CHAPTER IV MATERIALS AND METHODS

1. Site Selection

2. Sample Collection

2.1 Macroinvertebrate Sampling

2.1.1 Sampling methods

Samples were collected in the 12 sites for 2 seasons,on 26 May,95 in the dry season and on 12 October,95 in the rainy season. The Surber sampler was used in the shallow water bodies; stream sites(S1, S2 and S3) and in the dry season at irrigation canal sites(IC1, IC2 and IC3). In the rainy season with higher velocities and depths, Ekman grab sampling was done at these sites. The Ekman grab was also used at the Ping River sites(P1, P2 and P3) and at the sewage canal sites

(SC1, SC2 and SC3). Sampling was done two times at each stream and irrigation canal, so that there were 2 replications per site. The macroinvertebrate community per Surber frame (20cm x 20cm) at each site was then calculated.

The grab sampler had an aperture area of 15cm x 15cm.It was dropped suddenly from the bridge or at the side of the sites, so that it bit in vertically. There were 2 replications per site.

All specimens from each site were fixed with 4 % formaldehyde in the plastic boxes, and brought to the laboratory for sorting and identification.

2. 1.2 Sorting and Identification

All specimens were sorted in the laboratory from gravel and sand by visual inspection after the formalin was throughly washed out with flowing water while retaining the sample in a fine mesh net. After that,

macroinvertebrates were hand-sorted under a stereomicroscope and preserved in 70% alcohol. They were then counted and identified. In most cases identification was to family level, but in a few e.g. Acari,a higher taxonomic grouping was used and in a few e.g. *Gammarus sp.* , *Macrovelia beameri.* The references used for the identification included

- Pennak (1978),
- Mellanby(1963)
- McCafferty(1981),
- Needham(1969)
- Merritt and Cummins(1988),
- Lehmkuhl(1979)
- Williams(1991),
- Rajchapakdee(1992)

2.2 Water Sampling

All water samples were collected in BOD bottles or polyethylene bottles.The water samples for chemical parameters were fixed with

concentration sulfuric acid for nutrient analysis(NH_4, NO_3, PO_4) and kept in an icebox before analysis.

1. Physical-Chemical parameters measurement

Measurement of physical- chemical properties in all sites was carried out during the dry season and in the rainy season. The auto- reading equipment was used to measure **water temperature, conductivity, pH, dissolved oxygen**, and a velocity meter was used to measure the **velocity** of running waters.

Biochemical Oxygen Demand (BOD): water sample from each site was collected in a 300 ml dark BOD bottle at a depth of about 30 cm. Initial dissolved oxygen(DO) value was recorded using a DO meter.Then the bottles were kept in the incubator at 20°C for 5 days and the final DO value was taken. BOD_5 was then calculated using the formula:

$$BOD_5 = \text{Initial DO} - \text{Final DO}$$

Alkalinity was examined by titration with 0.02N H_2SO_4 until the pH at the end point was 4.3-4.5. Total alkalinity was computed using the formula:

$$\text{Total Alkalinity(mg/l } CaCO_3) = \text{ml0.02N}H_2SO_4 \times 10$$

Nitrate contents of water were measured employing the Cadmium Reduction Method using a powder pillow (Nitra Ver 5), Cadmium Reduction form AZO compound and FIA method.(Appendix L)

Reactive Phosphorus was determined using the Phos Ver 3 (Ascorbic Acid) Method, Molybpdenum Blue and FIA method (Appendix M)

Ammonia was determine using Nesslerization technique, Phenate Method. (Appendix N)

3. Habitat Assessment

3.1 The Score of Habitat Assessment :

The habitat was evaluated only at the three stream sites, using the methods of Rapid Bioassessment Protocols for Use in Stream and Rivers (Plafkin,1989). In this study the habitat assessment matrix is based on the Stream Classification Guidelines for Wisconsin developed by Ball(1982) and the Methods of Evaluating Stream, Riparian, and Biotic Conditions developed by Platts et al.(1983)(Appendix I).Three types of parameters were used to assess the habitat. The primary parameters, include characterization of the bottom substrate and available cover, estimation of embeddedness, and estimation of the flow or velocity and depth regime. These have the widest score range(0-20) to reflect their primary contribution to habitat quality. The secondary paramaters evaluate: channel alteration, bottom scouring and deposition, and stream sinuosity. The results of the evaluation have a score

range(0-15). The tertiary parameters evaluate riparian and bank structure and comprise: bank stability, bank vegetation, and streamside cover. The results of the evaluation have score range(0-10). All parameters are evaluated for each study site. The ratings are then totalled and compared to a reference to provide a final habitat ranking. Scores increase as habitat quality increases.

In this study the stream was divided into ten transects. The transect width is one meter(along the stream length). The transect lenght is depends on the stream width.(Figure 16). By this measurement, the stream width was recorded. After that, the structure of the stream, and riparian vegetation is drawn. Then, the scores of three types of parameters at each transect are recorded by visual evaluation. All parameters are evaluated for each stream site. Finally, the column total score are combined together. The scores increase as habitat quality increase.

3.2 PHYSICAL CHARACTERIZATION

The physical characterization indicates the structure of the stream sites. There are many kinds of measurement possible. In this study , the stream depth, width and velocity were measured. The percent distribution of organic and inorganic substrate components are included at each stream site.

3.3 THE HABITAT ORIENTATION OF THE MACROINVERTEBRATES FOUND

The families found at each stream site should be affected by the habitat quality of each site. The families that were present and absent from each habitat were determined.

3.4 THE HABITA QUALITY AND BIOLOGICAL CONDITION

The relationship between habitat quality and biological condition

(macroinvertebrates community condition) can be presented as a sigmoid curve with community response varying with habitat quality. In the upper segment of the curve, good quality habitat(supporting or excellent) will support high quality communites. The relationship can be quantified and subsequently used for discriminating water quality.

4. DATA ANALYSIS

The number of macroinvertebrate families at each sites was counted and analysed. Cluster analysis from the SPSS programme was used to classify the different running water sites based on their physico - chemical properties and benthos macroinvertebrate composition.

Cluster analysis is a classification technique for placing similar samples, objects or sites into groups or "clusters"(Ludwig and Reynolds, 1988) which are arranged in a hierarchical treelike structure called a dendrogram.

Cluster analysis can be either agglomerative or divisive. In agglomerative hierarchical clustering, clusters until all units are members of a single cluster.

The dendrogram is another way of visually representing the steps in a hierarchical clustering solution. The dendrogram shows the clusters being combined and values of the coefficients at each step. The dendrogram produced by the SPSS CLUSTER procedure does not plot actual distances but rescales them to numbers.However, the relationship between steps is preserved. The scale displayed at the top of the figure does not correspond to actual distance values.