CHAPTER 5

DISCUSSION

Mayfly larvae have been regarded as useful indicator organisms in monitoring the quality of the aquatic environments (Fremling, 1989). Very little is known about the use of macrobenthic invertebrates for bioindication purposes in the Asian region. According to Azrina *et al.* (2006), perhaps, owing to the lack of expertise and information, the DOE of Malaysia have not yet employed macrobenthic invertebrates as bioindicators of pollution for river pollution studies.

In Malaysia, mayfly nymphs have been in-cooperated as a freshwater biomonitoring tool in rivers of Bario, Kelabit Highlands, Sarawak (Shabdin and Abang, 1999), Linggi River in Negeri Sembilan (Omar *et al*., 2002), Crocker Range National Park in Sabah (Shabdin *et al*., 2002), Langat River, Selangor (Azrina *et al*., 2006), Semenyih River, Selangor (Yap *et al*., 2003) and Telipok River in Sabah (Kamsia *et al*., 2007).

However, little information was available on mayfly nymphs in Ulu Gombak Forest Reserve, especially on water quality and using these macroinvertebrates as an indicator. The area along Sg. Gombak (downstream) was rather exploited due to the activities carried out by students and individuals from the University of Malaya Field Studies Centre of the Ulu Gombak. This particular establishment has been used extensively as a research centre for researchers conducting biological and ecological studies and also as a training ground for students to experience their first fieldwork practice. Another sampling station, Anak Sg. Gombak flows along the Orang Asli settlement (Kampung Orang Asli Ulu Kemensah). Throughout the study period, villagers were frequently seen using the water body for bathing, laundry and even washing motorcycles. Such environment disturbance may have affected and reduce the water quality of the streams, thus impacting the data accuracy of the present study.

The present study is the first of its type of study in the area. Therefore, it is difficult to assess whether the diversity of the mayfly nymphs in the selected streams is increasing or decreasing. Thus, it is suggested that the area under the study should be continuously monitored to observe any changes in the diversity of mayfly nymphs, because the changes in the diversity can only be observed through continuous monitoring and comparing the data of every year.

The calculated values of Shannon's Diversity Index (H') ranged from 1.12 to 1.31 indicating low diversity. The value is lower than the range 1.5 to 3.5 (Margalef, 1972). Simpson's Index emphasises on the more abundant species in a sample and denoted by D. Simpson's Index is usually expressed as 1-D or 1/D (Sanders, 1978). Simpson's Index of Dominance, D ranged from 0.38 to 0.30. High dominance tendency among occurring ephemeropteran nymphs species were assumed as D is more than 0.25 (Stone & Pence, 1978; Yanez & Canaris, 1988). Nymphs in Sg. Batu 19 were found to have the highest dominance tendency (0.38). As D increases diversity of the nymphs community decreases (Table 2). Simpson's Index of Diversity ranges from 0.62 to 0.70, indicating high diversity with the highest diversity in Sg. Gombak and Anak Sg. Gombak, similarly to Shannon's Diversity Index (H'). Simpson's Index of Evenness, E_s ranges from 0.51 to 0.69, showing low equitability of ephemeropteran nymphs. These calculated values however contradict with those of Shannon's Diversity Index. Peet (1974) suggested that Simpson's index is of a type that is more sensitive to the common species in a sample.

Many studies have shown that changes in benthos community have good correlations with the water quality changes (Hellawell, 1986; Rosenberg and Resh, 1992; Mason, 1996). Results from the present study indicated that the abundance of the ephemeropteran nymphs appeared to respond to the water quality of the sampling stations. *Thalerosphyrus* sp. and *Campsoneuria* sp. from the Heptageniidae family were found to occur together in all three sites, similar to that described by Bishop (1973).

High abundance of mayfly nymphs at the upstream was associated with good quality water body conditions, while a lower abundance often signified environmental stress due to human activities. The poorer water quality at the downstream than at the upstream could be attributable to several man-induced activities such as mentioned earlier. Some species showed selective distribution by being found only at specific stations. This may be due to the variations in biotype or water quality gradient. A restricted distribution as indicated here could be used to characterise pollution tolerance.

Dissolved oxygen concentration can be an important factor affecting the distribution of species of aquatic insects (Ward, 1992). The amount of oxygen dissolved in the waters of Sg. Batu 19 was elevated by the lower temperature of the water (Manahan, 1991). The high water flows in Sg. Batu 19 also provides more oxygen to dissolve into the water. Kolar and Rahel (1993) established that nymphs of *Callibaetis montanus* cannot tolerate hypoxic conditions. One of the factors that may have contributed to the greater abundance of nymphs on sections of large rocks and boulders embedded in streams is higher dissolved oxygen concentration at the surface. Samways *et al.* (1996) reported that these nymphs occur near the water surface, to avoid the anoxic conditions and low temperatures of the bottom. This corresponds with the observation at streams in Ulu Gombak Forest Reserve, where *Baetis* sp. nymphs were mainly found clinging to the rocks and boulders at the streams. The ephemeropteran nymphs were found to be most abundant at the study site with the highest mean of dissolved oxygen, however, statistical data showed a significant negative correlation between dissolved oxygen measurements and ephemeropteran nymphs (Table 4.4).

Ephemeropteran nymphs are known to be sensitive to acidification, exhibiting reduced density, diversity or richness at sites with low pH (Allard and Moreau, 1987). *Baetis rhodani* and *Leptophlebia marginata* are among the species most investigated (Gerhardt, 1990). The disappearance of Ephemeroptera from acidified rivers was attributed to higher mortality at emergence (Bell, 1971), to inhibition of oviposition in acidic waters (Sutcliffe and Carrick, 1973) or to the avoidance (drifting or mortality) of acidic waters by early instars (Allard and Moreau, 1987). Pearson's correlation coefficient indicating a statistically significant linear relationship ($p \leq 0.05$) between *Baetis* sp. and pH from all three sampling station in this study is comparable to other studies. Smith *et al.* (1991) compared benthic invertebrate parameters between three streams of differing acidity in the Adirondack Mountains, New York, in an area heavily impacted by atmospheric acid inputs. Although the total invertebrate density was not significantly lower at the acidic site compared with the most other sites, Ephemeroptera was absent at the acidic site. *Baetis* and certain heptageniid mayflies (*Cinygmula*) were only found in streams with higher pH values (annual minimum >5.4). *Baetis* is generally reported to be intolerant of low pH values (Fiance, 1978).

Other abiotic elements such as illuminance, nitrate and ammonical nitrogen were measured, yet no correlation between these factors and number of nymphs and abundance were evident. Further study is needed to assess these relations. However the amount of ammonical nitrogen samples collected were within the World Health Organization (WHO, 1984) and Interim National Water Quality Standards (DOE, 1994) safety range.

There is little study regarding the water quality of streams in Ulu Gombak Forest Reserve. The constant physico-chemical parameters such as water temperature, conductivity and nitrate (Table 5.1) measured throughout this study were found to be in acceptable levels as compared to the criteria and standards in the Interim National Water Quality Standards for Malaysia (DOE, 1994). The lowest and highest measurement for pH indicated the water to be in Class II – III according to Interim National Water Quality Standards (DOE, 1994). The INWQS threshold range of pH for Malaysian rivers is 5.00 to 9.00 (DOE, 1994). The class categories for the environmental parameter along the three sampling stations varied from Class I to Class IV.

Due to the small sample size of this study, the result obtained was quite conservative but generally it suggested an acceptable overall rating for all three streams, classified as Class II and Class III if justified by median value of each stream. Sg. Batu 19 was classified as Class II which represents water bodies of good quality. Most existing raw water supply sources come under this category. Both Sg. Gombak and Anak Sg. Gombak were classified as Class III. Class III is defined with the primary objective of protecting common and moderately tolerant aquatic species of economic value. Water under this classification may be used for water supply with extensive or advance treatment. This class of water is also defined to suit livestock drinking needs (DOE, 1994). This is in agreement with the report by the DOE (2001) that the rivers in Malaysia were generally clean at the upstream and were either slightly polluted or polluted due to urban wastes and agricultural activities at the downstream (Azrina *et al.*, 2006).

Table 5.1. Proposed classification of water quality at stations in Ulu Gombak Forest Reserve

Parameter	Sg. Gombak		Anak Sg. Gombak		Sg. Batu 19		INWQS	
	Range	Class	Range	Class	Range	Class	Range	Class
pH	5.2-8.5	III	5.4-8.8	III	6.5-8.9	II	6-9 5-9	II III
Water Temperature (^{0}C)	21.3-34.5	II	23.0-27.7	II	20.7-28.1	II	Normal ± 2^{0}C	II
Dissolved Oxygen (mg/L O_2)	1.2-7.4	II-IV	1.09-7.35	II-IV	1.2-7.5	II-IV	5-7 <3	II IV
Conductivity (µS)	37.9-42.8	I	42.3-1087.7	I	34.7-48.2	I	1000	I
Nitrate (mg/L)	0.3-0.8	I	0.3-1.3	I	0.1-0.6	I	Natural levels	I
Phosphate (mg/L)	0.3-0.8	>II	0.1-1.0	>II	0.1-0.4	>II	0.1	II
Ammonical nitrogen (mg/L)	0.2-0.9	III	0.1-1.5	II-IV	0.1-0.3	II	0.1-0.3 0.3-0.9 0.9-2.7	II III IV
Overall Rating (Median Value)	III		III		II		-	

For further investigation the existing design of water quality can be adopted and modified to enhance accuracy in prediction and classification of the water bodies. Additional chemical and physical parameters, such as biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) can be recommended to provide a clearer relationship of the mayfly nymphs' population dynamics in the study area.

Parameters from the present study including the additional ones mentioned earlier may be evaluated according to the Water Quality Index (WQI) established by the DOE, Malaysia to provide a more conclusive classification of the streams at the study area. Continuous monitoring of the streams will also provide an indication of the long term water quality status and the appropriate category of water use of these streams.

Aquatic macroinvertebrates are commonly used in biological monitoring programs, but their use in biomarker studies has been limited. Studies have been conducted on acute toxicity of aqueous copper, cadmium, and zinc to *Rhithrogena hageni* nymphs from the Cache la Poudre River in Larimer County, CO, USA (Brinkman and Johnston, 2007), primary stressors impacting *Procloeon* sp. and other macroinvertebrates from the Salinas River in California, USA (Anderson *et al.*, 2006), discriminatory power of *Baetis rhodani* and *Baetis vernus* from Uper Silesia, Poland (Fialkowski and Rainbow, 2006), copper tolerances of *Adenophlebia auriculata* from Bergr/Palmiet River in Grahamstown, South Africa (Gerhardt and Palmer, 1998), distribution of heavy metal in *Hexagenia rigida* (Inza *et al.*, 2001), short term toxicity of iron (Fe) and lead (Pb) to *Leptophlebiu marginata* (Gerhardt, 1994) and *Baetis rhodani* and *Baetis vernus* nymph as biomonitors of trace metal pollution in Upper Silesia, Poland (Fialkowski *et al.*, 2003). However, there was no mayfly toxicity data found from any Malaysian publication, indicating the use of aquatic macroinvertebrates in biomarker studies for the purpose of river classification seems to be less widespread in Malaysia.

The biochemical part of the present study was designed as a short-term experiment under controlled laboratory conditions, to demonstrate toxic effects of selected inhibitor compounds on non-specific esterase, acetylcholinesterase (AChE) and glutathione-s-transferases enzyme activities and inhibition of *Baetis* sp. and *Campsoneuria* sp. Specific activity is a measure of enzyme purity. The value becomes larger as an enzyme preparation becomes more pure, since the amount of protein is typically less, but the rate of reaction stays the same or may increase due to reduced interference or removal of inhibitors) (Nelson & Cox, 2000). The enzymes specific activities were also investigated to identify suitable biomarkers for screening the biochemical level response of chemical exposure and thus applicable for *in situ* ecotoxicity monitoring. The measurement of specific activities of these enzymes in aquatic macroinvertebrates could be used as a biomarker of susceptibility to toxicity that could be linked to changes in population densities.

Several studies have used esterase and acetylcholinesterase inhibition to evaluate the biological impact of organophosphate and carbamate pesticides (Fourcy *et al*., 2002) or heavy metals (Dellali *et al*., 2001). *In vitro* inhibition of non-specific esterase activity shows that based on the I_{50} levels, esterases from *Campsoneuria* sp. were more sensitive to inhibition than those from *Baetis* sp. The most potent of the group of inhibitors are organophosphate pesticides while the most powerful single substance was Dichlorvos (DDVP). Copper and lead were less potent inhibitors.

Acetylcholinesterase is of interest because it is the target site nervous system, and its role in cholinergic synapses is essential for life (Fournier and Mutero, 1994), which maybe very useful in estimating the safety of river based on inhibition of AChE in mayfly nymph. Based on the values of I_{50}s and Ki (bimolecular rate constants for AChE inhibition), *Campsoneuria* sp.'s AChE was more sensitive to the inhibitory action of all tested inhibitors than that of *Baetis* sp. Dichlorvos (DDVP) exhibited high inhibition potency against both *Baetis* sp. and *Campsoneuria* sp. AChE, where I_{50} values were less than 1 nM. Organophosphates reduce AChE activity by reacting with a specific serine within the catalytic center of the enzyme to produce *o,o*-dialkyl phosphoserine. In this form, the enzyme is unable to hydrolyze choline esters (Kennedy, 1991).

In contrast with AChE, measurement of GST activity in *Baetis* sp. and *Campsoneuria* sp. does not appear to be sufficiently sensitive to be of practical use and may be influenced by stress that is not related to toxicant exposure (Callaghan *et al*., 2002). Few studies have been carried out on chemically induced levels of GST in freshwater invertebrates, but its responsiveness to organic xenobiotics is very low (Blat *et al*., 1988; Boryslawskyj *et al*., 1988). Therefore, because of the lack of specificity and qualitative response of some of the detoxication enzymes to contaminants, research is needed before they can be considered for use as biomarkers (Hyne and Maher, 2003).

From our results, we can deduce that ephemeropteran nymphs, namely *Campsoneuria* sp. and *Baetis* sp. are good indicators of river environmental quality, both with regards to the biological quality measured by several metrics, and the chemical quality of the water. The results also suggest that the biochemical parameters that were studied, such as the non-specific esterase, acetylcholinesterase and glutathione-s-transferases activity, may have considerable potential as an early warning signal of chemical stress in *Campsoneuria* sp. and *Baetis* sp.

Taking into account the ecological importance of ephemeropteran in the aquatic ecosystem, this study on the effects of pesticides and heavy metal on *Baetis* sp. and *Campsoneuria* sp. can provide information that may prove to be useful in determining the potential ecological consequences of these compounds.