 3.1 software (Kovach, 1999). The formula that was used to calculate Shannon Diversity Index (H') and evenness (E) are shown below.

Shannon diversity Indes, $H' = \sum_{i=1}^{S} - (P_i * \ln P_i)$

where:

H = the Shannon diversity index

P_i = fraction of the entire population made up of species i

S = numbers of species encountered

$\sum$ = sum from species 1 to species S

Evenness, $E = \frac{H'}{H_{max}}$

where:

H' = Shannon diversity index information function

H_{max} = The theoretical maximum value for H' if all species in the sample were equally abundant.

3.3.5 Cluster Analyses of Phytoplankton

Cluster analysis of study stations and sampling months based on species composition of phytoplankton was done using Multi Variate Statistical Package (MVSP) 3.1 software (Kovach, 1999). UPGMA Modified Morisita's Similarity was used to construct clusters of study stations and sampling months for each station. Analysis of the latter was divided into species composition of phytoplankton during low and high tides.

3.4 RAINFALL OBSERVATION

Rainfall observation was done started during the night before sampling until sampling period. Since the sampling was done in only 1 particular day of month, monthly rainfall data is not provided in this research. Rainfall was recorded as light rainfall or heavy rainfall. The unique weather of study site also does not have similar rainfall occurring in adjacent district which is the area of Kuala Langat.