ABUNDANCE AND DIVERSITY OF AQUATIC INSECTS IN RELATION TO THE PHYSICO-CHEMICAL PARAMETERS IN SEVERAL RIVERS FROM BUKIT MERAH CATCHMENT AREA

SITI HAMIDAH BINTI ISMAIL

UNIVERSITI SAINS MALAYSIA

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by

SITI HAMIDAH BINTI ISMAIL

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LIST OF ABBREVIATIONS

AFDM	Ash- Free Dry Mass
AN	Ammonia- nitrogen
APHA	American Public Health Association
BL	Body length
BMCA	Bukit Merah catchment area
BMWP	Biological Monitoring Working Party
BOD	Biochemical Oxygen Demand
CCA	Canonical Correspondence Analysis
Cd	Cadmium
CDF	Canonical Discriminant Function
CDHO	Coleoptera Diptera Hemiptera Odonata
cm	Centimeter
COD	Chemical Oxygen Demand
CPI	Cohort Production Interval
Cr	Chromium
Cu	Copper
D	Simpson’s Index
DM	Dry- mass
DO	Dissolved Oxygen
DOE	Department of Environment
E	Pielou’s Evenness Index
EPT	Ephemeroptera Plecoptera Trichoptera
ETOH	Ethyl alcohol
FBI	Family Biotic Index
Fe	Ferum
g	gram
GIS	Geographic Information System
GPS	Global Positioning System
H’	Shannon Wiener Diversity Index
Hg	Mercury
HNO_3	Nitric acid
HW	Head width

IDW	Inverse Distance Weighted
m	Meter
MARS	Microwave Accelerated Reaction System
mg/l	Milligram per liter
mm	millimeter
Mn	Manganese
m/s	Meter per second
ND	Not detected
NH_3	Ammonia nitrogen
NH_4^+	Ammonium
Ni	Nickel
NO_2	Nitrite
NO_3	Nitrate
OM	Organic matter
P	Significant value
Pb	Lead
pH	Potential hydrogen
ppm	Part per million
r	correlation
R	Richness Index (Menhinick Index)
SE	Standard error
SPSS	Statistical Package for Social Science
TDS	Total Dissolved Solid
TI	Total Inertia
TSS	Total Suspended Solid
TVE	Total Variance Explained
USEPA	United State Environmental Protection Agency
USGS	United State Geological Survey
WPL	Wing pad length
WQI	Water Quality Index
Zn	Zinc
^oC	Degree Celsius

KELIMPAHAN DAN KEPELBAGAIAN SERANGGA AKUATIK DAN HUBUNG KAIT DENGAN PARAMETER FIZIKO-KIMIA DI BEBERAPA SUNGAI DI KAWASAN TADAHAN BUKIT MERAH

ABSTRAK

Kelimpahan dan taburan serangga akuatik berhubung dengan beberapa parameter fiziko-kimia di empat sungai sekitar kawasan tadahan Bukit Merah telah dikaji. Sejumlah 13, 483 individu serangga akuatik dari 7 order telah dikutip setiap bulan dari Mei 2014 sehingga April 2015 menggunakan cara ‘Kick-net’ dan ‘drag’. Sungai Kurau sebagai tapak kawalan telah mencatatkan bilangan serangga akuatik yang tertinggi dengan 3941 individu diikuti oleh Sungai Ara (3922 individu), Sungai Ayer Itam (3112 individu) dan Sungai Jelai (2508 individu). Sungai Jelai mempunyai taburan dan kekayan serangga akuatik yang tinggi dengan nilai tertinggi dalam semua indek ekologi berbanding Sungai Ara, Sungai Kurau dan Sungai Ayer Itam. Kelimpahan serangga akuatik didapati berbeza di keempat-empat sungai (χ^2 = 20.03, P =0.00) dan di antara bulan persampelan (χ^2 = 189.61, P = 0.00). Berdasarkan Indek Kualiti Air (WQI), keempat-empat sungai telah dikategorikan mempunyai kualiti air yang bersih (Kelas II) manakala skor ‘Family Biotic Index (FBI)’ mengkelaskan keempat-empat kualiti air di sungai tersebut sebagai bersih, sangat bersih dan bagus. Biplot CCA menunjukkan kebanyakan serangga akuatik tidak dapat bertoleransi dengan kandungan (NH_3-N) AN, ‘Total Dissolved Solid (TDS)’ dan Ni yang tinggi. Walau bagaimanapun, *Baetis gombaki, B. illiesi, Thalerosphyrus* sp., *Gumaga* sp., *Naucoris* sp. dan *Tipula* sp. dapat bertoleransi dengan kandungan Cr, Cu, Pb dan organik yang tinggi. Selanjutnya, *Baetis* species dari order Ephemeroptera telah dianalisis menggunakan analisis IDW dari perisian ArcGIS 10.3. Daripada tujuh

spesies, *B. illiesi* (120 individu) merupakan spesies *Baetis* yang dominan diikuti oleh *B. idei* (119 individu). Berikutan kelimpahan tertinggi *B. illiesi* dan *B. idei* di empat sungai persampelan, kedua spesies tersebut telah dianalisis untuk kitaran hidup dan pengeluaran sekunder. Berdasarkan pengiraan Wilks lambda, sembilan peringkat instar (F-8 - F) telah terbentuk di plot berselerak Analisis Fungsi Diskriminan (DFA). *B. idei* dan *B. illiesi* mempunyai kitar hidup multivoltin di kesemua sungai. Sungai Ara mencatatkan pengeluaran tahunan yang tertinggi untuk *B. idei* (16.55 mg m^{-2} y^{-1}) dan *B. illiesi* (20.34 mg m^{-2} y^{-1}). Kadar pusingan (P/B) yang tertinggi untuk *B. idei* adalah dari Sungai Ara dengan 1.56 sementara Sungai Jelai dengan nilai 3.14 untuk *B. illiesi*. Berdasarkan kajian ini, Sungai Ara dan Sungai Kurau merupakan sungai yang paling digemari oleh semua order serangga akuatik dengan Sungai Ara telah diklasifikasikan sebagai kawasan yang paling digemari untuk semua *Baetis* sp. *Baetis* sp. lebih menggemari sungai yang mempunyai kelebaran yang lebih kecil dengan sebahagiannya dilitupi kanopi dan sedikit terdedah di mana 25-50% batu, kerikil dan partikel tanah terdedah di atas permukaan air.

ABUNDANCE AND DIVERSITY OF AQUATIC INSECTS IN RELATION TO THE PHYSICO-CHEMICAL PARAMETERS IN SEVERAL RIVERS FROM BUKIT MERAH CATCHMENT AREA

ABSTRACT

Abundances and distribution of aquatic insects in relation to several physico-chemical parameters from four rivers of Bukit Merah catchment area were studied. A total of 13, 483 individuals of aquatic insects from 7 orders were collected monthly from May 2014 until April 2015 using Kick-net and Drag method. Kurau River, as the control site, had the highest individual numbers of aquatic insects collected with 3941 individuals followed by Ara (3922 individuals), Ayer Itam (3112 individuals) and Jelai (2508 individuals) rivers. Jelai River had more diverse and richer aquatic insect assemblages with the highest value of all ecological indices compared to Ara, Kurau and Ayer Itam rivers. The abundances of aquatic insects were significantly different among the four rivers (χ^2 = 20.03, P =0.00) and between months of sampling (χ^2 = 189.61, P = 0.00). Based on Water Quality Index (WQI), all the four rivers were categorized as having good water quality (Class II) while scores of Family Biotic Index (FBI) classified the water quality of the four rivers as good, very good and excellent. CCA biplot showed that most of the aquatic insects could not tolerate with high (NH_3-N) AN, Total Dissolved Solid (TDS) and Ni. However, *Baetis gombaki*, *B. illiesi*, *Thalerosphyrus* sp., *Gumaga* sp., *Naucoris* sp. and *Tipula* sp. could tolerate with high Cr, Cu, Pb and organic contents. *Baetis* sp. from order Ephemeroptera was further analysed using IDW analysis from ArcGIS 10.3 software. Out of seven species, *B. illiesi* (120 individuals) was the dominant, followed by *B. idei* with 119 individuals. Due to highest abundances of *B. illiesi* and *B. idei* in the four sampling rivers, these

two species were further analysed for life history and secondary production. According to the Wilks lambda calculation, nine instar stages (F-8 – F) were formed on the Discriminant Function Analysis (DFA) scatter plot. *B. idei* and *B. illiesi* had multivoltine life cycles in all rivers. Ara River had the highest annual production for both *B. idei* (16.55 mg m^{-2} y^{-1}) and *B. illiesi* (20.34 mg m^{-2} y^{-1}). Besides, the highest turnover ratio (P/B) of *B. idei* was from Ara River with 1.56 while Jelai River had the highest for *B. illiesi* (3.14). Through this study, Ara and Kurau River were classified as the most preferred habitat for all the aquatic insects. In addition, Ara River was classified as the most preferred habitat and hotspot area for all the *Baetis* sp. *Baetis* sp. preferred smaller streams' width with partly shaded canopy cover and marginal embeddedness where 25-50% of cobble, gravel and sand particles were exposed on the water surface.

INTRODUCTION

1.1 Introduction

Aquatic insects can be found almost in all types of aquatic habitat throughout the world including lakes, rivers, highly saline pools, hot springs and coastal waters (Yule and Yong, 2004). Large number and wide variety of aquatic insects in the aquatic habitat make them of a great ecological importance (Yule and Yong, 2004). Aquatic insects play important roles in river ecosystem. They may feed on both autochthonous and allochthonous food resources and they, in return fed upon by many fish (Wantzen and Junk, 2000). Aquatic insects are important members of the food web and their optimum growth and diversity are reflected in the well- being of the higher forms such as fish.

Natural ecosystems all over the world had been adversely affected by human activities in the name of development. The most important natural resources such as water and soil have been polluted with all sorts of pollutants. Pollutants such as organic pollution, habitat degradation and pesticides usage causing major impacts on the abundances and distributions of aquatic macroinvertebrates in the rivers (Plafkin *et al*., 1989; Schulz and Liess, 1999). Habitat destruction such as deforestation, active land development, opening of recreational rivers, flood, land conversion for mechanized agriculture such as oil palm plantations (Mercer *et al*., 2013), industrial and domestic waste discharge (Azrina *et al*., 2006) often contribute to river water quality deterioration in the tropical regions and cause extinction of many aquatic insects (Yule and Yong, 2004).

Aquatic insects are very sensitive to any changes which occur in the environment and their degree of sensitivity differs among various groups (Hering *et* *al*., 2009). Aquatic insects are suitable for assessing site specific impacts due to their limited migration (Kripa *et al*., 2013). Besides, their assemblages that were made up of species that constitute a broad range of trophic levels and pollution tolerances also contribute to their suitability in assessing site specific impacts (Prommi and Payakka, 2015). Different genus of aquatic insects has different habitat preferences and pollution tolerances. However, water quality deterioration causes the absence of sensitive species and the presence of the tolerant species. Therefore, biological indices such as Family Biotic Index (FBI) have been used to evaluate the water quality.

There are several factors which affect the abundances and distributions of aquatic insects in the river. Requirements of nutrients, structure of vegetation, water quality and substrate components are different among species. Water quality parameter such as Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Total Suspended Solid (TSS), Total Dissolved Solid (TDS), pH, Ammonium nitrate (AN), water temperature and velocity as well as organic and heavy metal contents in the sediments also play an important role for the abundance and distribution of aquatic insects. In Malaysia, there are several studies done on the effects of anthropogenic activities such as agriculture (Azrina *et al*., 2006; Mercer *et al*., 2013), recreational activities (Wahizatul *et al*., 2006), industrial activities (Al-Shami *et al*., 2011), forest logging and fragmentation (Che Salmah *et al*. 2013), organic and heavy metal contamination (Ishadi *et al.*, 2014) on the abundance and distribution of aquatic insects.

In addition, one of the important groups of insects used in the bio-assessment of the rivers is Ephemeroptera (mayfly). This is due to their presence in a wide variety of habitats and substrates, and their increasing chances of detecting pollution impacts (Nerbonne and Vondracek, 2001). Ephemeropterans are widely distributed throughout the fresh water environment and they live in various types of standing and running waters (Elliott and Humpesch, 2010). Ephemeropterans are one of the major orders of lotic insects in streams. They are also known as mayflies. Mayflies spend more than 99% of their lives as larvae residing in the rivers, filling many crucial roles in freshwater ecosystems as they feed and grow (Merrit *et al.*, 2008). In addition, ephemeropterans also serve as an important food source for fish and other aquatic vertebrates in the food web (Parkyn *et al*., 2000). In Malaysia, ephemeropterans' larvae have been incorporated as freshwater biomonitoring tools in rivers of Telipok River, Sabah (Kamsia *et al*., 2007), Langat River, Selangor (Azrina *et al*., 2006), Semenyih River, Selangor, (Yap *et al*., 2003) and Kerian River Basin, Perak (Che Salmah *et al*., 2001). Out of all the ephemeropterans, genus *Baetis* is classified as the most sensitive group (Arimoro and Muller, 2010). *Baetis* species is the only tolerant and is able to survive within a specified range of water parameters. Besides, changes of water quality parameters in the rivers might alter the abundances and distribution of *Baetis* species as well. Therefore, in this study *Baetis* species was selected to provide clearer view on the effects of pollution towards its abundance and distribution by using Geographic Information System (GIS) application.

Geographic Information System (GIS) is a computer system that manages to process and analyse the spatial data. It has become an integral part of aquatic science and limnology (Johnson and Gage, 1997). Environmental parameters such as water quality, width and depth of the river, canopy cover, riparian vegetation, chemical parameters of soil and water in the river are constantly changing from time to time. To be able to keep up with these changes, technological advancements have provided scientists easier methods to enhance all aspects of scientific investigation, from geographical and spatial data to computer mapping of habitats. Agencies such as the Department of Wildlife and National Parks as well as other federal and state agencies are utilizing GIS to aid in their conservation efforts.

In addition, GIS also provides the availability for the information to be shared and updated at any time through the use of database collection. Spatial analysis, 3D analysis and network analysis are examples of several analysis provide by the GIS software (Huang *et al*., 2001). Spatial analysis is the common analysis used in aquatic science studies. In spatial analysis, spatial information or data are manipulated to extract new information and relationship from the original data (Huang *et al*., 2001). Spatial analysis can be used to obtain an output that fulfills the criteria of data layers. This analysis can be used to find out the sensitive and hotspot area for certain animals or species.

Baetis species and other aquatic insects play a central role in the flow of materials and energy through most terrestrial and benthic freshwater food webs. *Baetis* sp. larvae is a major group of ephemeropteran that dominantly contributes to total secondary production (Benke and Jacobi, 1986). Life history research has a long tradition in benthic biology because of its value in explaining patterns observed in nature, quantifying trophic relationships and energetic, and interpreting experimental results (Rosenberg and Resh, 1993). Good life history data of *Baetis* species is critical to the application of cohort methods for calculation on the secondary production and there is an extensive literature on Ephemeroptera life histories (Benke *et al.*, 1984; Benke and Jacobi, 1986; Benke, 1993). However, determination of life history and production for Ephemeroptera especially *Baetis* species is often difficult as a result of rapid growth and asynchronous development (Benke and Jacobi, 1986). Life history traits such as voltinism, growth rates, generation times and synchrony of development are related with the spatial and temporal heterogeneity of the environment (Townsend and Hildrew, 1994).

Response of these life history traits to shifts in environmental conditions will cause intraspecific changes to occur. Furthermore, seasonal variations in density, biomass and annual production are strongly influenced by life cycle parameters and this indicates the importance of correct life cycle information in production studies (Perán *et al.*, 1999).

Rivers in Bukit Merah catchment area are the water sources for Bukit Merah dam and the main purpose of the dam is to provide irrigation water and to meet the domestic demands. Recently, rivers in Bukit Merah catchment area have received different types of effluent based on the land use activities on the land nearby. These land use activities may have released organic and heavy metal residues into the water which might affect the aquatic insects. In this study, it was hypothesised that river with different land use activities at area nearby would have different abundances and compositions of aquatic insects. This study was the first of its type of study in the area. Thus, this research attempts to determine the effect of land use activities on the abundance and distribution of aquatic insects in Ara, Jelai, Kurau and Ayer Itam rivers in Bukit Merah catchment area as well as to classify the water quality status of four sampling rivers. Different types of effluent received in each river from the land use activities on the land nearby might affect the abundance and cause variation in the composition of aquatic insects' order. Furthermore, through this study, information about the preferable and hotspot area of *Baetis* species and also their distribution in selected rivers at Bukit Merah Catchment Area in Perak could be obtained via GIS application. Besides, this study also will provide data on life history and secondary production of *Baetis idei* and *B. illiesi* for further references. Information on the preferable habitat for each *Baetis* species was obtained through this study.

1.2 Objectives

This study was conducted on aquatic insects in four rivers from Bukit Merah catchment area, Perak with the following objectives:

1) To investigate the variation of aquatic insects communities in Bukit Merah catchment area of different habitat variability and physico-chemical parameters.
2) To map and analyse the abundances and distribution of ephemeropteran species using Geographic Information System (GIS).
3) To study the life histories and secondary production of selected ephemeropterans in several rivers from Bukit Merah catchment area.

LITERATURE REVIEW

2.1 Biological monitoring and aquatic insects as bio-indicators

Water is one of the most important resources and is the elixir of life. Fresh water has a pivotal role in sustenance of life of human beings and other organisms in the environment as well as maintaining the balance of nature. Rivers run through various regions of the earth like capillary blood vessels in the body. The characteristics of rivers differ depending on various factors such as geology, climate and topography. However, due to tremendous development of industry and agriculture nowadays, water ecosystem has become perceptibly altered in several aspects in these recent years. They are exposed to all local disturbances regardless where they occur (Gupta *et al*., 2013). The pollutants could be suspended particles, colloidal materials, heavy metals and could also be dissolved cationic and anionic substances. Man-made activities such as agriculture, domestic, industrial as well as recreational are the main sources of water pollution.

Aquatic insects are abundant and diverse groups that inhabit a variety of aquatic environments. They predominate in the trophic structure of the rivers (Wallace *et al*., 1997). These organisms are an important component of aquatic food webs because they break down and process organic matter and provide food for vertebrates and invertebrates (Wallace and Webster, 1996). Most of the aquatic insects undergo an aquatic immature stage followed by terrestrial adults. Physical appearances of the aquatic insects varies depend on the order and type of metamorphosis that the group undergoes. Insects with incomplete metamorphosis have immature stages that appear relatively similar to the adult (eg; Ephemeroptera, Hemiptera, Odonata and Plecoptera) (Bouchard, 2004). In contrast, insects with complete metamorphosis possess immature stages that are very different than the adults (eg; Coleoptera, Diptera and Trichoptera) (Bouchard, 2004). Apart from being important to aquatic food web, aquatic insects also are used for biomonitoring and act as bio-indicators of maintaining water quality in the rivers.

In an ideal situation, running waters should be assessed in physical, chemical and biological parameters in order to provide a complete spectrum of information for appropriate water management (Georgudaki *et al.,* 2003). Traditionally, the quality of waterways or stream health has been assessed by monitoring a range of water quality parameters. However, water chemistry parameter can vary markedly, both daily and seasonally (Humphries and Baldwin, 2003). In order to get the accurate trend of water quality parameters, the data need to be collected frequently over a long period of time and this can be time consuming and expensive, especially if numerous parameters are measured. Macroinvertebrates especially aquatic insects live almost continuously in water. Diversity and sensitivity of aquatic insects to environmental stressors such as organic and inorganic pollutants make them more suitable as effective estimators of overall (Rosenberg and Resh, 1993).

Biological quality can be assessed by different kinds of organisms such as diatoms, aquatic vegetation, invertebrates and fishes (Georgudaki *et al*., 2003). Stream macroinvertebrates have a range of environmental preferences and they represent a diverse group which integrates ecosystem changes over time. Therefore, they are widely used as the indicator of environmental disturbance (Metzeling *et al.,* 2003). Aquatic insects comprise a taxonomically diverse and ecologically important group of animals in fresh water systems. They are known to play a very significant role in the processing and cycling of nutrients as they belong to several specialized feeding groups such as shredders, filter feeders, deposit collectors and predators (Gupta *et al*., 2013).

Biotic indices are a numerical estimation of river's health based on the tolerance or sensitivity of aquatic insects towards an environmental gradient (organic or inorganic pollution) (Rosenberg and Resh, 1993). The representative samples are collected to evaluate the water. Quality of water can be assessed by observation of present-absent of some aquatic insects (Moreno and Callisto, 2006). The accurate taxonomy and insects' data are converted into water quality rating (Lenat, 1993). Aquatic insects have various levels of tolerance to pollution and this could be used as an indicator in water quality assessment. According to Azrina *et al*., (2006), relative abundances of aquatic insects have been used to make inferences about pollution loads.

Besides, concentrations of pollutant may vary radically with time. If the pollutants are discharged in clean stream, the chemicals can be detected in a very short period, but its effects may last for several months (Morse *et al*., 1994). Only tolerant species can live in environments with persistent pollution. A community of aquatic insects is very sensitive to stress. This characteristic can be used for detecting environmental perturbations resulting from any contaminants. Larvae of the aquatic insects have limited mobility and have relatively long life span. These characteristics are very useful to detect the contaminated pollution in water ecosystem because the discharged wastes would be difficult to detect by periodic chemical sampling (Buikema *et al*., 1982). Larvae of the aquatic insects are collected monthly and any changes on the present-absent of the abundances and composition of the aquatic insects were studied (Arimoro *et al*., 2007). Besides, larvae of the aquatic insects also had been used as biological indicator to detect the effect of human activities at the rivers towards the present-absent of certain genus or order of aquatic insects (Arimoro *et al*., 2007).

Foote and Hornung (2005) stated that diversity and abundances of odonata larvae are positively correlated with overall aquatic insects' diversity and abundance. He concluded that larvae community can be an accurate bio-indicator of diversity of overall aquatic insect's communities. Odonate species can be used as biological indices in a polluted river because of their sensitivity to human disturbances. Ephemeroptera, Plecoptera and Trichoptera also have been used as metal monitors in the previous heavy metal monitoring study (Sjobakk *et al.,* 1997). In addition, Plecoptera are very sensitive to pollution and habitat disturbance (Eaton and Lenat, 1991). On top of that, Diptera community pattern and diversity are affected by organic effluent (Arimoro *et al.*, 2007). In that study, the abundances and community structure of Diptera in the Nigerian Delta, especially Chironomidae, Culicidae and Syrphidae families showed strong evidence of impact from the abattoir effluents. The distribution of dipteran families very much follows their adaptation to specific habitat and suitability of water quality. Many dipteran families are indicators of polluted water because they are found in high abundance in such environments (Arimoro *et al*., 2007). Some of dipteran families (Simuliidae and Tipulidae) live in moderate clean to clean water (Allan and Flecker, 1993).

2.2 The importance and life history of Ephemeroptera

Mayflies are a vital link in the food web of freshwater ecosystems, making energy stored in algae and other aquatic plants available to higher consumers. Mayflies serve as food for many game fish. Habitat complexity correlates positively with increasing abundances of benthic invertebrates especially Ephemeroptera (Edmunds *et al.,* 1976). The importance of mayflies comes largely from their emergence and mating behavior. Mayflies assure the survival of their species using their "strength in numbers" approach (Rahel and Kolar, 1990). They coordinate their emergence and mating times (both time of year and time of day) so that they leave their safe habitats and emerge together in large numbers in a very short period of time (Rahel and Kolar, 1990). However, mayfly eggs are eaten by snails and caddisfly larvae. The larvae and subimagos may be eaten by fish, frogs, birds, flies, or water beetles (Rahel and Kolar, 1990). For their own nutrition, mayfly larvae move on the stones and weed surface to graze off bacteria (Merrit *et al.,* 2008). They may collect from sediments or feed on detritus (Merrit *et al.,* 2008). Most mayflies are collectors and scrapers (Edmunds *et al.,* 1976). In addition, the larvae also have symbiotic relationships with chironomids that could be commensal (Merrit *et al.,* 2008). Furthermore, ephemeropteran larvae especially *Baetis* sp. are usually microhabitat specialists as each species survives best on specific substrate at a certain depth under the water (McShaffrey and McCafferty, 1990).

The common name for Ephemeroptera is mayfly. The name comes from Greek word meaning ‘living a day’. This is due to the adults having a very short live (Miyairi and Tojo, 2007). The lifespan of an adult mayfly is very short, varying with the species. Ephemeroptera is a hemimetabolous insect having four distinct life stages; egg, nymph, subimago and adult (Barber-James *et al*., 2008). The life cycle of an Ephemeroptera starts when the eggs are laid in or on the fresh water or are stuck to plants or stones. The female often dips her abdomen as she flies to release the eggs (Waltz and Burian, 2008). Mayflies from family Baetidae pull themselves under the water to attach their eggs directly to the bed before being drowned by the current (Knopp and Cormier, 1997).

Then, the mayfly nymphs usually take between few days to a number of weeks to hatch depending on the water condition and the type of species (Edmund and Waltz, 1996). The nymphs will spend various lengths of time in the river before emerging as an adult fly. Nymph is the dominant life history stage of mayfly.

When it is time to emerge, the nymphs make their way to the surface. Then, the nymphs pull themselves free of their nymphal shuck and emerge as a sub-imago (Edmund and McCafferty, 1988). While resting to dry their newly exposed wings, they are at their most vulnerable to attack from fish. The soft-bodied subimagos are very attractive to predators (Edmund and McCafferty, 1988).

Lastly, the subimago fly to nearby plants or vegetation and molt again into adults or imago (Sivaramakrishnan and Venkatamaran, 1985). Adult mayflies are all terrestrial (Merrit *et al*., 2008) and the lifespan ranges from two hours to two weeks (Balachandran *et al.,* 2011). Most adults live within 48-72 hours. Adults and nymphs are an important source of food for fish and other macroinvertebrates.

Besides, the knowledge about the life history of many aquatic insect species is very important to evaluate the effects of environmental stress. Aquatic insects go through several developmental stages known as instars under the water. In some species the final immature instar forms a pupa which develops into a mature adult form (Petersen *et al*., 2004). However, some species have quite distinct larval stages. Instar determination can be determined by selecting the genus from the sample which consistently occur and in large numbers. The nymphs of the selected genus are collected for the analysis of body measurements and instar determination. Measurements will consist of head length and width, body width and lights of profemur (Miyairi and Tojo, 2007). Brown *et al*., (2004) stated that most aquatic species go through only 1-3 generation per year while having 2-3 generation per year are the most common in warmer climates. Due to the long life spans, the larvae of aquatic insects recover slowly from the effect of pollution. Pollution problems can be detected for a long period, at least three months after the stress had occurred.

Life history study of the aquatic insects reveals the detailed progress of their growth in the field (Che Salmah *et al.,* 2006). Generally, increases in body and head capsule lengths have been used as measures of growth (Corbet, 1957; Kormondy and Gower, 1965). Comparisons made on differences in the growth of similar species in different habitats or different species living in the same habitats (Benke, 1970) can give clues to natural regulations on food and space availability as well as cannibalism occurring in the environment (Hassan, 1975; Hopper and Crowley, 1996). Growth assessment are made on selected species to further compare the habitat suitability and the separation of niches among coexisting species in similar habitats (Che Salmah *et al.,* 2006).

2.3 Secondary production of Ephemeroptera

Secondary production refers to the formation of animal biomass over time (mass area $^{-1}$ x time $^{-1}$) (Benke, 1993). Annual secondary production is referring to the sum of all biomass produced by a population during one year, including production remaining at the end of the year and all biomass produced during this period. There are numerous methods exist for measuring secondary production and the relationship between the biomass-specific growth rates are implicit to all (Benke *et al.*, 1984).

When the production or immigration exceeds losses within a given area, the biomass of the river macroinvertebrates increase (Huryn and Wallace, 2000). Accumulating biomass may result in simultaneous positive and negative influences on rates of production because of its dual role as both an investment that contributes to future production and also as a burden because of the greater fraction of available energy needed for its maintenance (Brey and Gage, 1997). Taxa with long life cycles would have high levels of biomass production and long life cycles. Most studies of the production biology of river macroinvertebrates have focused on larval production as driven by growth rate and population biomass (Perán, *et al*., 1999; Carlisle and Clements, 2003; Frost *et al*., 2006).

2.4 Influence of environmental parameters on the abundance and distribution of aquatic insects

2.4.1 Physical parameters

Substrate suitability is one of the factors which affects the abundance and distribution of aquatic insects in the rivers. Substrate are classified according to their size into sand, gravel, cobble, boulder and bedrock (Barbour *et al*., 1999). Typically, at the downstream rivers, particle size decreases with larger bedrock and boulder on upstream river while sandy and stony substrate dominate downstream rivers. This is because upstream rivers transport smaller substrate material to the downstream rivers (Gomi *et al*., 2002). Besides, different types of substrates in the rivers also influence the abundance and diversity of aquatic insects. This is because habitat preferences differ among species (Collier, 2004). Previous study done by Georgian and Thorp, (1992) showed that Hydropsychidae (Trichoptera) prefers habitat with large and stable substrate while *Baetis* species (Ephemeroptera: Baetidae) and *Caenis* species (Ephemeroptera: Caenidae) prefer sandy substrate as their habitat. Heterogenous substrates, produced from various types of disturbances and physical conditions, act as patches for the population of aquatic insects in the river (Reice, 1994). Different types of substrate that exist together in a river create high richness and diversity of aquatic insects that live in the rivers (Godbout and Hynes, 1982).

Many freshwater animals (fishes and aquatic insects) inhabit rivers that flow through forested area. This is because rivers that flow through forested area have large and close canopy cover. Leaves from the surrounding forest or algae consist of organic matter. This particulate organic matter feeds the majority of aquatic insects in the rivers. Most of the fishes and aquatic insects use materials deposited from the forest for food and habitat. The input of detritus from the forests is important for the conservation and restoration of diverse river food webs (Wallace *et al.*, 1997). Partly shaded canopy cover is optimal for the growth of aquatic insects as the sunlight still can reach under water for photosynthesis processes. Contrary, shaded canopy cover might negatively affect the abundances of aquatic insects as the canopy cover blocks the sunlight from directly passing through the water. This phenomenon decreases the rate of photosynthesis and thus reduces the amount of dissolved oxygen in the water. Dissolved oxygen is crucially important for the respiration process of aquatic insects.

Water velocity varies at each river and it is not constant all the time. It keeps on changing depending on the weather. Current will be very fast during rainy season and slower during drought (Bunn and Arthington, 2002). Water velocity is directly important for regulating aquatic ecosystem. For example, high water velocity during heavy rain might carry away the aquatic insects especially those that burrow in the sediments (ephemeropterans) and thus reduce their abundances. However, high water velocity is important in maintaining high level of dissolved oxygen which is crucially required by macroinvertebrates especially aquatic insects for respiration. High water velocity with strong current may carry away all the suspended solid and fine sediment (Bilotta and Brazier, 2008). Therefore, light penetration can easily occur. Light penetration is important for the photosynthesis process to occur. Photosynthesis process provides sufficient amount of oxygen in the water. Therefore, rivers with relatively low velocity are often dominated with tolerant group of aquatic insects towards oxygen depletion such as *Chironomus* sp. (Diptera: Chironomidae) and *Culex* sp. (Diptera; Culicidae) (Popoola and Otalekor, 2011).

Besides, water temperature is one of the primary factors associated with aquatic insects' assemblages (Ward, 1992). Different species prefer different ranges of water temperature; for example, stoneflies (Plecoptera) prefer lower water temperatures than mayflies (Ephemeroptera) (Brittain, 1990), possibly because some order of aquatic insects are temperature dependent, and high temperature favors their feeding and metabolism rates. Optimal water temperature differs among species and optimal temperature for tropical freshwater ranges from 21^{o}C to 32^{o}C (Ayodele and Ajani, 1999). Previous study done by Oben (2000) showed that higher abundance of aquatic insects are collected in high temperature compared to low water temperature. Besides, water temperature has an inverse relationship with dissolved oxygen in the river (Popoola and Otalekor, 2011). When water temperature increases, respiration process occurs rapidly and thus decreases the level of dissolved oxygen in the water (Arimoro and Ikomi, 2008).

2.4.2 Chemical parameters

One of the chemical parameters that might affect the abundance and distribution of aquatic insects is dissolved oxygen (DO), which is the amount of free, non-compound oxygen that present in the water (Saudi *et al*., 2014). Non-compound or free oxygen is oxygen that is not bonded to any other element (Saudi *et al*., 2014). DO is an important water parameter used in assessing water quality due to its influence on the abundances of aquatic insects in the river. Besides, the amount of DO needed varies between each order of aquatic insects. Intolerant order of aquatic insects such as Ephemeroptera, Plecoptera and Trichoptera prefer high level of DO while moderately tolerant orders such as Diptera could inhabit in the river with low levels of DO. DO is an important parameter used by the aquatic insects for respiration. Different aquatic habitat with various changes in water current and altitude have different concentration of DO (Jacobsen, 2005). Too high or too low levels of DO could harm the aquatic insects and affect water quality in the rivers. DO concentration is higher in cold water and it decreases as the temperature increases (Murdoch *et al*., 2000). The levels fluctuate seasonally, over a 24-hour period. DO is carried into the rivers via runoff, entering in the flow of groundwater, through the air or as a plant by product (Allan and Castillo, 2007). Oxygen slowly diffuses across the water surface from the surrounding atmosphere. Besides, some of the DO is produced as a waste product from photosynthesis process of algae and other aquatic plants (Allan and Castillo, 2007). Resulting DO from photosynthesis might occur on the surface or under water. Large portion of DO in the river are produced under water. However, the amount of DO produced under water is limited to certain depth depends on the light penetration (Saudi *et al*., 2014).

Another chemical factor is the biochemical oxygen demand (BOD). BOD is the total amount of oxygen needed by aerobic microorganism in the water to breakdown or stabilizes the organic matter in the rivers (Parmar and Parmar, 2010). Organic matters that are discharged into the river serve as the food source for bacteria in the water (Meziane and Tsuchiya, 2002). After the break down process, the organic matters should be in the form of less complex organic substances. If the river is previously unpolluted, the river would be saturated with DO, where the bacteria involved in the breakdown process is aerobic types. These bacteria will multiply, degrade the organic waste and utilise the DO (Best *et al*., 2007). However, when the uptake of DO exceeds, in which the DO is replenished from photosynthesis or atmosphere, the water becomes anaerobic (Best *et al*., 2007). Therefore, high amount of organic waste in the water will result in higher reading of BOD. BOD is inversely related with the abundance of aquatic insects because aquatic insects require enough oxygen for their respiration process (Fleeger *et al*., 2003).

In addition, chemical oxygen demand (COD) is another factor which could affect the abundance and distribution of aquatic insects. Chemical oxygen demand (COD) is the amount of oxygen required to chemically oxidize organic and inorganic matter into carbon dioxide and water (DOE, 2003). Higher amount of pollution in the test sample will have higher reading of COD.

Total suspended solid (TSS) also is one of the factors affecting the abundance and distribution of aquatic insects. TSS in the water often occurs due to the natural causes. According to APHA (1992), total suspended solid (TSS) includes a variety of small particles such as silt, decaying plant and animal matter and sewage that can be trapped by a filter. Large rivers usually have high amounts of suspended organic and inorganic materials (Dagg *et al*., 2004). Organic and inorganic materials are easily suspended in the river from erosions or runoffs. The amount and size of the suspended solid in the river is dependent on the water flow (Corbet *et al*., 2004). In rivers of slow current, suspended solid usually consisted of fine sand, silt and clay. When the level of suspended solid is too high in the river, light cannot easily penetrate into the rivers, thus reducing the amount of dissolved oxygen produced by photosynthesis under the water (Cushing and Allan, 2001). Therefore, increased level of TSS would negatively affect the abundances of aquatic insects and thus reducing the primary productivity of the rivers.

Besides, other factor that might affect the abundance and distribution of aquatic insects in the river is ammonia- nitrogen (NH_3). Ammonia- nitrogen (NH_3) is a nitrogenous compound found in the river water. The sources of ammonia and nitrogen come from fertilizer, sewage and waste water treatment plants (Pielke *et al*., 2011). Ammonia- nitrogen (NH_3) originates from ammonium (NH_4^+). Ammonium is a non-toxic compound and it does not have any potential effect towards aquatic life especially aquatic insects (Camargo and Alonso, 2006). However, when the pH level is too high (exceeding 9), ammonia- nitrogen (NH_3) is released (Dohanyos *et al*., 2004). This unionized form of ammonia (NH_3) is extremely toxic at certain concentration to the aquatic life under specific temperature and pH.

pH or potential hydrogen ion concentration plays an important role in controlling the abundance and distribution of aquatic insects in the aquatic environment. pH is a measure of hydrogen ion concentration in water. In addition, pH reading can be used to classify the river water into acidity or alkalinity (Chapman, 1992). Majority of the aquatic insects prefer pH ranging from 6 to 9 (Chapman, 1992). When the pH water falls outside the range, the mortality rate for most of the aquatic insects will increases. However, extremely high or low pH values would increase solubility of toxic materials, thus making them mobile, and chances of toxic materials being absorbed by the aquatic insects increases (Tordoff *et al*., 2000). Previous study by Popoola and Otalekor, (2011) showed that aquatic groups of insects such as *Chironomus* (Diptera: Chironomidae), Corixidae (Diptera) and Zygoptera (Odonata) are highly affected by acidification while Ephemeroptera, Plecoptera and Trichoptera are acid sensitive groups and can only be found in alkaline water in nature.

Organic matters influence physical, chemical and biological properties of soils. Some of the properties influenced by organic matter include soil structure, soil compressibility and shear strength (Chenu *et al*., 2000). In addition, it also affects the water holding capacity, nutrient contributions, biological activity and water and air filtration rates (Chenu *et al*., 2000). Intolerant groups of aquatic insects such as Ephemeroptera, Plecoptera and Trichoptera could not tolerate with high organic content causing their limited abundances (Popoola and Otalekor, 2011). However, pollution tolerant groups such as *Chironomus* (Diptera: Chironomidae) are abundant in the river with high level of organic contents, possibly from leaf litter, recreational activity or untreated sewage (Popoola and Otalekor, 2011). Concomittantly, Loss on Ignition (LOI) is a common and widely used method used to determine the organic and carbonate content (% organic matter) of the soil samples (Bengtsson and Enell, 1986). The organic content is the ratio, expressed as a percentage of the mass of organic matter in a given mass of soil to the mass of the dry solids. This is relatively the simplest method compared to others used to determine % OM. This method does not involve the use of any chemicals, only the use of a muffle furnace.

Heavy metals are one of the serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems. Heavy metals from the fresh water sources are rapidly removed from the water body and deposited onto the sediments (Ryan, 1991). The concentration of heavy metal in organic residues (soil samples) is variable. It depends on the types of activity that run on land nearby. The concentration of heavy metal tends to be low in plant material and high in some residues from industries, animal production system or municipal wastes and industrial facilities (Tiller, 1989). Feeding and farm management system from the land nearby may lead to high Copper (Cu), Manganese (Mn) and Zinc (Zn) contents (Mattias *et* *al*., 2010). Maximum concentration of heavy metals permitted should be considered for the agricultural use since the risk of contamination of water and soil is always present (Kabata-Pendias, 2011).

In addition, the amount of heavy metals retained in sediments is also affected by the sediment characteristics such as the types of sediments, the quantities of organic matter and the size of the grain (Vertacnik *et al*., 1995). A study done by Moore *et al*., (1989) showed that the large amount of heavy metals is bound in the fine-grained fraction (< 63 um) of the sediment, mainly because of its high surface area-to-grain size ratio and humid substance content.

Cadmium (Cd), Copper (Cu), Lead (Pb), Mercury (Hg), Nickel (Ni) and Zinc (Zn) are several types of heavy metals that are usually traced in aquatic ecosystems (Zhou *et al*., 2008). The presence of the heavy metals in the water column may directly or indirectly affect the macroinvertebrates in the river (Courtney and Clements, 2002). Direct effects of heavy metals results in the declining in abundance and diversity of macroinvertebrates especially aquatic insects (Courtney and Clements, 2002). Meanwhile, indirect effects of heavy metals contamination cause alterations of species interactions (Courtney and Clements, 2002).

Currently, there are many devices and techniques to quantify heavy metal contents in soil samples but their efficacy is dependent on the digestion methods used. According to Azcue and Mudroch (1994), wet digestion in open systems is time consuming and subject to contamination and loss of some chemical elements by volatilization. Similarly, for the muffle furnace digestion, losses of Cadmium (Cd), Chromium (Cr) and Lead (Pb) may occur by volatilization and absorption of the chemical element onto the wall of the furnace. This is due to high combustion temperature, usually from 500- 550 ^{0}C. In contrast, in microwave digestion, time taken for the analysis can be greatly reduced. Microwave digestion also reduces contamination, lowers reagent and sample usage, reduces loss of volatile elements and enhances operator safety (Sandroni and Smith, 2002).

2.4.3 Ecological indices

Shannon-Wiener Index (H’) is widely used to assess the diversity of species in the sampling stations and it reflects the degree of species composition per unit area. The higher value of (H’), the greater the diversity and the cleaner the environments are (Metcalfe, 1989). Evaluations of water quality from Shannon-Wiener Index calculation are shown in Table 2.1.

$$H' = -\sum [(ni/N) \ln (ni/N)]$$

(Shannon and Weaver, 1949)

Where, H’ = Shannon-Wiener Index

n*i* = Total individuals belonging to the *i* species

N = Total individuals of population sampled

Table 2.1: Evaluation of water quality from Shannon-Wiener Index calculation (Shannon and Weaver, 1949).

Index value	**Indications**
>3.0	Clear water without disturbance
1.0 – 3.0	Moderately polluted conditions
< 1.0	Seriously polluted conditions

Besides, other ecological indices that are commonly used are Simpson's Diversity Index. Simpson's Diversity Index (1 - *D*) is derived from Simpson's Index (*D*). Simpson's Diversity Index (1 - *D*) represents the probability that any two individuals that were randomly selected from a sample will belong to different species.

$$D = 1 - \sum [\, n_i(n_i - 1) / N(N - 1) \,]$$

(Simpson, 1949)

Where, n_i = Number of individuals of genus i in the sample

N = Total number of individuals in the sample

On the other hand, Pielou's Evenness Index (E) is used to measure of how similar the abundances of different species are.

$$E = H' / H_{max} \qquad \text{or} \qquad E = H' / \ln S$$

(Ludwig and Reynolds, 1988)

Where, H' = Value derived from Shannon-Wiener Index

$H_{max} = \ln S$

S = Total number of species

In addition, another ecological index that is widely used to measure the number of species in a sample per unit area is Menhinick's Richness Index (R_2).

$$R_2 = S / \sqrt{n}$$

(Ludwig and Reynolds, 1988)

Where, S = Total number of species in the community

n = Total number of individuals sampled

2.4.4 Biological indices

There are several biological indices that are commonly used to classify the river water quality such as Biological Monitoring Working Party (BMWP), Family Biotic Index (FBI) and EPT taxa richness. Biological Monitoring Working Party (BMWP) is used to measure water quality using macroinvertebrates as biological indicators. This index is based on the principle that different family of macroinvertebrates have different tolerances to pollutant. The indications of BMWP index to classify river water quality are shown in Table 2.2.

$$\text{BMWP} = \sum [(ni \times ai) + (ni_2 \times ai_2) + \ldots\ldots\ldots\ldots + (ni_n \times ai_n)]$$

(Armitage *et al*., 1983)

Where, ni = Number of family sampled

ai = Tolerance value for each family

Table 2.2: Indication of BMWP index (Armitage *et al*., 1983).

Index value	**Water Quality**
>151	Very good
101 – 150	Good
51 – 100	Moderately good
15 – 50	Bad
0 - 10	Worst