DIVERSITY AND SOME ASPECTS OF BIOLOGY OF EPHEMEROPTERA IN STREAMS OF ROYAL BELUM STATE PARK IN PERAK

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By

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2) Wan Mohd. Hafezul, W. A. G., & Che Salmah, M. R. (2010). Functional Feeding Groups of Mayflies (Ephemeroptera): variation between selected rivers in Royal Belum State Park, Perak. Proceeding of The 7th IMT-GT UNINET and the 3rd Joint International PSU-UNS. Prince Songkhla University, Hat Yai, Thailand. 7-8 October 2010.

LIST OF ABBREVIATION AND SYMBOLS

asl	=	Above Sea Level
BOD	=	Biological Oxygen Demand
CCA	=	Canonical Correspondence Analysis
cm	=	Centimeter
COD	=	Chemical Oxygen Demand
CPOM	=	Coarse Particle Organic Matter
df	=	Degree of Freedom
DOE	=	Department of Environment
FFG	=	Functional Feeding Group
FPOM	=	Fine Particle Organic Matter
l	=	Liter
m	=	Meter
m/s	=	meter per second
mg	=	Milligram
mg/L	=	milligram per liter
NH_4-N_3	=	Ammonia-nitrogen
PCA	=	Principal Correspondence Analysis
ppm	=	Part per million
RBSP	=	Royal Belum State Park
SE	=	Standard Error
SPSS	=	Statistical Package for Social Science
TDS	=	Total Dissolve Solid

TSS	=	Total Suspended Solid
μm	=	Micrometer
ρ	=	Spearman’s Correlation Coefficient

KEPELBAGAIAN DAN BEBERAPA ASPEK BIOLOGI EPHEMEROPTERA DI SUNGAI TAMAN NEGERI DIRAJA BELUM, PERAK

ABSTRAK

Komuniti Ephemeroptera (lalat Mei) yang di selidiki di empat batang sungai, Sungai Kejar, Sungai Tan Hain, Sungai Mes dan Sungai Ruok di Taman Negeri Di Raja Belum (RBSP) mempunyai kepelbagaian yang tinggi secara relatif (H' = 1.407 – 2.166). Dua puluh genera daripada 10 famili diwakili oleh 10308 individu telah dikumpul melalui teknik 'kick sampling' bermula dari Februari 2009 hingga Februari 2010. Antara keempat-empat sungai, Sungai Tan Hain mempunyai kelimpahan tertinggi (3542) dan walaupun Sungai Ruok mempunyai kelimpahan terendah (1500), tetapi ia memberi skor indeks kepelbagaian (H' = 2.166, 1-D = 0.839) dan indeks kekayaan (R1 = 2.325, R2 = 0.465) tertinggi. *Baetis* sp. dominan di semua sungai dengan purata kepadatan 651.11 individu m^{-2} diikuti oleh *Habrophlebiodes* sp. (Leptophlebiidae) dengan purata kepadatan 187.76 individu m^{-2}. *Cinygmula* sp. *Drunella* sp. dan *Teloganella* sp. adalah jarang ditemui. Kebanyakan Ephemeroptera yang dikumpul di semua sungai adalah pemungut-kumpul; pemungut-turas kurang ditemui manakala pengoyak dan pemangsa adalah jarang ditemui. Skor Indeks Kualiti Air (WQI) mengkelaskan semua sungai di dalam Kelas I iaitu mempunyai kualiti air yang sangat bersih. Semua parameter persekitaran kurang mempengaruhi kelimpahan Ephemeroptera (Spearman's rho < 0.3, P=0.05) kecuali Keperluan Oksigen Kimia (COD). Plot taburan lebar kepala – panjang badan *Baetis* sp. mengasingkan peringkat nimfa kepada 9 instar (F – F-8). Kitaran hidupnya mengambil masa kira-kira sebulan daripada instar termuda hingga ke instar yang

terakhir. Kemunculan dewasa berlaku di setiap bulan, jadi *Baetis* sp. dispekulasikan mempunyai sejarah hidup yang multivoltin di kedua-dua Sungai Tan Hain dan Sungai Mes. Pengeluaran sekunder *Baetis* sp. dianggarkan 60.33 mg m^{-2} y^{-1} di Sungai Kejar dan 182.96 mg m^{-2} y^{-1} di Sungai Tan Hain. Kadar pusingan (produktiviti/biomas) sangat rendah di kedua-dua sungai dan mungkin tidak menggambarkan kadar sebenar kerana jumlah kohort tidak diketahui. Ephemeroptera dewasa lebih tertarik kepada keamatan cahaya yang tinggi. Sepuluh famili telah dikumpul pada 160 watts dari 2000 j hingga 0400 j dan hanya 7 famili ditemui pada 90 watts (1900 j - 0000 j), tetapi kelimpahan mereka didapati tidak berbeza secara signifikan (P=0.05) pada kedua-dua tahap keamatan cahaya.

DIVERSITY AND SOME ASPECTS OF BIOLOGY OF EPHEMEROPTERA IN STREAMS OF ROYAL BELUM STATE PARK IN PERAK

ABSTRACT

Communities of Ephemeroptera (mayflies) investigated in four rivers; Kejar River, Tan Hain River, Mes River and Ruok River of Royal Belum State Park (RBSP) were found to be relatively diverse (H' = 1.407 – 2.166). Twenty genera of 10 families were represented by 10308 individuals collected by the kick sampling technique from February 2009 until February 2010. Among the four rivers, Tan Hain River had the highest abundance (3542) and although Ruok River had the lowest abundance (1500), it scored the highest Diversity Index (H' = 2.166, 1-D = 0.839) and Richness Index (R1= 2.325, R2 = 0.465). *Baetis* sp. was dominant in all rivers with an average density of 651.11 individuals m^{-2} followed by *Habrophlebiodes* sp. (Leptophlebiidae) of 187.76 individuals m^{-2}. *Cinygmula* sp., *Drunella* sp. and *Teloganella* sp. were rare. Most ephemeropterans collected in all rivers were collector-gatherers; collector-filterers were scarce and scrapers and predators were uncommon. The scores of Water Quality Index (WQI) classified all rivers into Class I of very clean water quality. All environmental parameters were weakly associate with the abundances of ephemeropteran species (Spearman's rho < 0.3, P=0.05) except the Chemical Oxygen Demand (COD). The head width-body length scatter plots of *Baetis* sp. separated the nymphal stages into 9 instars (F-F-8). Its life cycle took approximately a month from the youngest instar to the final instar. Adult emergence took place every month, thus *Baetis* sp. is speculated to have a multivoltine life history in both Tan Hain and Mes rivers. The secondary production

of *Baetis* sp. was estimated at 60.33 mg m^{-2} y^{-1} in Kejar river and 182.96 mg m^{-2} y^{-1} in Tan Hain River. The turnover rate (productivity/biomass) was very low in both rivers and might not reflect the true rate because the cohort number was not known. Adults Ephemeroptera were more attracted to high light intensities. Ten families were collected at 160 watts from 2000 h – 0400 h and only 7 families were encountered at 90 watts (1900 h - 0000 h) but their abundances were not significantly different (P = 0.05) at both levels of light intensities.

CHAPTER 1

INTRODUCTION

1.1 Background

Ephemeroptera are unique group of aquatic insects as they are ubiquitously distributed in both tropical and temperate aquatic habitats. They are an important component of the aquatic food web as well as energy flow of the riverine ecosystem. In addition to their importance in ecosystem functioning and stability, mayflies have been intensively applied in assessment and biomonitoring of polluted rivers (Xiaoyu & Yunjun, 2008).

Investigations of biological and ecological aspects of Ephemeroptera inhabiting the headwater streams are state of the art in aquatic ecology research. Their role in relation to ecosystem functioning and integrity of the aquatic habitats requires thorough understanding (Baptista *et al*., 2001; Schmera *et al*., 2003). Similar to other aquatic insects, distribution and abundance of mayflies in headwater streams depend on several abiotic and biotic factors. Different anthropogenic activities including forest management, agricultural land use, civilization and road construction are well known for their adverse effects on the richness and abundance of Ephemeroptera in tropical (Douglas *et al.*, 1993; Dudgeon, 2000a; Dudgeon, 2000b; Iwata *et al*., 2003; Sodhi and Brook, 2006; Dudgeon, 2006; Boyero *et al*., 2009; Gucker *et al*., 2009; Sodhi *et al.*, 2010) and temperate aquatic ecosystems (Effenberger *et al.*, 2006; Tessa *et al.*, 2007; Rohasliney & Jackson, 2008). Timber logging, agricultural activities and road construction change physical and chemical characteristics of the streambeds such as deposition input and sedimentation (Billy *et al*., 2000; Kreutzweiser *et al*., 2005; Pond, 2010). Ultimately, these changes in the aquatic ecosystems affect the community structure of aquatic organisms including mayflies (Kreutzweiser *et al*., 2005). Habitat disturbance is the major problem affecting the distribution and abundances of mayflies, however streams normally are either resistant or resilient to the perturbation by (Fuller *et al*., 2008).

Generally, headwaters range from steep, swift, and cold mountain streams to warm, low-gradient, swampy tributaries (Meyer *et al*., 2007). The headwaters and downstream rivers differ significantly in their physical, chemical and biotic attributes such as hydrological conditions, water quality, proportions of allochthonous and autochthonous organic matter, and riparian vegetation (Cowell *et al*., 2004). Usually, the headwater and downstream rivers vary in their biological components such as producers' community structures, which are affected by difference in physical, chemical and nutrient levels in the streams. These variations consequently influence the aquatic macroinvertebrate communities living in both ecosystems (Fuller *et al*., 2008). Secondary production of Ephemeroptera was sustained in the rivers although the abundance of food source changed seasonally because of the contribution of allochthonous and autochthonous food to ephemeropteran nymphs throughout the year (Salas and Dudgeon, 2003). Study of secondary production provides more information to other population parameters such as biomass, individual growth rate, survivorship, and development time of Ephemeroptera (González *et al*., 2001).

Ephemeroptera is one of the most abundant insect taxa in the forested headwater rivers and can constitute over 88% of the macroinvertebrate assemblage (Dudgeon, 2006). Among the three most sensitive insect groups, Ephemeroptera, Plecoptera and Trichoptera (EPT), Ephemeroptera showed the highest abundance in at least six tropical regions previously studied: Sulawesi, Hong Kong, Papua New Guinea, Ecuador Pacific, Ecuador Amazon and Bolivia (Dudgeon, 2008). Therefore, ecological research concerning various biological and ecological aspects of Ephemeroptera in tropical streams is receiving more attention. Despite these positive developments, Ephemeroptera biogeography and ecology is poorly known and data on Ephemeroptera diversity is sparse and disconnected both at the local and regional scale (Dudgeon, 2000a; Dudgeon, 2006; Boyero *et al*., 2009). In general, there is an incomplete understanding about Ephemeroptera in the tropics and their roles in ecosystem stability and function (Boyero *et al*., 2009). Knowledge of ephemeropteran adults is lacking especially in the tropics (Webb and McCafferty, 2008; Boyero *et al*., 2009). Adult mayflies have two main functions: mating and oviposition (Brittain, 1982). However, swarming and mating behavior of ephemeropteran adults has received more attention from researchers (Brodsky, 1973; Edmunds and Edmunds, 1980; Brittain, 1982; Peckarsky *et al*., 2002)

Biological assessments have emerged as an important empirical tool in monitoring environmental health primarily of the aquatic ecosystem. High population growth in developing countries adds tremendous pressure on water resources (Soldner *et al*., 2003). Together with the application of conventional water quality monitoring techniques, the application of the response living organisms to determine quality of the environment is one of the most valuable tools in biomonitoring (Mandaville, 2002). Biomonitoring has been proven to be an inexpensive, easy and time saving tool for quick and effective assessment of the environment. In development countries with limited funding resources, biological monitoring of water quality is an ideal approach (Soldner *et al*., 2003) due to its relatively low cost. In addition, the conventional chemical analysis of river water can only identify possible contaminants whereas biological communities readily interact with the abiotic or biotic factors in their environment which consequently reflect the impact of habitat, community, or ecosystem changes (Hodkinson & Jackson, 2005). Ephemeropteran roles in the environment such as nutrient cycling can be explained by their life cycles, distributions, abundances and their ability to adapt in disturbed habitat (Gupta and Michael, 1992; Chung, 2005; Arimoro, 2009)

In Malaysia, literature on the biodiversity and abundance of Ephemeroptera, Plecoptera, Trichoptera (EPT) in northern peninsular Malaysia is very scarce. Che Salmah *et al*. (2007) reported that the distribution of the EPT in Temenggor Lake feeder streams and the number of ephemeropterans recorded were considerably high. A preliminary study of the EPT in Kerian River Basin in Perak reported that the distribution of Ephemeroptera (mayflies) was the widest in comparison to that of Plecoptera and Trichoptera (Che Salmah *et al.*, 2001). Since Ephemeroptera typically live in clean water, the distribution of Ephemeroptera in various ecosystems was documented in relation to their role as bioindicators based on community distribution in various chemically and physically disturbed habitats (Che Salmah & Wahizatul Afzan, 2005).

1.2 Objectives

This study was conducted in Royal Belum State Park (RBSP) in Perak investigating various biological and ecological aspects of Ephemeroptera to achieve the following objectives:

1. To investigate the diversity of Ephemeroptera community in the streams of Royal Belum State Park in Perak.

2. To assess the influence of water quality and the role of habitat diversity in determining the abundances of the Ephemeroptera community.

3. To describe the life histories and secondary production of the most abundant species of the Ephemeroptera in the headwater streams in Royal Belum State Park.

4. To investigate biological aspects of the adult, such as flight activity and light intensity preferred by adult populations of Ephemeroptera.

CHAPTER 2:

LITERATURE REVIEW

2.1 Introduction

Mayflies are insects in the Order Ephemeroptera (from the Greek 'ephemeros' = short-lived and 'pteron' = wing), referring to the short lifespan in adults. Ephemeroptera is unique because they are the nearest representative to early pterygote ancestor (Miyairi & Tojo, 2007) dating back to the late Carboniferous or early Permian periods, which is 250 million years ago (Barber-James *et al*., 2008). The highest diversity of Ephemeroptera in ancient time is believed to be during the Mesozoic era (Brittain and Sartori, 2003). They have been placed into an ancient group of insects known as the Palaeoptera, which also includes dragonflies and damselflies. This group is considered as the sister group of all other existence mainly winged orders (Kukalová-Peck, 1991).

The Ephemeroptera is a large order consisting about 3000 known species worldwide. They belong to 42 families with more than 400 genera (Barber-James *et al*., 2008). Many studies on this group of insects have been conducted in temperate countries, especially in North America (Brittain & Sartori, 2003). With increasing interest on the group, studies are widely in progress, especially in tropical, subtopical and temperate regions (Boyero *et al*., 2009; Dugeon, 2000; Soldan, 2001). In the Oriental region, Soldan (2001) reported as many as 19 families, 104 genera and about 540 species occur in the region and its transition areas (Soldan, 2001).

A typical nymph of Ephemeroptera has seven pairs of lateral abdominal gill (Plate 2.1). At the tip of the abdomen are two cerci and one median terminal filament. However, a few genera such as *Platybaetis* and *Epeorus* have no median terminal filament (Ghee, 2004; Waltz & Burian, 2008). A mayfly nymph can be easily differentiated from other aquatic insects by characteristics such as the unsegmented tarsus, a single tarsal claw, larger mesothorax than prothorax and presence of gills at the lateral sides of the abdominal segments (Ghee, 2004).

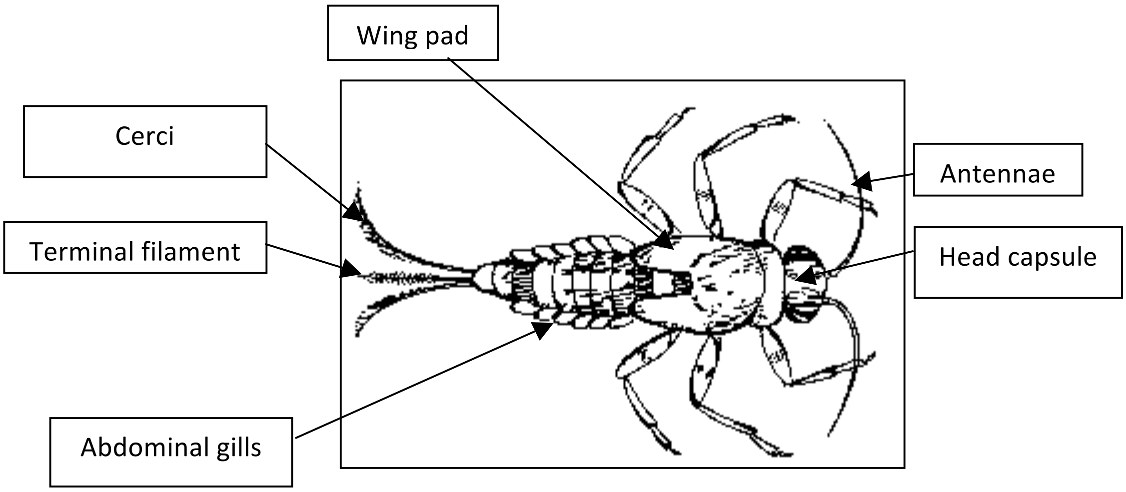

Plate 2.1: General characteristics of an ephemeropteran nymph. Most of the nymphs have a pair of cerci and one median terminal filament (Barber-James *et al*., 2008).

The unique characteristic of Ephemeroptera is that seven eighths of its lifespan is spent as immature (nymph) in aquatic environment. The nymphal stage undergoes a series of moult from one instar to as many as 50 instars (Morse *et al.,* 1994; Ruffiex *et al.,* 1996). Temperature strongly influences the duration of ephemeropteran life history that can be as short as 10-14 days and up to 1- 2 years for some species (Edmunds, 1996). The life cycle starts with eggs that are normally deposited on the water surface with special sticky covering to anchor to its habitat (Koss & Edmunds, 1974). In addition, egg structure is useful in taxonomic and phylogenetic analyses. In the temperate region, the eggs of some species take 7-9 months before hatching or can stay dormant for over 11 months and hatch in spring (Edmund, 1996). Specific hatching temperatures are species dependent and these variations lead to wide distribution of ephemeropteran species in different habitats and regions.

2.2 Life Cycle of Ephemeroptera

Ephemeroptera is a hemi-metabolous insect having four distinct life stages: egg, nymph, subimago and adult.

2.2.1 Eggs

Adult mayfies are all terrestial (Merritt *et al*., 2008). Adult female goes close to the water to lay eggs or to oviposit. Since the life span in the adults' stage is very short, the development of the eggs in the ovary has started even during the nymphal stages (Dominguez & Cuezzo, 2002). The females lay eggs on the water surface without parental care or egg-guarding behaviors (Encalada & Peckarsky, 2007). They dip their abdomens a few times to lay all eggs in one or two clusters (Waltz & Burian, 2008). Eggs of the mayflies are covered by sticky layer and for some species, the egg structure is further specialized by anchoring devices (Koss & Edmunds, 1974). Koss (1968) described the external morphology of the Ephemeroptera eggs in details. The egg has four major external structures, a polar cap, an attachment structure, a micropyles and chorionic sculpturing. Variations in egg morphology are very useful for the species identification in taxonomic studies.

The hatching times of eggs are dissimilar for different species. Inside the eggs, embryonic development normally takes a few weeks (Waltz & Burian, 2008). However, suspended development of the embryo is common in many species living in the temperate region. Temperature plays a significant role in the development of the eggs. According to Elliot (1972), in some species, for instance *Baetis rhodani*, the period from oviposition until hatching times are different at different temperatures. At $3^{o}C$, the eggs hatch within 17 days whereas it only takes 1 week at $22^{o}C$. However, for some species, Friesen *et al*. (1979) reported that the lower temperature limit for the eggs hatching is between $8^{o}C$ to $12^{o}C$ and the upper temperature limit is between $32^{o}C$ to $36^{o}C$.

2.2.2 Nymphs

Most of the studies on Ephemeroptera are focused on the nymphal stages (Barber-James *et al*., 2008; Bispo *et al*., 2006; Ghee, 2004; Stitt *et al*., 2006). Ephemeropteran nymphs spend most of their lives in aquatic environment. Similar to their eggs, the length of this stage is influenced by water temperature. It can be as short as fourteen days for some Baetidae and up to six months for other species (Edmund & Waltz, 1996). However, *Hexagenia limbata* in the temperate habitat takes two years before it becomes an adult (Waltz, 2008).

2.2.3 Subimago and adults

Ephemeroptera is a unique living insect because it molts after becoming fully winged. This stage is known as preadult stage or subimago (Edmunds & McCafferty, 1988). The subimagoes are similar to the adults because they are winged and terrestrial except they are dull in color and some characteristics of the adults are less developed. Ephemeropteran subimagoes are weak and clumsy fliers (Edmunds & Edmunds, 1980), hence, highly vulnerable to predators (Sivaramakrishnan & Venkatamaran, 1985).

After molting, the subimagoes fly and rest on riparian vegetation to harden their bodies as well as avoiding predation when they are less exposed to predators in comparison to actively flying subimagoes (Sivaramakrishnan & Venkatamaran, 1985). The subimago undergoes another molting process, which transforms it into an adult or imago (Edmunds & Edmunds, 1980). After molting, most adult males will initate aerial mating aggregations or swarm to attract female imagoes (Peckarsky *et al*., 2002). Sivaramakrishnan and Venkatamaran, (1985) described male swarming as a kind of wedding dance. The adult females fly into the swarm and copulate in the air (Peckarsky *et al*., 2002; Sartori *et al*., 1992; Sivaramakrishnan & Venkatamaran, 1985). Usually the number of females is high because many of them die during oviposition because they are more exposed to predation by aquatic predators (e.g, fish) and flying predators (e.g., predatory insects or birds) (Elliot & Humpesch, 1983). Some of them drown when they fly very near to the water (Edmunds *et al*. 1976; Elliott & Humpesch 1983).

2.3 Functional Feeding Group of Ephemeroptera

Functional feeding groups (FFG) of Ephemeroptera are classified based on their food acquisition mechanisms. The distribution of the ephemeropteran FFG in rivers collectively reflects the process-level in an aquatic ecosystem (Rawer-Jost *et al*., 2000). FFG distribution can also indicate the quality of the aquatic environment. According to Barbour *et al.* (1999), the presence of herbivores such as shredders, collectors and scrapers in the streams indicates good water quality. Gatherers and filterers are more tolerant to pertubations (Barbour *et al*., 1999; Rawer-Jost *et al*., 2000), hence are found in disturbed habitats.

Waltz and Burian (2008) found that most ephemeropteran nymphs are collector-gatherers that feed on detritus and algae (FPOM). The second common feeding group of the nymphs is scrapers and collector-filterers. Only a few families are predators or engulfers such as Acantharmetropodidae and Siphlonuridae. These families are found in North America.

Classification of the nymphs according to their FFG is related to the River Continuum Concept (RCC) in which the order of the rivers can be identified. Based on the RCC, most ephemeropteran nymphs inhabit second to fourth order stream (Greathouse & Pringle, 2006; Vannote *et al*., 1980), while most shredders inhabit the first order river. However in Malaysia, no ephemeropteran shredder has been recorded (Yule *et al*., 2009).

2.4 Secondary production of Ephemeroptera

Secondary production is defined as consumption of the producer and the generation of body tissue by consuming such producers. This includes the movement of energy and organic material between trophic levels. According to Jackson and Fisher (1986), aquatic insects such as ephemeropteran nymphs exchange nutrients and energy in the form of dissolved substances and particulate matter. The term 'production rate' was described by Clarke (1946) as the amount of tissue elaborated per unit time in a unit area.

In upper streams, Benke and Jacobi (1986) reported that ephemeropteran nymphs are the major component of stream benthos which contributes substantially to total secondary production. However, when the water temperature is higher than 15^{o}C such as in tropical and subtropical areas, the estimation of the growth rate or secondary production becomes difficult because the ephemeropteran nymphs grow very fast. Both Benke and Jacobi (1986) and Boyero *et al.* (2009) suggested the application of an alternative approach to estimate the production of the nymphs. Usually the productivity of ephemeropteran nymphs varies widely among regions due to the differences in the river substrates, foods, water chemistry and the biota (Neves, 1979).

Estimation of the secondary production of ephemeropteran nymphs is important because productivity is always integrated with life history, survival, growth and voltinism of the nymphs (Dudgeon, 1996). These factors are useful when the study is collaborated with the determination of water quality of the rivers (Krueger & Waters, 1983) and habitat suitability for other animals since ephemeropteran nymphs are one of the major components of river food chain.

The differences of ephemeropteran nymphs production in rivers are related to the differences in the amount and type of terrestrial photosynthesis input in the water (Krueger & Waters, 1983). Riparian vegetation has been found to contribute to river productivity. As such, rivers in tropical areas have higher production due to dense growth of riparian plants along riverbanks in comparison to the rivers in temperate region. Krueger and Waters (1983) suggested that riparian vegetation in temperate regions did not have obvious differences in terrestrial input among streams. Petersen and Cummins (1974) supported the finding when they reported that leaves from riparian vegetation were rapidly processed in the temperate stream ecosystem, consequently they do not contribute to rivers' input.

2.5 Distribution of the Ephemeroptera

Ephemeroptera is an important component of zoobenthos community in running water (Obrdlik *et al*., 1979). The distribution of the Ephemeroptera is strongly influenced by biotic and abiotic factors. Predation and competition are the most important biotic factors that affect the distribution of the Ephemeroptera. Meanwhile Obrdlik *et al*. (1979) found that Ephemeroptera distribution is influenced by abiotic factors such as water temperature, content of dissolved oxygen, flow rate, water quality, stream character, and food availability.

The distribution of Ephemeroptera is reflected by the regional faunal diversity. However, in many studies, its diversity pattern is linked with the sampling effort (Barber-James *et al*., 2008). Based on the most recent study, Ephemeroptera in the Holartic region represented the highest Ephemeropteran species diversity. Many studies also were done in this region especially in North America (Merritt *et al*., 2008). The Holartic realm is divided into two zones: Nearctic and Palearctic. These two regions support the highest diversity of Ephemeropteran species in the world but have the lowest generic diversity.

In regions other than Holarctic, diversity of Ephemeroptera are mostly underestimated because these areas, especially Oriental and Afrotropical regions, are still not properly explored. Furthermore, ecological studies in India and South East Asia are very scarce (Gupta & Michael, 1992). However, it was reported by Sartori *et al.* (2003) in a recent study of the ephemeropteran fauna in a tropical forest of Borneo led to the finding of more than ten genera and more than ten new species. In Antartica, the Ephemeroptera is still undiscovered.

The lowest diversity of Ephemeroptera species has been recorded from the Australasian and Palaearctic regions (Barber-James *et al*., 2008). Many genera are endemic to certain regions. Peters *et al*. (1978) and Peters & Peters (1980) reported that 18 genera and 37 species of Leptophlebiidae were endemic to Pacific components, more specifically in New Caledonia.

2.6 Distribution of Ephemeroptera in Malaysia

The distribution of Ephemeroptera in Malaysia is scarcely reported. However, Ephemeroptera dominated most upstream rivers (Che Salmah *et al.,* 2007, Azrina *et al.,* 2006). Since Ephemeroptera preferred to live in clean water and some species are very sensitive to changes in water quality, they are most diverse in rocky-bottomed, second and third order streams (Edmunds & Waltz, 1996). Their distribution also varies according to types of microhabitat. They prefer the stable habitat for colonization and food source availability (Che Salmah *et al.,* 2001).

According to Edmunds and Polhemus (1990), in Malaysia, more than 50 ephemeropteran genera which belong to 15 families have been described. In Perak's Kerian River Basin, Ephemeroptera is the dominant order with 10 described genera in 10 families (Che Salmah *et al.,* 2001). In Selangor, the central state of peninsular Malaysia, Azrina *et al.* (2006) reported that 16 Ephmeroptera genera dominate the upstream Langat River. The number of Ephemeropteran genera is the highest in Perak's Temenggor Catchments located in northern peninsular Malaysia. Nineteen genera had been described from the area (Che Salmah *et al.,* 2007). However, fewer families (9 families) were recorded there in comparison to Langat River and Borneo with 10 families each (Azrina *et al.,* 2006) and 15 families respectively (Edmunds & Polhemus, 1990). In comparison, thirteen families were collected from 10 southernmost provinces in Thailand. However, from 28 genera, twelve genera are reported for the first time in Thailand (Sites *et al.* 2001).

2.7 Influence of physico-chemical parameters on the distribution of ephemeropteran nymphs

2.7.1 Microhabitat

Habitat for ephemeropteran nymph communities in riverine ecosystems could be materialized within the framework of various spatio-temporal scales (Subramanian & Sivaramakrishnan, 2005). They range from as fine as silt (<0.06 mm) up to the biggest of boulder (>25 cm). Fenoglio *et al.* (2008) reported that common ephemeropteran microhabitats consisted of coarse particle such as boulders, cobble and gravel. Pools (Subramanian & Sivaramakrishnan, 2005) and leaf packs (Lamouroux *et al.,* 2004) are considered as the habitat for some ephemeropteran nymphs. Stones of various sizes provided suitable habitats for many ephemeropteran nymphs (Fenoglio *et al.,* 2008). Boulders are stones with diameters ranging between 26 cm and 45 cm, cobble ranges between 6 cm and 25 cm, and gravel ranges between 6 mm and 60 mm (Fenoglio *et al.,* 2008). Boulders provide stable habitat for the nymphs because they are difficult to be carried away by water current. Half exposed or half embedded boulders in the river substrate are stable for colonization by ephemeropteran nymphs. In contrast, cobble and gravel are exposed to fast water currents (Sweeney *et al.,* 1995), therefore less stable as habitats for the nymphs.

2.7.2 Dissolved Oxygen

Dissolved oxygen in aquatic environment is contributed by diffusion from surrounding air, aeration or rapid movement of the water that traps the oxygen into the water. Photosynthesis of aquatic plants contributes oxygen into the water as waste product. Aquatic macroinvertebrates including ephemeropteran nymphs need oxygen for their locomotion, growth and reproduction.

Water that has contact with the atmosphere has a partial pressure of dissolved oxygen similar to that of the air. Concentration of dissolved oxygen in the aquatic ecosystem is approximately 10 mg O_2 1^{-1} at 15^{o}C. In comparison, oxygen in the air amounts 299 mg O_2 1^{-1} (Kramer, 1987). The amount of dissolved oxygen in aquatic environment is low corresponding to increase of temperature and salinity. Therefore the amount of the oxygen is low in the warm water river compared to cold water river (Fry, 1971).

For aquatic animal such fish and macroinvertebrates, their activities are dependent on the amount of dissolved oxygen in the ecosystem. For terrestrial insects, relatively high concentration of oxygen in the air prevents them from oxygen limitation, and their activities are more likely to be limited by food availability (Kramer, 1987). In response to this limiting factor, aquatic insects including ephemeropteran nymphs have adapted to the situation by changing their activities, such as increased their breathing and increased use of aquatic surface respiration. Those species of aquatic insects with minimal energy respire through anaerobic pathway (Hochachka, 1982).

2.7.3 pH

pH of water in rivers is the measure of how acidic or basic the water is on a scale from 0 to 14, with neutral being 7. The results from many studies suggested that the distribution of ephemeropteran nymphs changes due to changes of water pH (Al-Shami *et al.,* 2010; Pond, 2010). Most ephemeropteran nymphs prefer to inhabit neither acidic nor basic water (Kamsia *et al.,* 2008). Rohasliney and Jackson (2008) reported that ephemeropteran nymphs tended to be correlated with relatively moderate to high pH. However, some families like Baetidae, Ameletidae, Heptageniidae and Isonychidae are more tolerant to slightly acidic water (Pond, 2010).

2.7.4 Temperature

Temperature is the factor most frequently reported to have influenced the life cycle of the Ephemeroptera (Sweeney, 1984). Temperature not only influences the growth of ephemeropteran nymphs, it indirectly influences the availability, quantity and quality of food source in aquatic ecosystem (Giberson & Rosenberg, 1992) by influencing the algal production and microbial growth rates. According to Waltz and Burian (2008), the length of the ephemeropteran nymphal life varies with water temperature. In temperate regions, the life history of nymphs is longer in comparison to those in tropical regions. For instance, the development of *Hexagenia limbata* (Ephemeridae) varies from 1 to 2 years in winter, yet this species may develop less than 17 months in warmer streams (Waltz & Burian, 2008).

2.7.5 Total Suspended Solid (TSS) and Total Dissolve Solid (TDS)

Total Suspended Solid (TSS) is used to quantify concentrations of suspended solid-phase material in the surface of the water in riverine ecosystems. The TSS comprise of a variety of materials such as silt, animal matter, sewage and decaying plants (aquatic and terrestrial) that are trapped by a filter (APHA, 1992).

Total Dissolve Solid (TDS) is used to measure dissolved organic matter, inorganic salt and other dissolved materials in the river (U.S. Environmental Protection Agency, 1986). The concentration of TDS in natural waters is determined by habitat geology and water drainage, water balance (evaporation-precipitation) and atmospheric precipitation. Mean salinity in the rivers generally is approximately 120 mg L^{-1} and most commonly, anions are found in the rivers as bicarbonate (68 mg L^{-1}) followed by sulfate (20 mg L^{-1}). Usually, calcium is the cat-ion found in rivers (21 mg L^{-1}), and silica and sodium are found in lesser amount (each one is 9 mg L^{-1}) (Weber-Scannell & Duffy, 2007). Brackish water contains more than 1000 mg L^{-1} TDS. Changes in TDS usually relates to pollution or industrial effluent. However, most upstream rivers have low TDS because they are far from human activities. High

TDS limits species biodiversity and increase tolerant species in the riverine ecosystem. High TDS increases salinity and leads to the decline of ephemeropteran nymphs in the river (Weber-Scannell and Duffy, 2007). However, some species such as *Hexagenia bilineata* (Ephemeridae) can survive in water with TDS up to 2000 mgL^{-1} (Woodward *et al.,* 1985).

2.7.6 Velocity

Distribution of ephemeropteran nymphs in the rivers largely regulated by water velocity (Ciborowski, 1982) which also controls the availability of food and distribution of the river substrate. Allocthonous organic matter which serves as important food for the ephemeropteran is carried by the water current to other parts of the river. Along with food availability and substrates, water flow is important for the nymphs to escape from predators by drifting in the current (Hildebrand, 1974). Fast flowing water may sweep the ephemeropteran nymphs from their habitat. For instance, Bishop (1973) discovered that harsh water during flood and spate washed all the aquatic insects in Gombak River to its downstream areas.

2.7.7 Ammonia-Nitrogen NH_4-N_3 (NH_4)

Ammonia-nitrogen is the waste product of animals including aquatic insects. Ammonia-nitrogen is directly excreted into the water as one of the important sources of nitrogen for aquatic plants. In rivers, ammonium undergoes a two step process before being transformed into nitrate compound which produces nitrite as the intermediate product (Beketov, 2004). Since ammonia-nitrogen, nitrate and nitrite are toxic to living organisms, their amounts in the water correlates with river water quality. The amount of ammonia-nitrogen influences the colonization of ephemeropteran nymphs. However, different species of the nymphs respond differently to different concentrations of ammonia-nitrogen in the water (USEPA, 1999).

2.8 Ephemeroptera as the bioindicator of the rivers

Mayflies play an important role in almost all aquatic ecosystems: Ephemeropteran nymphs are accepted as worldwide bioindicators of water quality and ecological integrity (Barbour *et al*., 1999; Bauernfeind & Moog, 2000). Mayfly assemblages and diversity in their natural habitats correlated with water quality thus implies their high suitability to live in polluted environment. Availability of simple sampling technique and identification keys of both nymphs and adults make them very useful and reliable as tool for biomonitoring (Bauernfeind & Moog, 2000). Krno (1990) has demonstrated that ephemeropteran nymph community structure effectively reflects aquatic environmental situation in river ecosystems. However, human activities lead to the pollution of the water sources or rivers. Ephemeropteran nymphs which assemble in clean water habitat provide sufficient data for river biomonitoring assessment. Buffagni (1997) proposed the operational unit (O.U.) of ephemeropteran nymphs on the basis of their sensitivity to water quality or habitat alteration. This is an example on how the nymphs are manipulated as a useful tool for biomonitoring.

In Malaysia, there is no exclusive study of ephemeropteran as a single bioindicator for assessment of water quality. Few studies conducted used it in combination with other macroinvertebrates such as EPT as a tool for biomonitoring (Al-Shami *et al*., 2010; Azrina *et al*., 2006; Che Salmah *et al.*, 1999). However, an exclusive study on Ephemeroptera as bioindicators might be useful for biomonitoring as they have the highest significant correlation with all water parameters (Kamsia *et al*., 2008).

CHAPTER 3:

DISTRIBUTION AND ABUNDANCES OF EPHEMEROPTERA NYMPHS IN RIVERS OF RBSP

3.1 Introduction

Ephemeroptera is widely distributed throughout fresh water environment, especially in pristine areas (Bauernfeind & Moog, 2000). They live in various types of standing and running waters. Ephemeroteran nymphs dominated most habitats in the Kerian River Basin, Malaysia (Che Salmah *et al*., 2001). Many of them inhabit boulders and gravel while some burrow into sand, fine sediment and detritus (Subramanian & Sivaramakrishnan, 2005). Ephemeroptera plays an important role in aquatic habitat, and is one of the major components of the food web (Vannote *et al*., 1980, Tamanova *et al*., 2006; Mantel *et al*., 2004; Dudgeon *et al*., 2010). Recently, Ephemeroptera together with Plecoptera and Trichoptera have become more frequently used as a biological monitoring tool to assess water quality, particularly in headwaters (Bispo *et al*., 2006). Ephemeropteran diversity in rivers provides information on the status and condition of the rivers (Bauernfeind & Moog, 2000). Human activities such as logging and mining have caused tremendous disappearances of ephemeropteran genera from their habitat (Che Salmah *et al*., 2007).

In general, most ephemeropterans in tropical streams including in Malaysia, are collector-gatherers, scrapers and collector-filterers (Merritt *et al*., 2008). Very few are shredders (Rosemond *et al*., 1998; Yule *et al*., 2009). Collector-gatherers are dominant in forested areas (Medhurst *et al*., 2010; Rosemond *et al*., 1998) implying their important contribution to leaf breakdown dynamics. Similar to collector-gatherers, shredders are relatively abundant at shaded areas in headwater (Bottorff & Knight, 1989; Dudgeon, 1989). They live on coarse particulate organic matter (CPOM) (>1 mm) and very important in undisturbed forested watershed because smaller headwater streams receive allocthonous matter from riparian vegetation that provides their food source (Boyero *et al*., 2009).

In Malaysia, studies on the distribution and abundance of ephemeropteran nymphs are lacking. Previous reports included this group of insects as part of the Ephemeroptera, Plecoptera and Trichoptera (EPT) fauna (Jongkar, 2000; Ghee, 2004; Che Salmah *et al*., 2001; Che Salmah *et al*., 2007). Jongkar (2000) had documented the fauna of aquatic insects in feeder streams of Temenggor Lake, (adjoining lake, south of RBSP). A few years later, Che Salmah *et al*. (2007) reported higher diversity of the Ephemeroptera in the streams compared to two other dominant orders, Plecoptera and Trichoptera.

This study was carried out to investigate the diversity of the Ephemeroptera community in the rivers of Royal Belum State Park in Perak and possible factors that influenced their abundance.

3.2 Materials and methods

3.2.1 Study Site

This study was carried out in four selected rivers in Royal Belum State Park (RBSP) in Perak State, northern peninsular Malaysia; Kejar River, Mes River, Tan Hain River and Ruok River. Kejar River is located at the northern part of the Royal Belum State Park (Figure 3.1) and Mes River is approximately one kilometer south of Kejar River. Ruok River is located near to the entrance of the park which is guarded by an army post, and Tan Hain River is situated at the middle of the park. Insect sampling was conducted monthly for thirteen months, starting from February 2009 until February 2010. Preliminary sampling was carried out in December 2008 and January 2009.

Kejar River (N05°48'52.4", E101°25'49.0"), located at the northern part of the park at 273 m asl, flows into Perak River. The river was surrounded by dipterocarp trees and herbaceous plants that were dominant along the margins. River substrates comprised 20% boulder, 30% cobble, 30% gravel and 20% sand.

Mes River (N05°47'59.4", E101°24'51.0") is a relatively short river, located about 1.5 km from Kejar River at 261 m asl about 200 meter from Kejar Hilir Orang Asli (aborigin) Settlement. Thirty families of Orang Asli (aborigines) lived in the settlement and they used the water from Mes River for daily consumptions. There was a waterfall at the upper part of the river and a site approximately 100 meters downstream was available for sampling of ephemeropterans. The main vegetation on the river banks included dipterocarp trees and herbaceous plants. River substrates comprised of boulders, cobbles and gravel. Several riffles and runs were observed in this river whereas pools were relatively uncommon.

Tan Hain River, located at 290 m asl (N05°44’49.6”, E101°23’03.6”) drained through a wider undisturbed, forested watershed as compared to other rivers. Similar to Mes River, it was surrounded by dipterocarp trees and herbaceous plants. Some grasses grew along river margins. Approximate 30% of the river substrates comprised moderate to large sizes boulders. The width of this river approached 15 meters in some parts. Water flow was fast and very few pools (5%) occured in the river.

Ruok River (N05°36’18.7”, E101°25’15.9”), at 295 m asl, is located at the southern part of the park near the entrance of the RBSP. River substrate consisted of mainly sand with many fallen leaves (leaf packs). The surfaces of boulders and cobbles in this river were rough. The water was partly shaded by relatively heavy growth of dipterocarp trees and herbaceous plants on both banks.