CHAPTER 1

INTRODUCTION

1.1 The origin of mayfly

Mayflies belong to the order Ephemeroptera (in Greek: *ephemeros* = living for a day; *ptera* = wings) (Khoo, 2004). This is a reference to the short lifespan of most adult mayflies. Because of the brief duration of the adult stage, mayflies are frequently characterized in biodemography (Carey, 2001) and gerontology (Finch, 1990) literature as the quintessence of a short-lived organism with the life span of adult usually reported as 1-2 days. Mayflies are common insects found in almost all freshwater habitats, as well as some brackish ones. According to Elliott and Humpesch (1983), there are over 2,000 named species in 200 genera and 19 families. They are considered to be part of the clade Uniramia which includes silverfish and dragonflies, among others. Ephemeroptera and Odonata are the only extant orders of winged insects in the infraclass Paleoptera. All other insects with wings are in the Neoptera, and are characterized by a wing articulation (joint) that allows them to fold their wings back over their abdomens at rest (Carpenter, 1992).

1.2 The anatomy and life cycle of mayfly

Ephemeroptera are aquatic insects that often go through many nymph stages (living in water) and two flying stages (the subimago and the imago). Mayfly nymphs can easily be distinguished from nymphs of other aquatic exopterygote insects by an unsegmented tarsus, bearing usually a single tarsal claw at the end, a mesothorax that is larger than the prothorax or the metathorax, a ten-segmented abdomen bearing three caudal filaments (occasionally the median filament is very reduced or absent) and the presence of abdominal gills in varying combinations between segments 1 to 7 (Khoo, 2004). When the nymphs hatch from the eggs, they are less than 1 mm long, have no gills at first and their body shape varies according to habitat. For example, those that burrow (such as *Ephemera*) have more cylindrical bodies, whereas those that slide under rocks (such as *Heptagenia*) are flatter. Those in the genus *Caenis* crawl on mossy stones and vegetation, so they have short bodies with squat legs (Riek, 1973). Ephemeropteran nymphs may grow between 4 mm to 3 cm long. They are generally camouflaged against their background. The number of molts a nymph goes through on its way to becoming an adult does not depend on its nutrition, but the increase in size that comes with each molt does (Harker, 1989) (Figure 1.1).

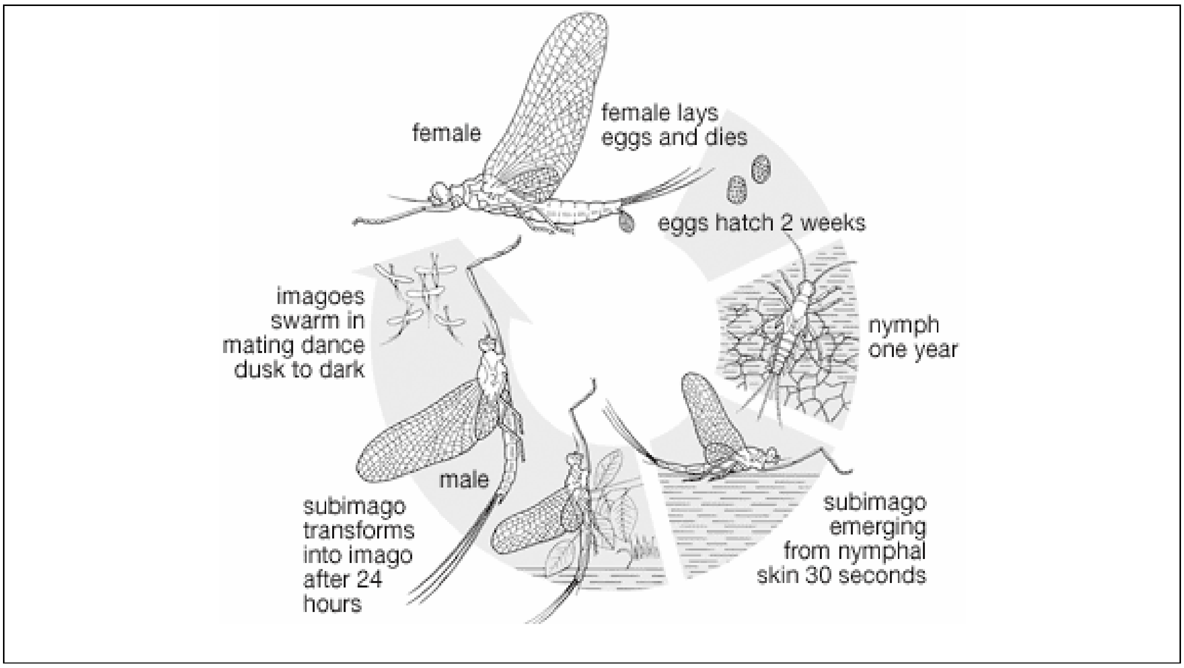

Figure 1.1. Life cycle of the mayfly (Ephemeroptera) (Source: Encyclopedia Britannica, 2008)

1.3 Habitat of mayfly

Mayfly nymphs occur in a variety of aquatic habitats ranging from standing to running waters. They are absent in severely polluted waters but they are very common in running waters especially in hill streams where the oxygen concentration is high due to the relatively low temperature and water turbulence. Mayfly nymphs usually live in crevices among stones and gravel at the stream bottom or among trailing roots of plants along the sides of streams (Khoo, 2004).

1.4 Diversity of mayfly nymphs

Diversity is the central theme of ecology and its measures are frequently seen as indicators of the wellbeing of the ecological system. A co-efficient of diversity is a convenient way of demonstrating the variety of species present in a habitat or a sample and abundance of individuals within the species. The measure of diversity of the fauna will represent the number and the available niches present in the environment. If niche heterogeneity is great, it will support a more diverse fauna and thus will result in a higher co-efficient or index of diversity (May, 1975). Diversity indices may provide a good measure of community structure and changes that correlate with both its richness and evenness values (Omar *et al.*, 2002). Bishop (1973) described the mayfly zoome of Gombak as remarkably diverse for a small river, a function of the wide spectrum of microhabitats available.

1.5 Aquatic system as ecology indicator

Over the past decade, biodiversity has become an important focus of scientific research and a foundation for conservation policy and practice. Today, surveys of biodiversity are useful tools in the identification and assessment of environmental degradation. Measures of diversity made in closely associated sites can provide an indication of relative health and stability of local ecosystems. Biological systems are sensitive to pollution and environmental stress and this sensitivity can be used for the biological monitoring of the environment (Munawar *et al.*, 1995). Aquatic systems are particularly important as early indicators of ecological decline. One group of aquatic organisms that exhibits measurable variation in diversity, and is easy to sample, is the community of benthic macroinvertebrates. As biological monitoring subjects, benthic macroinvertebrates have a number of advantages (Barbour *et al.*, 1999): macroinvertebrate assemblages are good indicators of localized conditions, they integrate the effects of short-term environmental variations, they are made up of species that constitute a broad range of trophic levels and pollution tolerances, thus providing strong information for interpreting cumulative effects, they are abundant in most streams and are relatively easy to identify to family (many taxa intolerant of human-induced stresses can be identified to lower taxonomic levels with relative ease), sampling is relatively easy and has minimal detrimental effect on the resident biota. The census of these species has been practiced to the point that indices of water quality based on the presence or absence of pollution tolerant taxa are commonly calculated (Moody *et al.*, 2000).

1.6 Pollution and pesticide

Contaminants in aquatic ecosystems, such as heavy metals, polychlorinated biphenyls and organochlorine pesticides, have become a matter of concern because of their toxicity and tendency to accumulate in food chains (Marcotrigiano and Storelli, 2003). Surface water bodies are contaminated with many anthropogenic toxic chemicals that can affect their natural communities. Among the anthropogenic chemicals, pesticides may cause the most serious problems because they are designed specially to kill organisms (both the noxious target organisms and other non-target ones) and they are released into the natural environment intentionally (Hanazato, 2001).

Pesticides include insecticides, herbicides, fungicides, molluscides and nematicides (Hayes, 1975) and they are non-biodegradable and accumulate in the food chain. Mostly they are prone to affect the nervous system causing tumors in living organisms. They are not only neurotoxic but also affect other systems and have shown a high degree of impact on metabolism by inhibiting enzymes like acetyl cholinesterase (O’Brien, 1967; Matsumura, 1975). It has been widely documented that pesticide concentrations in the natural environment are often high enough to kill certain organisms (Hatakeyama *et al.*, 1991, 1994) and affect the structure and function of natural communities (Helgen *et al.*, 1988; Hatakeyama *et al.*, 1990). Elevated levels of organochlorine compounds such as Dichloro-Diphenyl-Trichloroethane (DDT) and polychlorinated biphenyls (PCBs) are toxic to all animals and can cause bioaccumulation in tissue, cause tumors, and cause hormonal and behavioral problems. They can also suppress the immune and respiratory systems and cause abnormal development in aquatic species. The primary effect on aquatic communities is reduction of numbers of sensitive species, allowing species that are more resistant to contaminants to become dominant (Harte *et al.*, 1991). Pesticides exert their impacts at multiple levels - including molecules, tissues, organs, individuals, populations and communities - and a variety of ecotoxicological tests have been designed to assess these effects (Cairns and Niederlehner, 1985).

Pesticides used in the present study are Dichlorvos (DDVP), Malathion and Fenitrothion. DDVP is a very volatile organophosphate, evaporating quickly but with acute toxicity and quick knockdown. Acting as a cholinesterase inhibitor on insects that it contacts, DDVP attacks the nervous system of the target organism blocking the enzyme required for proper nerve functioning (Bennett, 2001).

Malathion is an organophosphorus pesticide, widely used for both domestic and commercial agricultural purposes. It is considered to be one of the safest organophosphate insecticides (EXTOXNET, 1995). Various aquatic invertebrates are extremely sensitive, with EC50 values from 1 ug/L to 1 mg/L (Menzie, 1980). Malathion is highly toxic to aquatic invertebrates and to the aquatic stages of amphibians. Because of its very short half-life, Malathion is not expected to bioconcentrate in aquatic organisms.

Fenitrothion is a broad spectrum, non-systemic organophosphate insecticide that is used for the control of insect pests such as grasshoppers, locusts, weevils, beetles, moths, mealworms and grubs. It is used on rice, cereals, pastures, fruits, vegetables, stored grain, and on structures such as flour mills and poultry houses. Like other organophosphate pesticides, fenitrothion acts by inhibiting the activity of the enzyme acetylcholinesterase, which is important in the conduction of impulses to nerves and muscles (APVMA, 2004).

When fenitrothion concentration in streams was up to 6.4 mg/L after an aerial spraying in New Brunswick (Eidt and Sundaram, 1975), mortality of Ephemeroptera (*Baetis* spp.) and Plecoptera (*Leuctra* spp., *Amphinemoura* spp.) was high. The dead component of the drift increased from 26% to 90% (Heliövaara and Väisänen, 1993).

1.7 Heavy metal

The term heavy metal is a general collective term applying to the group of metals and metalloids with an atomic density greater than 6 gcm^{-3} (Alloway and Ayres, 1993). However it is generally used to refer to elements that are commonly associated with pollution and toxicity problems. Metals are continuously released into aquatic systems as a result of the natural weathering of rocks and soils, and by human activities such as mining, industries and agriculture, as well as the release of sewage (Depledge *et al*., 1994). Most of the micronutrients, for example copper, zinc and nickel owe their essentiality to being constituents of enzymes and other important proteins involved in key metabolic pathways. Hence, a deficient supply of the micronutrient will result in a shortage of the enzyme that leads to metabolic dysfunction causing disease (Alloway and Ayres, 1993).

All metals are, however, toxic to aquatic organisms when present at elevated levels, causing direct or indirect effects such as histological damage, or a reduction in the survival, growth and reproduction of the species it influences (Heath, 1987). Non-essential metals such as cadmium, lead, mercury, and silver also can accumulate in the tissues of aquatic organisms and cause adverse biological impacts in aquatic organisms (Lau *et al*., 1998). Elevated levels of arsenic, cadmium, lead, mercury, and zinc can affect the nervous, respiratory, circulatory, and reproductive systems of aquatic organisms, as well as affect their development and feeding habits (Rand and Petrocelli, 1985).

According to Moore and Ramamoorthy (1984) the discharge of heavy metal wastes into waters may result in numerous chemical, physical and biological responses, namely two main categories: effects of the environment on the metal and the effect of the metal on the environment. The first category emphasises that conditions in receiving waters may lead to a change in the speciation and toxicity of the metal (Moore and Ramamoorthy, 1984), and consequently the bioavailability of the metals to aquatic organisms (Abel, 1989). Metals exist in water in equilibrium between free metal ions, metal bound in inorganic and organic complexes, and metal bound to organic and inorganic particulate matter. The chemical composition of the water strongly influences the speciation of metals (Calow, 1994).

Biological responses under the second category are often equally diverse. Depending on environmental conditions, there may be direct or indirect effects such as histological damage; or a reduction in the survival, growth and reproduction of the species that were influenced (Heath, 1987; Timmermans, 1993; Calow, 1994). There may also be a change in population density, diversity, community structure, and species composition of populations (Moore and Ramamoorthy, 1984).

1.8 Insects and trace metals

Insects represent one of the most common and diverse groups of freshwater animals known to readily accumulate trace metals (Hare, 1992). Trace metals, even essential ones, are toxic when present above threshold availabilities, and may pose a significant threat to the biota streams affected by mining and industrial run-off (Rehfeldt and Söchtig, 1991; Gower *et al*., 1994). The use of aquatic organisms, particularly benthic invertebrates, as biomonitors of the local availabilities of potentially toxic trace metals has become increasingly widespread (Cain *et al*., 1992; Hare, 1992; Phillips and Rainbow, 1993). Benthic deposit-feeder larval stages of aquatic insects are of particular interest because they can accumulate metals via two potential routes: via the gut wall from fine-grained sediment consumed as food and via respiratory surfaces from the ambient water (Saouter *et al.*, 1991a, 1993; Hare *et al.*, 1991; Odin *et al.*, 1994, 1995a, b; Andres *et al.*, 1998).

1.9 Biomarker

According to McCarthy and Shugart (1990), biochemical biomarkers are increasingly used in ecological risk assessment of aquatic ecosystems to identify the incidence of exposure to, and effects caused by, xenobiotics such as pesticides because of their potential as rapid early warning systems of potentially damaging effects at higher levels of biological organization. Biomarkers were originally defined as any biochemical, histological, or physiological alterations or manifestations of environmental stress (NRC, 1987). Peakall and Shugart, 1993 have classified them as biomarkers of exposure to a toxicant, biomarkers of effects of exposure, or biomarkers of susceptibility to the effects of exposure. This definition has been challenged by several authors (Adams, 1990; Engel and Vaughan, 1996; McCarty and Munkittrick, 1996) and the term biomarker is now more commonly used in a more restrictive sense, namely biochemical sublethal changes resulting from individual exposure to xenobiotics (Hyne and Maher, 2003). A working definition of 'biochemical biomarker' is 'biochemical sublethal changes resulting from individual exposure to xenobiotics' (Lagadic *et al.*, 1994).

1.10 Acetylcholinesterase (AChE)

Two such biochemical biomarkers are acetylcholinesterase (AChE) and glutathione-s-transferase (GST). The inhibition of AChE by neurotoxic compounds has been widely used as a sensitive biomarker in wildlife and humans (Crane *et al.*, 1995; Thompson, 1991). This enzyme is important in nerve transmission; nerve impulses travel down the presynaptic cholinergic nerve axon, provoking the release of acetylcholine (ACh), which then crosses the synpactic cleft and binds to the acetylcholine receptor (AChR) triggering excitation of the post-synpatic neuron (Schriever *et al.*, 2008). AChE terminates the process by breaking down ACh into acetate and choline (Schriever *et al.*, 2008).

AChE is inhibited by phosphate and carbamate esters that are commonly used as insecticides (Crane *et al.*, 2002). These insecticides bind the enzyme, leading to the accumulation of acetylcholine in the synapse, resulting in the disruption of normal nervous system function (Habig and DiGiulio, 1991). Since this reaction is substantially irreversible for many pesticides percentage inhibition of acetylcholinesterase activity can be used as an indicator of exposure of an organism to organophosphorus pesticides for a considerable period after the contaminant itself is metabolized or eliminated from the organism's body (Hyne and Maher, 2003). Sibley *et al.* (2000) demonstrated in a microcosm study that AChE activity could be used as a reliable biomarker of exposure and mortality at the individual organism level and had the potential to predict responses at the population level for zooplankton.

1.11 Glutathione-s-transferases (GST)

GSTs are a diverse group of enzymes with widely differing specificities and are well characterised in insects, because they have been shown to be responsible for insecticide resistance (Wang *et al.*, 1991; Lagadic *et al.*, 1993; Usui *et al.*, 1997; Willoughby *et al.*, 2006). GST is a family of detoxicating enzymes that catalyze the conjugation of reduced glutathione (GSH) with a group of compounds having electrophilic centres (Crane *et al.*, 2002). These can include nitrocompounds (Usui *et al.*, 1977), organophosphates (Motoyama and Dauterman, 1977; Usui *et al.*, 1977), and organochlorines (Clark *et al.*, 1986). The GSH conjugation products become less toxic and more water soluble so that they can be easily excreted from cells after further metabolism (Crane *et al.*, 2002).

1.12 Mayfly nymphs as bioindicators

Since the 1970s, many toxicological studies have been done using mayfly nymphs (Wielgolaski, 1975; Van Wijngaarden, 1993; Admiraal *et al.*, 2000; Fialkowski *et al.*, 2003). *Hexagenia limbuta* has been used since the late 1970s in sediment toxicity evaluations and is sensitive to the presence of toxicants, both in laboratory and field surveys (Giesy *et al.*, 1990; Burton, 1992). Although these aquatic invertebrates have been routinely used as biological indicators, biochemical markers in ephemeropteran have been largely neglected. It has been proposed that metabolic pathways and reactions that allow aquatic invertebrates to persist under adverse environmental conditions can be considered as biomarkers to characterize either the exposure situation itself or the effects induced by stresses (Triebskorn *et al.*, 2002). In a review of metal bioaccumulation by freshwater invertebrates of different feeding guilds, Goodyear and McNeil (1999) concluded that concentrations of cadmium, copper, lead and zinc in mayfly larvae are directly proportional to those in sediments, reflecting their ability to reflect ambient metal availabilities in accumulated body concentrations.

1.13 Objectives and rationale of the study

The objectives of this thesis are as the following:

- To present an analysis and results of a 12-month study that generated an average abundance and diversity indices of mayfly nymphs from selected streams at the Ulu Gombak Forest Reserve.

- To assess water quality and abiotic parameters at the study area. The physical-chemical data obtained were further used for water quality classification of streams in comparison with the Interim Water Quality Standards for Malaysia (INWQS).
- The environmental parameters were also used to evaluate the potential of mayfly nymphs and parameter correlation. This part of the study was designed to see whether interactions between the abundance of mayfly nymphs and environmental parameters might enhance or reduce the effects of the latter.
- To expose nymphs of *Baetis* sp. and *Campsoneuria* sp. to various organophosphate pesticides and heavy metal, measure and investigate subsequent effects on biochemical biomarkers.

Adaptation of this study will be able to provide general guidance in the development of a water quality monitoring plan for any particular water body in Malaysia. The presence of enzymatic biomarkers in mayfly nymphs could be useful in surveying water quality index of a particular aquatic area as well to detect the presences of heavy metal.