ABUNDANCE AND DIVERSITY OF EPHEMEROPTERA, PLECOPTERA, TRICHOPTERA (INSECTA) IN RELATION TO ENVIRONMENTAL QUALITY OF UPSTREAM RIVERS IN KEDAH, MALAYSIA

by

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Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

July 2011

ACKNOWLEDGEMENT

I sincerely thank the Universiti Sains Malaysia and Ministry of Higher Education Malaysia for financial support (ASTS under SLAI program), with which the completion of my Ph.D. work is possible.

I would like to express my hearty gratitude to my supervisor, Prof. Che Salmah Md Rawi for choosing me as a Ph.D. candidate, her excellent supervision, valuable suggestion and support, great patience and encouragement during the conduction of this work. I am thankful to Prof. Donald C. Jackson from Misssissippi State University, Mississippi, USA for consenting to be the co-supervisor.

I express my thanks to my understanding and supportive husband, Adi bin Abdullah for his permission and courage, not forgotten my precious jewels of my life that illuminate every breath I take through their very existence. I am often moved and encouraged by their love, intelligence, diligence and kindness.

For generously sharing their wisdom, love and divinity, I pay homage to my amazing family whom is always by my side and whose love and support knows no limits; mother, Abidah Abd Wahab, father, Ab. Hamid Sulaiman, my siblings, parents and family in-law, nephew and niece.

I am also grateful to Dr. Hamady Dieng for his valuable guidance, many helpful ideas and constructive discussions, and Prof. Abu Hassan Ahmad as the dean of the School of Biological Sciences. Not forgotten, those who had a touch of life in my research world: Prof. Yeon Jae Bae (Seoul, Korea), Prof. Richard L. Brown (Mississippi, USA), Prof. Bill P. Stark (Mississippi, USA) and Dr. Rodolfo Novelo Gutteirez (Veracruz, Mexico).

Thanks must be given to Salman, Kumara, Nur Aida, Hazzeman and Huda for their friendly behavior and help in some way or another in all phases of my work. Special acknowledgements are given to the valuable technical assistance in laboratory work of School of Biological Sciences staff; Pn. Siti Khadijah, En. Muthu, En. Shukri, En. Mohd Hafizul, En. Nordin and En. Hadzri.

Gratitude is also extended to all other colleagues: aquatic lab members. Adibah, Wan, Rina, Zul, Aiman, Yus, Shafiq, Mohd Rasdi, Hamaseh, Madziatul, Amelia and many others who could not be mentioned here for their readiness to help and creating a friendly atmosphere during my studying at the aquatic entomology lab, School of Biological Sciences, USM. Last but not least to Zarul Hazrin and Russell Barabe for lending a hand during my stay in Mississippi, USA for attachment program.

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LIST OF ABBREVIATIONS

Abbreviation	Caption
BOD	Biochemical Oxygen Demand
CCA	Canonical Correspondence Analysis
COD	Chemical Oxygen Demand
CPOM	Coarse particulate organic matter
DO	Dissolved oxygen
DOE	Department of Environment
EPT	Ephemeroptera, Plecoptera and Trichoptera
FFG	Functional feeding group
FPOM	Fine particulate organic matter
GJFR	Gunung Jerai Forest Reserve
ISI	Important Species Index
NH_4-N	Ammonia-nitrogen
PCA	Principal Component Analysis
SOM	Self-Organizing Mapping
TSS	Total Suspended Solid
WQI	Water Quality Index

LIST OF PUBLICATIONS

1) **Suhaila, A.H.** and Che Salmah, M.R. (2008). Water Quality of Three Recreational Rivers In Gunung Jerai Forest Reserve. Proceedings of 4th Life Sciences Postgraduate Conference in 2nd USM Penang International Postgraduate Convention. Universiti Sains Malaysia, Penang. Malaysia. 18-20 June 2008.

2) **Suhaila, A.H**. and Che Salmah, M.R. (2008). Effects of Embeddedness and Canopy Cover on EPT Diversity and Abundance at Three Rivers From Gunung Jerai, Kedah, Northern Peninsular Malaysia. Proceedings of XXIII International Congress of Entomology (ICE 2008). International Convention Centre, Durban, South Africa. 6-12 July 2008.

3) **Suhaila, A.H**. and Che Salmah, M.R. (2009). Diversity and Abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) Immature with Implication To Water Quality of Rivers from Gunung Jerai Forest Reserve, Kedah, Malaysia. Proceedings of Malaysia Biological Simposium 2009. Equatorial Hotel, Bangi, Selangor. 17-18 November 2009.

4) **Suhaila, A.H**. and Che Salmah, M.R. (2010). Temporal Variation of Adult Ephemeroptera, Plecoptera And Trichoptera Collected in Tupah River, Kedah, Northern Peninsular Malaysia. Proceedings of The 7th IMT-GT Uninet and the 3rd Joint International PSU-UNS Conferences. Prince of Songkla University, Hat Yai, Songkhla, Thailand. 7-8 October 2010.

5) **Suhaila, A.H.** and Che Salmah, M.R. (2010). Population Dynamic and Life History of *Thalerospyrus* spp. (Ephemeroptera: Heptageniidae) at Two Rivers in Gunung Jerai Forest Reserve of North Peninsula Malaysia. Proceedings of International Conference on Wetland Ecosystem Services 2010. Charoenthani Princess Hotel, Khon Kaen, Thailand. 17-21 November 2010.

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8) **Suhaila, A.H.** and Che Salmah, M.R. (2011). Aquatic Insects Assemblages in Relation to Environmental Quality of Upstream Rivers in Kedah. Proceedings of 5th Biocolloquium School of Biological Sciences. Universiti Sains Malaysia, Penang. 22-23 June 2011.

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11) **Suhaila, A.H**., Che Salmah, M.R., Hamady Dieng, Abu Hassan, A., Tomomitsu, S. and Fumio, M. (2011). Seasonal Changes in Mayfly Communities and Abundance in Relation to Water Physicochemistry in Two Rivers at Different Elevations in Northern Peninsular Malaysia. *Wetland Science* (In press).

KELIMPAHAN DAN KEPELBAGAIAN EPHEMEROPTERA, PLECOPTERA DAN TRICHOPTERA (SERANGGA) DAN KAITANNYA DENGAN KUALITI PERSEKITARAN SUNGAI-SUNGAI TANAH TINGGI DI KEDAH, MALAYSIA

ABSTRAK

Sebanyak 17,315 individu daripada order Ephemeroptera, Plecoptera dan Trichoptera telah disampel dari Sungai Tupah, Batu Hampar dan Teroi, Hutan Simpan Gunung Jerai. Sepanjang persampelan bulanan bermula dari September 2007 hingga Ogos 2008 menggunakan teknik ‘kick-net sampling’, 29 genus daripada 19 famili EPT telah dicamkan. Struktur komuniti dan kepekaan khusus setiap genus EPT tidak dipengaruhi oleh kualiti air sungai (Indeks Kualiti Air – Kelas 1-II) yang mana lebih daripada 20 taxa telah disampel di setiap sungai. Kelimpahan EPT adalah tertinggi di Sungai Teroi namun kepelbagaiannya adalah terendah. Sungai Tupah pula merekodkan sebaliknya. Kelimpahan EPT tertinggi dicatatkan di Sungai Teroi (9,667) diikuti dengan Sungai Tupah (4,298) dan Sungai Batu Hampar (3,350). Nilai daripada Indeks Kekayaan taxa EPT (>10) menunjukkan kesemua sungai tidak tercemar dengan aktiviti manusia atau gangguan semulajadi. Biplot CCA menunjukkan taburan *Etrocorema, Lepidostoma, Hydropsyche*, *Diplectrona* dan *Chimarra* dipengaruhi oleh suhu air yang tinggi. Taburan *Cheumatopsyche* berkadaran dengan nilai pH yang tinggi sementara *Marilia* dan *Thalerospyrus* dengan nilai BOD yang tinggi. *Centroptilum*, *Rhyacophilia* dan *Platybaetis* cenderung kepada nilai COD yang rendah. Kelimpahan Plecoptera tidak dipengaruhi oleh perubahan musim tetapi kelimpahan Ephemeroptera adalah tinggi pada musim hujan manakala Trichoptera didapati mempunyai kelimpahan yang tinggi pada musim kering (z = -6.096, P = 0.000). Fungsi komuniti EPT di Sungai Tupah yang diukur dengan kadar pereputan daun, didapati lebih cepat pada dua spesis daun (28 hari) berbanding pada satu spesis daun (35 hari). Pada pek daun yang tidak dimasuki EPT, dua spesis dan satu spesis daun masing-masing mengambil masa 35 dan 42 hari untuk mereput sepenuhnya. EPT telah mengurangkan tempoh pereputan daun sebanyak tujuh hari pada kedua-dua pek daun. Berdasarkan taburan panjang badan, *Thalerospyrus* sp. (Ephemeroptera: Heptageniidae) mempunyai kitar hidup trivoltin pada altitud rendah seperti di Sungai Tupah dan Batu Hampar tetapi bivoltin pada altitud tinggi (Sungai Teroi). Perangkap cahaya untuk menangkap serangga dewasa EPT mencatatkan kelimpahan dan kepelbagaian Trichoptera adalah yang tertinggi di Sungai Tupah. Lapan famili dikenalpasti dengan kelimpahan tertinggi direkodkan daripada famili Hydropsychidae, Philopotamidae dan Leptoceridae. Dikalangan Ephemeroptera, famili Baetidae sering ditemui manakala famili Ephemerellidae pula jarang ditemui. Plecoptera hanya diwakili oleh dua famili iaitu Perlidae dan Nemouridae. Populasi serangga dewasa EPT memuncak pada bulan Mei, Jun dan Disember 2008 iaitu lebih tinggi pada musim kering (Januari-Julai 2008) berbanding pada musim hujan (Ogos-Disember 2008). Trichoptera didapati dominan pada musim kering namun Ephemeroptera lebih mendominasi pada musim hujan. Walaupun kesemua sungai ini terkenal sebagai kawasan rekreasi, air yang mengalir di sungai-sungai ini masih dianggap bersih dan mempunyai habitat yang kondusif serta sesuai untuk EPT.

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ABSTRACT

A relatively rich assemblage of Ephemeroptera, Plecoptera and Trichoptera (EPT) (17,315) (Insecta) immatures were collected from Tupah, Batu Hampar and Teroi rivers in the Gunung Jerai Forest Reserve. From monthly collections starting September 2007 until August 2008 using a kick-net sampling technique, 29 genera representing 19 families of EPT were identified. The EPT community structure and specific sensitivity of EPT genera were not influenced by river water quality (Class I - II of WQI) of which more than 20 taxa were collected from each river. The EPT was most abundant but less diverse in Teroi River while the reverse was recorded from Tupah River. The highest EPT abundance was recorded from Teroi River (9,667) followed by the Tupah River (4,298) and Batu Hampar River (3,350). High scores of EPT taxa richness (>10) indicated that all rivers were not impacted by human activities or natural disturbances. The CCA biplot showed that distribution of *Etrocorema*, *Lepidostoma*, *Hydropsyche*, *Diplectrona* and *Chimarra* were characterized by high temperature. *Cheumatopsyche* was regulated by high pH while *Marilia* and *Thalerospyrus* by high BOD. *Centroptilum*, *Rhyacophylia* and *Platybaetis* preferred low COD. The abundance of Plecoptera was not affected by seasonal changes but Ephemeroptera was more abundant in the wet season while more Trichoptera occurred during the dry season ($z = -6.096$, $P = 0.00$). EPT community function in Tupah River, measured as leaf breakdown rate, was faster in the two species leaf (28 days) than in single species leaf (35 days). In cages without these insects, the two-species leaf and single-species leaf

took 35 and 42 days respectively to completely decompose. The EPT reduced the decomposition time by 7 days in both leaf packs. Based on distribution of body length, *Thalerospyrus* sp. (Ephemeroptera: Heptageniidae) had a trivoltine life cycle at lower altitude in Tupah and Batu Hampar rivers but a bivoltine life cycle occurred at higher altitude in Teroi River. Light trapping of EPT adults showed that Trichoptera was the most abundant and diverse in Tupah River. Eight families were identified with high abundances of Hydropsychidae, Philopotamidae and Leptoceridae. Among the Ephemeroptera, Baetidae was very common while Ephemerellidae was rare. Plecoptera was only represented by two families, Perlidae and Nemouridae. Collectively, the populations of EPT adults peaked in May, June and December 2008 and their abundances were higher in the dry season (January-July 2008) compared to wet season (August-December 2008). Trichoptera was dominant during the dry season but more Ephemeroptera were collected during the wet season. Although all rivers were popular recreational areas, the water was considerably clean and together with conducive physical habitat, they were very suitable for the EPT.

CHAPTER 1

GENERAL INTRODUCTION

1.0 Introduction

Insects constitute a large proportion of local biodiversity particularly in tropical regions. Over one million insect species described, with approximately 30,000 species are aquatic, living in freshwater (Bonada *et al.*, 2006). Although there are 30-31 insect orders, only 13 orders have aquatic representatives (Merritt and Cummins, 1984; Merritt *et al.*, 2008) and majority of them are in freshwater communities. In the aquatic environment, aquatic insects are widely used in water quality assessment (Lenat, 1993).

Aquatic insects are excellent overall indicators of both recent and long-term environmental condition. According to Hodkinson and Jackson (2005), aquatic insects live almost continuously in the water and respond to all environmental stressors, including synergistic combinations of pollutants (acting together with greater total effect than the sum of their individual effects). Insects sensitive life stages will response quickly to environmental stress, endure the disturbance, adapt quickly or die and replaced by more tolerant species communities (Morse *et al.*, 2007). In most streams, lakes and rivers, insect larvae dominate the benthic macroinvertebrates community. As in the benthic macroinvertebrates assemblages, integration of structural or compositional and functional characteristics provides the best means of assessing impairment (Barbour *et al.*, 1999).

The utility of invertebrates for assessing environmental conditions in aquatic ecosystems has long been recognized and this has spawned a variety of biological monitoring tools that use aquatic invertebrates (Norris and Thoms, 1999). These biological assessment methods are often used to complement traditional chemical analyses in the assessment of river water quality.

Great attention has been paid to the loss of biodiversity in tropical Asian streams along with rising concerns over the fate of tropical rain forests because of the recent increase of anthropogenic influences in the region (Dudgeon, 2000). At present, the ecology of aquatic insects in Asia including Malaysia is not well-understood (Morse *et al*., 1994). In Malaysia, earlier studies on aquatic macroinvertebrates including insects compared the macroinvertebrate fauna of an urban river (Langat River and Semenyih River) with pristine river (Yap, 2005; Yap *et al*., 2003). Azrina *et al.* (2006) examined both clean and polluted sites on the Langat River over four months. However, these two researchers focused on all macroinvertebrates at both clean and polluted river, without emphasis on Ephemeroptera, Plecoptera and Trichoptera (EPT).

A preliminary study of EPT in river basin in northern region was carried out in Kerian River Basin (Che Salmah *et al.,* 2001) while a more comprehensive study in northern region was done in the Temenggor catchment area in Perak (Che Salmah *et al*., 2007). The distribution of EPT from rivers at forest reserve areas in the northern areas of peninsular Malaysia such as in Ahning Lake, Kedah (Che Salmah *et al.,* 2002) and Pantai Acheh Forest Reserve, Penang Island (Che Salmah *et al.*, 2004) have been studied previously. Concomitantly, the survey of aquatic insects especially EPT in Gunung Stong Forest Reserve, Kelantan, eastern state of peninsular Malaysia was carried out by Che Salmah *et al.* (2005). Results from short studies made during expeditions did not accurately represent the abundance of aquatic insects in those rivers or lakes. Therefore, the assessment of water quality based on those communities of insects did not represent the values generated from very short surveys.

Most interestingly, freshwater invertebrate species particularly the EPT vary in sensitivity to organic pollution and thus their relative abundance have been used to make inferences about pollution loads like other macroinvertebrates. The concept of the biological indicator using the EPT is based on their diversity, abundance and the distribution in relation to the physical and chemical conditions of the habitats. These primary aquatic groups (EPT) have highly adapted to live in upstream rivers. The presence of a particular species in a habitat indicates that the given determinant or parameter is within the tolerance limits of that species (Hellawell, 1989). Thus, the information provided by indicator species is useful for estimating the degree of environmental impact and its potential threat to other living organisms (Soldner *et al.*, 2004). Bustos-Baez and Frid (2003) had used the concept of indicator species based on presence or absence of characteristic taxa to determine the degree of community change due to the effects of pollution.

The finding of this study provides a new alternative in assessment of disturbances specifically in the upstream water bodies and for the conservation efforts of the aquatic environment. Furthermore, upstream diversity is always underestimated because insects in this habitat remain undescribed. The major focus of this study was to assess the suitability of native aquatic insects as key indicator or flagship genera representing the overall health of headwater ecosystem.

Headwaters are unique components of catchments as they usually support a rich and diverse aquatic fauna (Meyer *et al.*, 2007; Miyairi and Tojo, 2007). Headwater streams are the most varied of all running-water habitats because their catchments are usually small and easily influenced by small differences of disruption (Meyer *et al*., 2007). A study by Stout and Wallace (2003) discovered that the diversity of EPT and other insect taxa increased with distance from the source of disturbance. Moreover, changes in water quality are difficult to detect chemically (Chutter, 1972) whereas biological studies can detect toxic, intermittent or mild organic pollutions. In other words, measure the actual effects on biota (Metcalfe, 1989).

According to Morse *et al.* (2007), many aquatic insects especially EPT are found in second order tributaries such as small springs and streams. EPT assessment is crucial for determining aquatic ecosystem health in upstream rivers. The presence of leaf litter (Vehvilainen *et al.*, 2007), various substrate (Rae, 1985), canopy cover (Tiziano *et al.*, 2007), water quality (Death and Winterbourn, 1995; Kohler, 1992) in these habitats were highly correlated with changes in EPT species composition, population size and hydrologic process. Good assemblages of EPT are found in habitat with ample food supply, shelters to escape from predators and other factors that can guarantee reproductive success (Silveira *et al.*, 2006). Rosenberg and Resh (1993) suggested that high density, diversity, small body size and short life cycle of aquatic macroinvertebrates such as EPT when compared to other organisms favour their use in aquatic-ecosystem monitoring, complementing the physical, chemical and physico-chemical evaluation of the environment.

In addition, seasonal changes do influence the EPT community structures (Robinson and Minshall, 1986). In tropical rivers, seasonal changes mainly represented by variation in precipitation, play important role for changes in the EPT community assemblages. In this study, hypothesis that EPT assemblages were significantly different in different environmental characteristics of rivers in the Gunung Jerai Forest Reserve (GJFR). The studies were carried out to determine the real condition of EPT in clean upstream rivers at northern peninsular Malaysia.

1.1 Objectives

The Ephemeroptera, Plecoptera and Trichoptera were studied in three selected rivers of GJFR with the following objectives:

1) To compare the abundance and diversity of EPT immatures in relation to environmental parameters of Tupah, Batu Hampar and Teroi rivers.
2) To evaluate water quality based on the EPT assemblages and to compare them with the water quality classification of the Malaysian Department of Environment (DOE).
3) To study the seasonal influence on the abundance and diversity of EPT (immature and adults) communities in the rivers.
4) To investigate the life history of *Thalerospyrus* sp. (Ephemeroptera: Heptageniidae) in the field.
5) To study the preferences of EPT community to leaf species in Tupah River.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Since the beginning of civilization, rivers have played a major important role in shaping and influencing the development of the nations and cultures of its people (Chan and Nitivattananon, 2006). Almost all major towns in Malaysia are located along rivers (DOE, 2001). Rivers are valuable natural resources for human life, environment and national development (Chan and Nitivattananon, 2006). There are 150 river systems in the country with 100 of them in the peninsular Malaysia and the rest are in Sabah and Sarawak (Department of Irrigation and Drainage, 2008). These river systems consist of 1800 rivers with a total length of 38,000 km (Department of Irrigation and Drainage, 2008).

Significant progress has been made in recent years in the understanding of tropical rivers with most subjects receiving at least some attention at one or more tropical locations (Maloney and Ferminella, 2005). However, in tropical and subtropical areas of the developing world, the knowledge of stream ecology is still extremely limited (Dudgeon, 1999) and little specific information is available for the majority of rivers (Lim, 1987). At the same time, these rivers are increasingly influenced by human activities (Morse *et al*., 1994). Besides, the ongoing research on biodiversity in Asia is inadequate as well as inappropriate for the policy requirements (Gopal, 2005). Rapid population growth in developing nations exerts a tremendous pressure on the water resources of these countries. Furthermore, found there were strong relationships between human activities and disturbances of the environment (Hodkinson and Jackson, 2005).

The biological diversity in Asian aquatic ecosystems is very rich (Dudgeon, 2000). Among all aquatic ecosystems, streams are excellent systems for identifying indicators of land use because they are intimately linked to their catchments and thus integrate catchments-scale ecological process and cumulative responses to disturbance (Dudgeon, 2000). The interaction of these factors determines some gradients in stream invertebrate species richness from the local to regional scales (Cereghino and Lavandier, 1998). The species richness of stream invertebrates is also strongly influenced by anthropogenic disturbances that may lead to losses of taxa (Compin and Cereghino, 2003) and cause spatial discontinuities in predictable gradients.

The greatest threats to the biodiversity both upstream and downstream habitat are the constructions of dams and barrages that affect the regulation and diversion of river flows (Poff *et al.*, 1997). Farnsworth and Milliman (2003) found that extensive deforestation, agriculture and urbanization of the watersheds up to the headwaters of most rivers in Asia contributed to high sediment load in the rivers. Many aquatic insects species are threatened and on the verge of extinction. In the southern Appalachian Mountains, review on the status of Ephemeroptera, Plecoptera and Trichoptera, found that the fauna are vulnerable, and at risk based on whether they were rare, inhabited isolated habitats and identifiable threats (Morse *et al.*, 1993).

2.2 Biological monitoring and Ephemeroptera, Plecoptera and Trichoptera (EPT) as bioindicators

Bioindication or biomonitoring uses organisms that live within natural ecosystems to monitor the impact of disturbance and the knowledge is adapted in the management of the ecological system. Hodkinson and Jackson (2005) define bioindicator as a species or group of species that readily reflects the abiotic or biotic state of an environment, represents the impact of environmental change on a habitat, community or ecosystem.

Butcher *et al*. (2003) listed four traditional approaches to bioassessment using aquatic insects (the saprobic system, diversity indices, biotic indices and community comparison indices). According to Che Salmah and Abu Hassan (2002), biological organisms that were used to evaluate ecosystem health can be measured quantitatively. As biological indicators, aquatic insects have been used effectively to determine environmental conditions of stream ecology (Hynes, 1970a).

Bioindication can be used in urban settings and in agricultural communities as well (Jeanneret *et al.*, 2003). In that case, biodiversity indicators are used to measure the diversity including character richness, species richness, level of endemism and genetic diversity (Hodkinson and Jackson, 2005). To measure species reaction towards environmental qualities, biological diversity parameters such as presence/absence, abundance, growth, and recruitment rates of indicator species are used (Mcdowall and Taylor, 2000).

Some indicator species may continue to exist even in a polluted environment but suffer physiological stress as that resulted in diminished rate of growth, impaired reproductive capacity or modified behavior (Hellawell, 1986). This is essentially a ‘bioassay’ of the environmental contamination and in order to detect the change and perhaps estimate its intensity; the indicator has become a ‘bio-sensor’ for that pollutant or stressor (Norris and Thoms, 1999). Furthermore, different aquatic insects live in different microhabitats and occur very close together (Voshell, 2003).

Nonetheless, the use of aquatic insects for bioindication is rather seems unpopular in the Asian region although this technique provides a cheaper but good methodology in river classification (Dudgeon, 2000). Biological method using aquatic insects as bio-indicator is environmental friendly, less expensive and less time consuming (Rosenberg *et al*., 1986; Cairns and Pratt, 1993). According to Hilsenhoff (1988), biomonitoring has been widely used in rivers the northern American and European regions.

Currently, the Department of Environment (DOE) of Malaysia has not yet employed aquatic insects as bioindicators of pollution for river pollution studies (DOE, 2002). The DOE principally uses Water Quality Index (WQI) based on physico-chemical water parameters for monitoring water quality purposes.

Aquatic insects are not only numerous but also divergent in their taxonomic composition consisting of the orders Ephemeroptera (mayflies), Odonata (dragonflies, damseflies), Plecoptera (stoneflies), Blattodea (cockroaches), Trichoptera (caddisflies), Hemiptera (water bugs), Megaloptera (alderflies, fishflies, dobsonflies), Neuroptera (spongillaflies, owlflies), Coleoptera (beetles), Lepidoptera (moths), Hymenoptera (wasp), some Diptera (midges) and semi aquatic orthoptera (Merritt and Cummins, 1984). Aquatic insects’ assemblages are made up of species that constitute a broad range of tropic levels and pollution tolerances thus providing strong information for interpreting cumulative effects (McGeoch, 1998).

Among all insect groups, Ephemeroptera, Plecoptera and Trichoptera (EPT) are good indicators of environmental conditions in streams (Rosenberg *et al.*, 1986). EPT insects are ubiquitous in freshwater habitats and are found in all the continents except in Antarctica (Parker *et al*., 2007). Interestingly, EPT species vary in sensitivity to organic pollution and thus their relative abundance has been used to make inferences about pollution alarms. Many EPT insects are sedentary thus they can be use to assist in detecting the precise location of pollutant sources (Hellawell, 1989). This provides both a facility for examining temporal changes and integrating the effects of prolonged exposure to intermittent discharges or variable concentration of a pollutant (Bonada *et al*., 2006).

These insects' groups of EPT reach their maximum development in streams and contain families that are entirely or almost confined to running water and have limited mobility (Harper, 1990), making them a good indicator of watershed health (Hodkinson and Jackson, 2005). The concept of biological indicator using EPT is based on their diversity, abundance and the distribution in relation to the physical and chemical conditions of the habitats (Resh and Jackson, 1993; Che Salmah and Abu Hassan, 2002). According to Bonada *et al*. (2006), qualitative sampling of EPT is relatively easy, the methodology is well developed and equipment is simple. Taxonomic keys are available for most groups although certain 'difficult' taxon exists. However, taxonomical studies of the young stages of insects have become increasingly unfashionable and neglected in recent times (Wiggins, 1996a).

EPT shows response towards disturbance (environmental stress) at different levels of organization and the individuals demonstrated their response to environmental stress in their behavior or physiology (Hodkinson and Jackson, 2005). For example, mayflies and stoneflies, move their body parts (behavior) more rapidly to get more gas exchange when oxygen levels is depleting in the water (Eriksen *et al.*, 1996).

The other level of response is at species-population level. Multiple individuals (population) response to changes in environment by reducing rates of recruitment or mortality (Hodkinson and Jackson, 2005). Moreover, Frati *et al.* (1992), Benton and Guttman (1990) and Benton and Guttman (1992) showed that the quality of the individual in the Ephemeroptera and Trichoptera population might change through damaging impacts on growth or through genetic selection when they are exposed to chemical contaminants such as heavy metal exposure.

Responses of insects at community level involved responses of many populations of insect species. This complex response involves a number of species present, the relative abundances of the different species and presence of important species (Hodkinson and Jackson, 2005). Such complexity requires necessary to work with subsets of taxa to show representative for the whole community. Example given by Resh and Jackson (1993) was the EPT index. The subset of EPT is monitored together as a single richness variable (Resh and Jackson, 1993). However, each taxonomic group responds to a distinct combination of environmental factors (Passy *et al.,* 2004). Rawer-Jost *et al.* (2000) suggested using functional groups or guilds of organisms rather than taxonomic entities. However, feeding functional structure itself is not a strong indicators (Barbour *et al.,* 1999), so combination of feeding habits with other biological traits such as body size, voltinism and fecundity (Statzner *et al.,* 2004) have been shown to show better results for detecting changes in community structure (Charvet *et al.*, 2000; Gayraud *et al.,* 2003).

2.3 Biology of EPT

2.3.1 Ephemeroptera

Up to October 2005, Ephemeroptera are represented by 42 families with a little over 3000 described species in 400 genera (James *et al.*, 2008). Out of that, 14 families can be found in Malaysia (Khoo, 2004). Ephemeroptera, commonly known as mayflies, spend most of their lives as nymphs with a very brief adult stage (2 hours-3 days) (Lenat and Penrose, 1996). Their nymphs are characteristics of shallow streams and littoral areas of lakes and are widely distributed. In general, ephemeropterans are small insects (Appendix A: Plate 1). Their sizes range from a few millimeters to a few centimeters.

Ephemeroptera is an ancient order of fragile insects with many cases of convergent and parallel evolution (Brittain, 1980). Early workers used unstable characters of adults like the colors of the body for identification. Modern workers prefer to use nymphal characters as they were more prominent. Fourteen Ephemeroptera families recorded in Malaysia are listed below as adapted from Edmunds and Polhemus (1990):

Family Baetidae
Family Caenidae
Family Ephemerellidae
Family Heptageniidae
Family Oligoneuriidae
Family Leptophlebiidae
Family Neoephemeridae
Family Polymitarcyidae
Family Tricorythidae
Family Behningiidae
Family Ephemeridae
Family Euthyplocidae
Family Prossopistomatidae
Family Potamanthidae

2.3.2 Plecoptera

Plecoptera are primitive group of insects also known as stoneflies or salmonflies. The diversity of Plecoptera declines rapidly from temperate Asian latitudes (nine families) to tropical latitudes (four or fewer families). The only diverse stonefly family in the Malaysian region is the Perlidae. Comparative to their temperate counterparts, tropical stoneflies are incompletely understood (Sheldon and Theischinger, 2009) although these regions have the highest diversity of stoneflies (Zwick, 2000). Asian stoneflies diversity is much greater than that of Europe or North America but the knowledge of the enduring Asiatic areas is extremely poor (Fochetti and Tierno de Figueroa, 2008). In Malaysia, no systematic taxonomic work has been undertaken. Sivec and Yang (2001) estimated there are approximately 350 Plecoptera species in countries forming the Oriental Region excluding Southern China.

Among the EPT, Plecoptera is a small order of hemimetabolous insects with more than 3497 described species (Fochetti and Tierno de Figueroa, 2008). Generally, Plecoptera is highly diverse at higher altitudes especially in temperate regions as the nymphs are most commonly found in cool, fast flowing and rocky rivers. Plecoptera are cold water specialists and probably one of the most endangered groups of insects (Fochetti and Tierno de Figueroa, 2008). They are good indicator species as the nymphs are intolerant to pollution (Sivec and Yule, 2004). Among the organism sensitive to water quality, Plecoptera occupy an outstanding position for their vulnerability to environmental impacts. Many methods for the evaluation of water quality consider the stoneflies as good indicators of clean waters (Oliveira and Froehlich, 1997). Numerous stoneflies species are being reduced to small isolated populations and many others have already gone extinct due to the growing pollution and alteration of water courses.

Plecopteran nymphs and adults can be easily distinguished from other insect groups by the presence of a pair of long cerci at the end of the abdomen; the antennae are very long and robust (Appendix A: Plate 2). Nymphal taxonomy of Southeast Asian stonefly species is completely unknown and only four families have been recorded in this region:

Family Leuctridae
Family Nemouridae
Family Peltoperlidae
Family Perlidae

2.3.3 Trichoptera

Caddisflies belong to the order Trichoptera and are closely related to butterflies and moths (order Lepidoptera) (Appendix A: Plate 3). The larvae have a single pair of abdominal prolegs, which are located on the terminal segment and each are equipped with an apical anal claw. Trichoptera larvae are best known for their intricately designed cases and fixed shelters, which are species-specific. Their diversity and richness are high in natural pristine water (Armitage *et al.,* 1983). However, few species are associated with stagnant water at lower altitude (Maltchik *et al.*, 2009). In adult stage, the Lepidoptera form membranous wings rather hairy wing and the venations are generalized with few cross veins. Trichoptera have a widespread distribution and show the highest species diversity in Oriental and Neotropical regions. There are about 12,627 species distributed into 610 genera and 40 extant families around the world (Moor and Ivanov, 2008).

Caddisflies larvae can be very good bioindicators of water quality and ecological changes since many of them only survive in rivers or streams of good water quality (Dudgeon, 1984; Hynes, 1976; Chapman, 1996; Azrina *et al.*, 2005). Composition and distribution of caddisflies larvae are determined by their physical-chemical tolerance to an array of environmental factors (Dudgeon, 1984). The most important factors influencing the occurrence of attached organisms like caddisflies in running waters are substrate types, velocity, erosion and deposition, light, temperature and dissolved oxygen (Chapman, 1996; Wagner and Schmidt, 2004; Wagner *et al*., 2006). Their growth, reproduction and survival are strongly influenced by water temperature. Caddisfies are also important in the trophic of wetland systems as they serve as food for several species of fishes and waterfowl (Maltchik *et al.,* 2009).

The Trichoptera larvae range in size from 2 mm to over 40 mm. Larvae are soft-bodied and usually cream-colored or greenish with the head and thorax variously colored from tan to dark brown or black. Trichopteran larvae can construct cases. These cases are often intricate in design and usually important in the identification of a particular group. Below are the 26 families of the order Trichoptera listed in Malaysian/Bornean (Morse *et al*., 1994).

Family Brachycentridae

Family Calamoceratidae

Family Dipseudopsidae

Family Ecnomidae

Family Glossosomatidae

Family Goeridae

Family Helicopsychidae

Family Hydrobiosidae

Family Hydropsychidae

Family Odontoceridae

Family Philopotamidae

Family Phryganeidae

Family Polycentropodidae

Family Psychomyiidae

Family Stenopsychidae

Family Rhyacophilidae

Family Seriscotomatidae

Family Xiphocentronidae

Family Hydroptilidae | Family Uenoidae
Family Lepidostomatidae | Family Apataniidae
Family Leptoceridae | Family Molannidae
Family Limnephilidae | Family Limnocentropodidae

2.4 Life cycle of EPT

2.4.1 Life cycle of Ephemeroptera

Ephemeroptera are hemimetabolous insects; their life cycle includes the egg, nymph and adult stage. The adult wings (two to four in number) represent a very primitive condition in insects, which are held vertically, unfolded above their body and the presence of two or three long, slender tails. The males are easily differentiated by their enlarged compound eyes, which allow them to quickly spot and mate with females of the same species during flight. Above streams and rivers, swarms of males can be seen in up and down flight pattern, while females fly through the swarm until males intercept them. Then, the mated females will deposit their fertilized eggs on the water surface or submerge themselves and lay eggs underwater.

After hatching, tiny nymphs disperse in a wide range of aquatic microhabitats. The nymphs go through a large number (as many as 30 to 40 times) of molts as they grow; with most species having 15-25 instars (Triplehorn and Johnson, 2005). Life forms of Ephemeroptera nymph are diverse, but they fall into three broad categories: burrowing, swimming and creeping. The nymph fills its stomach with water before its transition to an adult, later replaced by air (Needham *et al.*, 1935). Ephemeroptera have two winged stages, namely the subimago and the imago (Khoo, 2004). The subimago or dun is a short lived, rather dull in appearance compared to the glossy imago (Khoo, 2004). Ephemeroptera nymphs are characteristic of shallow streams and littoral areas of lakes. The nymphs are truly aquatic but the adult stages are terrestrial (McCafferty, 1981).

2.4.1.1 Life history of Ephemeroptera

In order to link species traits to ecosystem process, knowledge of the life histories of freshwater invertebrates is crucial (Gonzalez *et al*., 2003). Insect development or growth involve progressive changes in size, morphology and physiology of the insect. When the number of instars and the degree of development for instars are known, understanding of the biology of many insects will greatly improved. Ruffieux *et al.* (1996) elucidated that information on the number of instars could clarify some important phenomena of the Ephemeroptera life history, such as the size differences between individuals of different cohort or generations. Ephemeroptera instar determination is particularly difficult due to the generally large number of instars and prolonged immature life stages (Flannagan *et al.,* 1990) as observed in the temperate countries.

Throughout the year, many species appear and disappear as different broods completed their development in synchrony with seasonal climatic factors (Kondratieff and Voshell, 1980). The environmental conditions, especially the water temperature (Vannote and Sweeney, 1980; Rosillon, 1988; Giberson and Rosenberg, 1992) affect the development and the number of instars in Ephemeroptera. Habitats with warm temperature usually have small sizes insects because their growth and life cycles complete quickly (Huryn and Wallace, 2000). According to Fink (1980), the rate of development and number of instars are probably controlled by genetic and environmental factors and generally, poorer nutrition seems to result in a greater number of instars.

The Heptageniidae is widely distributed in Palaearctic and Oriental stream (Dudgeon, 1999) but poorly known in Asia. The genus *Thalerospyrus*, among the 28 genera of Heptageniidae inhabits cleans freshwater. The common genera in Malaysia are *Epeorus, Thalerospyrus* and *Campsoneuria* (Khoo, 2004). The head capsule of Heptageniid nymphs are flattened and hides all the mouthparts (Khoo, 2004). They crawl on hard surfaces such as cobble and boulder in the water (Kondrateff and Voshell, 1980) but they cannot swim. Preliminary data provided by Dudgeon (1996) showed this family had an asynchronous growth and the cohort cannot be distinguished. Life histories of Malaysian Heptageniidae have not been studied. Even parts of life history such as voltinism and life cycle were not studied in the tropical region.

2.4.2 Life cycle of Plecoptera

A typical stonefly life cycle includes an egg, nymph and adult. Stoneflies are terrestrial as adults and the nymphal stages are strictly aquatic. The eggs can be in masses up to 1000 and always deposited in water (Gullan and Cranston, 2005). The nymphal instars, ranging from 10 to over 30, occur in one to three years (Triplehorn and Johnson, 2005). Prior to emergence, nymphs crawl to the stream bank or some emergent object like rocks or logs (Sweeney, 1993) and moult into adults which resemble the nymphs except for the presence of wings. They can live from one to four weeks (Triplehorn and Johnson, 2005). According to Merritt *et al.* (2008), the adults' males and females use species-specific form of communication or 'drumming' to locate each other. The males tap or rub their abdomens on resonant substrate like a tree leaf to send initial drumming signals for attracting the females. Receptive females respond to the male signal with her own call. The males locate the females' signal using sensory structures called tympanum on its legs and mate.

2.4.3 Life cycle of Trichoptera

Trichoptera undergo a complete metamorphosis (holometabolous) which includes an egg, larva, pupa and adult. Eggs are laid on sticky masses attached to twigs or rocks in the water (Spanhoff, 2005). The larval and pupal stages inhabit a wide range of freshwater habitats. The pupa is exarate and in case-building species, develop within the larval case after it has been secured to the substrate and sealed with silk (Morse, 2004). Pupae have functional mandibles that they use to chew their way out of the pupal case once they are ready to emerge as adults (Triplehorn and Johnson, 2005). Once they emerge, their mandibles degenerate and become nonfunctional (Petersen *et al.*, 1999). Adult Trichoptera differ from moths in their wing venation and structure of the mouthparts (Triplehorn and Johnson, 2005). Adult Lepidoptera being covered by scales while Trichoptera wings are typically clothed with hair and have a roof top shape when resting (Morse, 2004). The larval stages construct cases using silk enabled them to adapt a more diverse habitat. Trichoptera life cycle can vary from a few months to a couple of years with the adult stage being very short-lived.

2.5 Tropic roles of EPT

EPT trophic structure was described using functional feeding groups (Thompson and Townsend, 2003) which demonstrated some advantages (McShaffrey and McCafferty (1986; 1988). A behavioral arrangement for obtaining specific feeding resources was only allowed by using morphological adaptations of insect mouthparts (Arens, 1990). Merritt and Cummins (1996) divided the EPT functional feeding groups into four groups: a) collector-filterers (CF) - filtering material from the flow using constructed nets, b) collector-gatherers (CG) - feed on organic deposits on the streambed c) predators (P) - feed on other invertebrates and d) shredders (SH) - feed on coarse organic material.

The ephemeropterans nymphs of most genera are generally CG. Some of the examples are *Thalerospyrus*, *Baetis* and *Caenis* (Merritt *et al*., 2008). Some genera are filterers (*Isonychia*) or scrapers (*Habrophlebiodes*) and few genera are predators, especially those inhabiting large rivers. One genus, *Ameletopsis* is known to be carnivorous. Meanwhile, adults of this order do not feed.

Generally, Plecoptera nymphs are either shredders or predators. Some groups are been reported to be herbivorous or detritivorous in their early instars. Leaf breakdown and allocthonous input process was studied using plecopteran detritivorous shredders (Gessner *et al.*, 1999; Fenoglio *et al.*, 2005; Aggie and Dudgeon, 2009). Some Plecoptera are predaceous in their late instars. Tikkanen *et al.* (1997) used predaceous plecopterans as model in prey-predator studies in streams.

Most of Trichoptera larvae are filter feeders especially Hydropsychidae and Philopotamidae, but few are predatory (Rhyacophilidae) (Triplehorn and Johnson, 2005). Some Trichoptera are gatherers such as Leptoceridae that use silken nets to collect seston or catch prey and preferentially removing more nutritious foods into their nets (Moor and Ivanov, 2008). Meanwhile, shredder (Odontoceridae) larvae feed on fresh vegetative material or detritus (Merritt *et al.*, 2008).

2.6 Functional feeding groups

According to Gullan and Cranston (2005) identification to species level is sometimes inadequate to be used as it needs expertise to do it. Therefore, they suggested a solution by subsuming taxa into functional feeding groups. In the stream ecosystem, instead of studying hundreds of taxa, a group of organisms can be studied collectively based on their function in mechanisms for obtaining food.

Functional feeding group (FFG) is a classification approach based on morpho-behavioral mechanisms of food acquisition rather than taxonomic group (Merritt and Cummins, 1996). It involves the use of information on feeding habits of benthic taxa (Rawer-Jost *et al*., 2000). Gullan and Cranston (2005) used mouthpart morphology as a guide to categorizing feeding modes. The categorization of stream macroinvertebrates by functional feeding group has shown considerable assurance as a tool for assessing spatial changes in lotic communities based on environmental conditions (Blasius and Merritt, 2002).

The FFG categories include scrapers, collectors, shredders and predators. A scraper removes algae that are attached on the surface of rocks or substrates. Collectors are divided into two sub-groups; collector-filterers and collector-gatherers. Collector-filterers use their long hairs on their head, legs, or silk net to filter small particles out of the water. Collector-gatherers use their mouthpart to gather fine particles and shove into their mouths (Merritt and Cummins, 1996). Most shredders shred pieces of vegetation using their mouthparts. Predators prey on other living animals and often have special structures such as sharp teeth or spiny legs for catching (Voshell, 2003).

According to Gullan and Cranston (2005), the notion that linkages subsist in riparian headwater streams was supported by the functional feeding group analyses; coarse particulate organic matter (CPOM) with shredders, fine particulate organic matter (FPOM) and collectors. The primary productivity is connected to scrapers. The functional feeding group has shown extensive assurance as a tool for assessing spatial changes in lotic communities (Blasius and Merritt, 2002). CPOM is reduced to FPOM by shredders in upland streams and is made available to numerous collectors downstream (Compin and Cereghino, 2007). Moreover, Bispo *et al.* (2006) noted that shredder and scraper insects are expected to be found at higher abundance in lower order streams (first and second order).

2.7 Economic importance of EPT

Ephemeroptera is a secondary consumer in many systems (Adler and Currie, 2008). They are also use as fish baits. Both adults and nymphs are important food of many freshwater fish. In adult stage, Ephemeroptera also serves as food for many animals including birds, amphibians and spider. Moreover, the Ephemeroptera fauna of an aquatic habitat may serve as an indicator of disturbance of that habitat because the nymphal stage is restricted to particular types of habitat (Triplehorn and Johnson, 2005).

Meanwhile, Plecoptera provide a valuable food source for a wide variety of vertebrates. Nymphs and adult stages are eaten by many species of fish and amphibians (Petersen *et al.*, 1999). Actively dispersing adults' Plecoptera are a valued and plentiful food source for bats and birds that feed at dusk on flying insects (Fochetti and

Tierno de Figueroa, 2008). Moreover, Sweeney (1993) observed that mammals such as shrews and raccoons also eat on Plecoptera nymphs as well as their emerging adults.

Trichoptera larvae may serves as food for fish and other aquatic vertebrates and they are often used as bait by fisherman. Some are nuisance during emergence since the insects are attracted to outdoor lights; human allergies to the hairs on their wings have also been reported (Wiggins, 1996a). On the beneficial side, many hydropsychids larvae prey on black fly larvae (Diptera: Simulidae). According to Burton and McRae (1972), *Hydropsyche* and *Cheumatopsyche* (Trichoptera: Hydropsychidae) are predators seizing on black fly larvae outside the retreat. Adults Simulidae are rather harmful because they often bite to acquire blood for egg maturation and because of this habit, they become agents transmitting various filarial, protozoan and viral diseases to domestic animals and wildlife (Adler and Currie, 2008).

2.8 Influence of environmental parameters on diversity and abundance of Ephemeroptera, Plecoptera and Trichoptera

2.8.1 Physical parameters

Biology of insects is correlated with environmental factors (Fink, 1984). Many aspects of physical stream environment affect the composition and abundance of EPT. Their population distribution is ultimately determined by physical-chemical tolerance to an array of environmental factors (Che Salmah *et al*., 2004) such as substrate type (Erman and Erman, 1984), embeddedness, velocity, temperature (Allan, 1995; Bale *et al.,* 2002; Brodersen and Anderson, 2002) and altitude (Marchant *et al*., 1995).

2.8.1.1 Substrate suitability

The suitability of a substrate for colonization by aquatic insects depends on its particle size, its mixture of sizes, the size of pore spaces, and its surface topography (Cain *et al.*, 1992; Rempel *et al.*, 2000; Fowler and Death, 2001). Thus, the distribution of sediment sizes along a stream influences the distribution of organisms in a river. Biological activity in coarser substrates is dependent upon the maintenance of inter-gravel flow rates for the replenishment of nutrients, oxygen and the removal of metabolic wastes (Sarriquet *et al*., 2007). Gravel-bed stream, which filled with silt, may display a shift in the insect species compositions from Ephemeroptera and Trichoptera towards Diptera, which, in turn, can affect fish species compositions (Milhous, 1982).

Eriksen's (1968) study showed that mayfly *Hexagenia limbata* burrow into fine sediment while *Ephemera simulans* choses gravel as refuges. Meanwhile, Plecoptera such as Leuctridae and Capniidae burrow into the gravel to avoid harsh water current (Hynes, 1970b). Scott (1958) found most of the Trichoptera species on large stones but *Glossoma boltoni* prefer on medium sized stones because the larger stones usually have moss while the small stones are least stable.

2.8.1.2 Water velocity

The water velocity of rivers increases and water level begin to rise with the arrival of the rains or a flood from a distant part of the catchment area (Payne, 1986). Inherent need for current can be seen in many invertebrates either because they rely on it for feeding purposes or because their respiratory requirements demand it (Hynes, 1970b). Allen (1951) in New Zealand observed caddisflies *Helicopsyche* is larger in swifter reaches. Furthermore, Voshell (2003) stated that aquatic insects especially EPT group favored the sections of streams and rivers where the water is flowing fast enough to splash (riffles). The ephemeropteran from Hepatgeniidae family can cling to rocks in very swift current. Their head and bodies are very flat and the current just passes over without flushing them off.

2.8.1.3 Water temperature

In streams and rivers, the temperatures vary but quite often; this variation is over a much smaller range than that of at least the shallower parts of still water (Hynes, 1970a). Furthermore, in the tropics seasonal changes of rainfall have little change of temperature (Hynes, 1970a). Langford and Daffern (1975) concluded that temperature increases did not have a significant effect on the total numbers and overall emergence period of Ephemeroptera, Trichoptera and Megaloptera. deKozlowski and Bunting (1981) found *Ephemerella invaria* and *Stenonema ithaca* (Ephemeroptera) to be the most sensitive species to heated water. *Caenis latipennis* (Ephemeroptera: Caenidae) were found dead according to Puckett and Cook (2004) at temperature between 36.7ºC and 38.5ºC. In contrast, *Hydropsyche* (Trichoptera) are considered tolerant of heated water (Gaufin and Hern, 1971), for example *Helicopsyche borealis* (Trichoptera: Helicopsychidae) have been found in streams at 34ºC or more (Wiggins, 1996b).

2.8.1.4 Canopy

Canopy cover can be a factor in the ecology of stream invertebrates. Some prefer shaded while other prefer an open environment. *Baetis rhodani* (Thorup, 1963), *Centroptilum*, *Cheumatopsyche* and *Hydropsyche* (Hughes, 1966) were more abundant in unshaded areas than under trees but caddisflies *Wormaldia* showed an opposite correlation (Thorup, 1963). Shading by full riparian canopy cover could suppress