# HABITAT CHARACTERIZATION, TROPHIC POSITION AND SEASONAL INFLUENCE ON AQUATIC INSECTS IN THE SELECTED FEEDER STREAMS OF BELUM-TEMENGOR FOREST COMPLEX (BTFC), PERAK


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**By**

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## LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| ANOVA | = Analysis of Variance |
| BOD | = Biological Oxygen Demand |
| BTFC | = Belum-Temengor Forest Complex |
| C | = Carbon |
| CCA | = Canonical Correspondence Analysis |
| COD | = Chemical Oxygen Demand |
| CPOM | = Coarse Particulate Organic Matter |
| DO | = Dissolved Oxygen |
| DS | = Dry Season |
| EA-IRMS | = Elemental Analysis-Isotope Ration Mass Spectrometry |
| EPT | = Ephemeroptera Plecoptera Trichoptera |
| ETOH | = Ethanol |
| FFGs | = Functional Feeding Groups |
| FPOM | = Fine Particulate Organic Matter |
| GJFR | = Gunung Jerai Forest Reserve |
| IUCN | = International Union for Conservation of Nature |
| L | = Liter |
| m | = Meter |
| mg | = Miligram |
| MMD | = Malaysian Meteorological Department |
| N | = Nitrogen |
| $NH_3$ | =Ammonia |
| $NH_3$-N | = Nitrate |
| OM | = Organic Matter |
| P | = Proton |
| PBRRC | = Pulau Banding Rainforest Research |

| | |
|---|---|
| | Centre |
| $PO_4^{3-}$ | = Phosphorus |
| PSPC | = Perak State Parks Corporation |
| RCC | = River Continuum Concept |
| S | = Sulfur |
| SCA | = Stomach Content Analysis |
| SIA | = Stable Isotope Analysis |
| SPSS | = Statistical Package for the Social Sciences |
| TI | = Total Inertia |
| TSS | = Total Suspended Solid |
| TVE | = Total Variation Explained |
| VPDB | = Vienna Pee Dee Belemnite |
| WS | = Wet Season |
| $\alpha$ | = Alpha |
| $\beta$ | = Beta |
| $\beta w$ | = Beta Diversity |
| $\gamma$ | = Gamma |
| $\delta$ | = Delta notation |
| $\delta^{13}C$ | = Stable Carbon Isotope Signatures |
| $\delta^{15}N$ | = Stable Nitrogen Isotope Signatures |
| $\Delta_{dt}$ | = Diet-tissue Discrimination |
| $^{0}/_{00}$ | = Part per thousand |

**PENCIRIAN HABITAT, KEDUDUKAN TROFIK DAN PENGARUH MUSIM KE ATAS SERANGGA AKUATIK DI BEBERAPA SUNGAI PEMBEKAL TERPILIH DI KOMPLEKS HUTAN BELUM-TEMENGOR (BTFC), PERAK**

## ABSTRAK

Kajian serangga akuatik dan tindak balas mereka terhadap keadaan persekitaran telah digunakan di seluruh dunia sebagai pemantauan biologi kesihatan sungai. Oleh itu, tujuan kajian ini adalah untuk menentukan pengaruh ciri-ciri fizikal sungai terhadap kelimpahan dan kepelbagaian invertebrata akuatik dan bagi menentukan sumber makanan yang berpotensi untuk organisma air tawar. Dalam kajian ini, lima sungai pembekal ke Kompleks Hutan Belum-Temengor (BTFC) telah dipilih sebagai tapak persampelan. Dengan menggunakan prosedur pengukuran substrat berbatu dan pengelasan substrat, substrat utama yang didapati di kawasan kajian adalah kelikir kasar (17 - 32 mm) tetapi kelimpahan serangga akuatik yang tinggi ditemui pada batu bulat kecil (51.54 %). Terdapat korelasi yang tinggi di antara kekasaran permukaan (kepelbagaian susunan saiz substrat dan taburannya di dasar sungai) dan kelimpahan serangga akuatik serta kepelbagaiannya (kedua-dua korelasi Spearman’s Rho dan ujian regresi berganda pada $p = 0.05$). Hubungan rangkaian trofik dalam ekosistem akuatik dikaji melalui analisis isotop stabil berasaskan bahan organik yang dikutip di kawasan kajian. Anggaran kedudukan trofik berserta model rantaian makanan mendedahkan bahawa nilai $\delta^{13}C$ tertinggi yang diperoleh adalah daripada alga berfilamen( - 22.68 ± 0.126 $^{0}/_{00}$) dan $\delta^{13}C$ terendah pada pek daun alokton ( - 31.58 ± 0.187 $^{0}/_{00}$). Nilai $\delta^{15}N$ tertinggi telah diperolehi daripada ikan (8.45 ± 0.177 $^{0}/_{00}$) dan paling rendah $\delta^{15}N$ dicatatkan pada makrofit akuatik autokton (2.00 ± 1.234 $^{0}/_{00}$). ). Berdasarkan keputusan kandungan $\delta^{15}N$, terdapat tiga aras trofik didalam sungai ini dan dicadangkan aras trofik bermula dengan bahan organik diikuti dengan kumpulan serangga dan berakhir dengan ikan (bahan organik < serangga < ikan). Kesan perubahan bermusim (basah dan kering) terhadap taburan serangga akuatik telah menunjukan penurunan ketara (pengurangan 45 %) bagi kelimpahan dan kepelbagaian (98 genera) pada musim basah berbanding musim kering (114 genera) ($p = 0.000$; Ujian Mann-Whitney U). Penurunan yang mendadak pada kelimpahan subfamili Limoniinae dan *Ceratopsyche* sp., menunjukkan taksa ini sangat dipengaruhi oleh arus air yang deras pada musim basah. Suhu air, kedalaman, BOD, DO dan COD pula berbeza secara ketara antara musim (ujian Kruskal Wallis, $p < 0.05$). Fosforus dan nitrogen-ammonia sangat mempengaruhi kelimpahan serangga akuatik pada musim kering manakala taburan hujan adalah faktor utama yang mengawalatur komuniti serangga pada musim basah. Hasil daripada kajian ini menekankan pentingnya komponen substrat sungai, interaksi biologi antara komponen di dalam rantaian makanan akuatik dan perubahan parameter air mengikut musim terhadap taburan serangga akuatik. Gabungan di antara pengetahuan ini dengan pelan pemuliharaan akan dapat meningkatkan pemuliharaan biodiversiti dalam ekologi perhutanan.

**HABITAT CHARACTERIZATION, TROPHIC POSITION AND SEASONAL INFLUENCE ON AQUATIC INSECTS IN THE SELECTED FEEDER STREAMS OF BELUM-TEMENGOR FOREST COMPLEX (BTFC), PERAK**


## ABSTRACT

The study of aquatic insects and their response towards environmental conditions has been used worldwide for biomonitoring of river health. Thus the purposes of this study were to determine the influence of physical characteristics of the streams towards abundance and diversity of aquatic macroinvertebrates and to ascertain potential food sources of freshwater organism. In this study, five feeder streams of Belum-Temengor Forest Complex (BTFC) were chosen as sampling sites. Using stony substrate measurement and substrate classification procedures, the major substrate occupied the study areas was coarse gravel (17 – 32 mm) but high abundance of aquatic insects was found on small cobble (51.54%). There was a strong correlation between bed roughness (arrangement of different mean grain size and their distribution in riverbed) and aquatic insect abundance as well as its richness (Spearman's Rho correlation and multiple regressions tests both at $p=0.05$). The linkages of the trophic chain in the aquatic ecosystem were determined by analyzing stable isotope-based organic materials collected at the study areas. Estimated trophic position in a food web paradigm revealed that the highest $\delta^{13}C$ value was acquired from filamentous algae ($-22.68 \pm 0.126\ ^{0}/_{00}$) and the lowest $\delta^{13}C$ was in allocthonous leaf packs ($-31.58 \pm 0.187\ ^{0}/_{00}$). The highest $\delta^{15}N$ value was obtained from fish ($8.45 \pm 0.177^{0}/_{00}$) and the lowest was recorded in autochthonous aquatic macrophytes ($2.00 \pm 1.234^{0}/_{00}$). Based on the $\delta\ ^{15}N$ contents, there were three trophic levels in these streams and it was suggested that the trophic chain began with organic matter followed by group of insects and ended with fish (OM<insects<fish). Plausible effect of seasonal change (wet and dry) on the distribution of aquatic insects was indicated by significant decrease (45% reduction) in their abundance and richness (98 genera) in the wet compared to dry seasons (114 genera) ($p = 0.000$; Mann-Whitney U test). Large reduction in abundance of the subfamily Limoniinae and *Ceratopsyche* sp., implied that these taxa were the most affected by heavy water flow in the wet season. Water temperature, depth, BOD, DO and COD were significantly different between seasons (Kruskal Wallis test, $p<0.05$). Phosphorus and ammonia-nitrogen affected the abundance of aquatic insects the most in the dry season while rainfall was the main factor regulating the insect communities in the wet season. The outcome of this study highlighted the importance river substrate components, biological interaction among components of aquatic food chain and seasonal variation of water parameters on the distribution of aquatic insects. Incorporation of this knowledge into the conservation planning would enhance the preservation of biodiversity in the forested ecosystem.

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Background

Tropical region is made up of high diversity of biota associated with conducive environment. Insect populations dominate in large proportion of local biodiversity especially the aquatic insects (Clarke *et al*., 2008) in freshwater ecosystem (Bonada *et al.*, 2006). Out of 30-31 orders of insects, 13 orders of aquatic insects have been described by Merrit *et al*., (2008) in the North American freshwater rivers. The freshwater insects in Malaysian region also constitute almost the similar number of orders (11 orders) (Yule and Yong, 2004) but may vary by the numbers of families compared to other regions. The high number of insect population in an ecosystem is highly associated with the availability of habitat. Most of them inhabit wide range of aquatic microhabitats from lakes to rivers and from tiny water container to stagnant water (Voshell, 2009).

In running waters such as rivers, the composition of aquatic insect communities inhabiting headwater and downstream reaches are different based on available microhabitats (Meyer *et al*., 2007). Many earlier studies on the assemblages of aquatic insects in headwater have found most pollution-sensitive insects such as ephemeropterans, plecopterans, trichopterans and coleopterans inhabit upper catchment that provide healthy environment (Hodkinson and Jackson, 2005; Bispo *et al*., 2006; Hughes, 2006; Miserendino, 2006). Meanwhile, these groups of insect also can be found in the lowland rivers but in different quantity of taxa and abundance with an addition of other communities such as snails, oligochaetes, fly larvae and others. The relationship of different habitat characteristics and taxa composition has been exploited for water quality assessment using aquatic biota in freshwater ecosystem.

The usage of aquatic insect as an indicator of water quality is strongly related to spatial and temporal distribution which closely follows the physical, biological and chemical conditions of the river (Merritt *et al*., 2008). Spending most of their life cycle in the water and having limited mobility, they are easy to collect and identify thus aquatic insects are suitable to be used in river assessment (Kamsia *et al*., 2008). Most of aquatic insects prefer a healthy river habitat with riparian vegetation that may provide shades and filter the pollutants before entering the river. In addition, insect assemblages in river are also determined by some of environmental factors for shelter such as rocks, overhanging tree limbs, logs and roots, vegetation as a place to breed and hatch their young and sufficient preys to eat (Stuart *et al*., 2008). In addition, physicochemical parameters of water bodies such as specific velocity, depth and temperature are important factors for aquatic living insects.

Fluvial geomorphology also has become one of the important tools in river management (Thorne *et al.*, 1997; Newson *et al.*, 2002; Downs and Gregory, 2004). Hydromorphological features of a river can reflect a long term climatic and geomorphological processes being influenced by the anthropogenic impacts and global changes (Syrovatka *et. al*., 2009). Lancaster and Hildrew (1993) have examined the relationship between the fluctuation of flow and microdistibution of aquatic macroinvertebrates in structuring the benthic community. Concomitantly, the benthic community composition, abundance and distribution are affected by river hydraulic and substratum (Quin and Hickey, 1994), including source availability (Richardson, 1993), water chemistry (Giberson and Hall, 1988), temperature (Bournaud *et al.*, 1987) and light (Robinson and Minshall, 1986).

Aquatic and terrestrial habitats are interdependent and have been described as intricate and complex continuum of linked habitat by VanDongen *et al*. (2011). The river continuum concept introduced by Vannote *et al*. (1980) explained that the river from headwaters to downstream provide different continuous physical gradient of water depth, river width, water velocity and temperature which also progressively increase with energy inputs and rate of entropy gain. Thus, it is important to understand interaction among smaller habitats within the whole environment. The interaction between terrestrial and aquatic habitats can be quantified by measuring the rates of energy exchange between trophic levels and proximate habitat using stable isotopes analysis (Ballinger and Lake, 2006).

Stable isotopes analysis (SIA) has been used as a tool to answer questions in plant and animal physiology, biogeochemistry, migration patterns, diet composition, trophic level estimations and food web functioning (Fry, 2006; Bouillon *et al*., 2012). In this study, SIA has been applied to determine the trophic base of food webs in aquatic systems. Salas and Dudgeon (2001) reported that the analysis allowed for the determination of relative contribution of allochthonous and autochthonous sources toward the production of consumer biomass. The organic carbon in rivers is derived from either allochthonous (terrestrial) sources, primarily plant litter, or from autochthonous sources such as primary producers within the river itself. According to

Dudgeon and Bretschko (1996), low order rivers in tropical Asia are generally considered to be heterotrophics, with high allochthonous inputs and primary production limited by riparian shading and scouring caused by spates.

Another important factors influencing biological assessments programs of aquatic ecosystem are seasonal fluctuation and climatic change. In tropical country, seasonal changes cause by variation in precipitation (Flecker and Feifarek, 1994) play important role for the aquatic insect assemblages especially the ephemeropterans, plecopterans and trichopterans (EPT) (Robinson and Minshall, 1986). In addition, the quantity of precipitation is closely related to the climate change.

According to Milly *et al*. (2005) and Kundzewics *et al.* (2008), future climatic impacts on global freshwaters are important and there will be an increase in temperature and decrease in rainfall (Lawrence *et al*., 2010). Furthermore, these impacts will be compounded by increasing water withdrawal (Palmer *et al.,* 2008). However, Feio *et al*. (2010) stated that temporal instability of invertebrate communities in rivers over long time scale (> 10years) is not well understood. Short term interval research done by Reynoldson *et al*. (1997) on community bioassessment might be less accurate. The temporal variability is a serious issue under a global climate change scenario in which the benthic communities are expected to drift as temperature changes and extreme events increase in frequency and intensity (Easterling *et al*., 2000; Diffenbaugh *et al.,* 2005; DeToffol *et al*., 2009). Recently, several studies have begun to describe some long-term shifts in freshwater macroinvertebrates assemblages towards directional climatic changes (Daufresne *et al*., 2004; Mouthon and Daufresne, 2006; Burgmer *et al*., 2007; Durance and

Ormerod, 2007). A study by Hickling *et al.* (2005) showed climatic changes had affected the phenology and distribution of aquatic macroinvertebrate community.

Many Malaysian river ecologists have focused on the use of aquatic macroinvertebrates as bioindicator in running water since the last decade. The study of habitat deterioration and water quality assessment on diversity and distribution of benthic macroinvertebrates have expanded all over Malaysian waters such as at Linggi River, Negeri Sembilan (Ahmad *et al*., 2002), Langat River, in Selangor (Azrina *et al*., 2006), Belum-Temengor catchment, in Perak (Che Salmah *et al*., 2007), Telipok River, in Sabah (Kamsia *et al*., 2008), Juru River, in Penang (Al-Shami *et al*., 2010; Al-Shami *et al*., 2011) and Kerian River Basin, in Kedah (Nurul Huda, 2011; Nur Adibah, 2011). The studies of benthic invertebrates encompassed urban rivers and especially pristine forested upstream rivers. Anthropogenic disturbance becomes a serious threat to the loss of biodiversity in tropical Asian rivers (Dudgeon, 2000b) and thus receive more attention from many researchers.

Belum-Temengor Forest Complex (BTFC) is one of the largest protected areas in northern Peninsular Malaysia, located in state of Perak. BTFC has been gazetted into a state park in order to conserve and protect the natural heritage in the rain forests. The areas can be divided into three parts; lower Belum forest reserve, Temengor forest reserve and Royal Belum State Park. Except for the Royal Belum State Park, the other two protected areas are allowed for logging and hunting activities through official permits issued by Perak State Parks Corporation (PSPC). BTFC comprises of a man-made Temengor Lake due to damming of several rivers for the purpose of generating hydro-electric power and water supply into Perak River. In 1996, BTFC was developed as one of the ecotourism areas in Malaysia (MOCAT, 1996). As a consequence, more development and forest clearing were carried out to meet the plan, threatening its pristine environments.

Jongkar (2000) has initiated a study of aquatic insect diversity in Temengor Dam, Perak and verified that the distribution of aquatic insects was influenced by the seasonal changes and the physicochemical characteristics of water. In the same areas, several other studies had produced important findings on the relationships of habitat and physicochemical characteristics of river ecosystems with communities of ephemeropterans (Wan Mohd Hafezul, 2011), trichopterans (Mariam Zhafarina, 2011) and plecopterans (Wan Nur Asiah, 2011).

To further understand the factors influencing the assemblages of aquatic insects living in pristine environment, this study examined; i). The influence of fluvial geomorphology on distribution of aquatic insects ii). The components of trophic level which linked to the aquatic food chain and iii). The importance of seasonal variation on aquatic insect distribution in forested river environment. The knowledge gained from this study would contribute to sustainable development of pristine areas in Malaysia.

## 1.2 Objectives

The study of aquatic insects in selected rivers of BTFC, Perak was focusing on the following objectives:

1. To identify the importance of physical characteristics of the rivers on the abundance and diversity of aquatic macroinvertebrates in feeder streams of Belum – Temengor Complex, Perak.
2. To determine the food sources of aquatic macroinvertebrates and pattern of nutrient flow along the trophic levels.
3. To determine the impacts of physico chemical parameters on macroinvertebrate communities in different rivers and seasons.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Aquatic macroinvertebrate distribution

Macroinvertebrates are organisms that are large (macro) enough to be seen with naked eyes and lack of backbone (invertebrate) (Jacobsen *et al*., 2008). Macroinvertebrates are important links between primary producers and higher tropic levels in aquatic food chains (McIntosh, 2007) and their role in the environment has been studied widely. Their habitats are closely associated with riparian vegetation, aquatic plant, biotic and abiotic factor and hydrological factors (Mesa, 2010). They inhabit all types of running waters, from fast-flowing mountain rivers to slow-moving muddy rivers. Most of their live parts or life cycles are attached to submerged rocks, logs, and vegetation. Examples of aquatic macroinvertebrate are insects in their larval or nymphal forms, crayfish, clams, snails, and worms. Freshwater macroinvertebrate assemblages may vary at upstream and downstream sites of the same river because they response to longitudinal gradients in the physical environment and hydrologic conditions, changes in water quality, proportion of allochthonous and autochthonous organic matter and riparian vegetation (Cowell *et al*., 2004).

The tropical river faunas usually have distinctive features in relation to the abundance and richness orders compared to higher latitudes rivers. It has its own unique characteristics in seasonality patterns, geographical evolution, temperature and humidity (Helson *et al*., 2006; Gopal, 2005). Specifically, the Asian tropical river ecosystems are inhabited by a diverse fauna composition especially aquatic insect (Jacobsen *et al*., 2008) with the availability of various habitats and ecological patterns (Dudgeon, 2000a; Dudgeon, 2000b). However, in terms of abundance, more insects are found in the temperate region than in the tropics (Dudgeon *et al*., 2006).

Despite variation between regions, a lowland river and upland river also are inhabited by different insect communities. In tropical high-altitude river, most of the faunas are dominated by insects especially Plecoptera, Ephemeroptera, Coleoptera, Trichoptera and Diptera (Jacobsen, 2008). Meanwhile, the tropical lowland rivers are relatively rich with crustaceans, snails, insects such as Odonata, Heteroptera and Trichoptera (Jacobsen *et al*., 2008). Variation of insects communities in different part of rivers are determined by various geographical, biological and physicochemical factors.

The distribution and abundance of aquatic macroinvertebrate in rivers are mainly determined by the association of individuals with the changes in the aquatic environment. Many earlier studies on freshwater aquatic ecosystem worked on the relationship of relative abundance and species assemblages with the physical gradients (Slack, 1955; Minshall, 1968; Ziemer, 1973; Platts, 1979). Later, Vannote *et al*. (1980) came out with a River Continuum Concept (RCC), describing the structure and function of communities along a river system. Generally, the concept proposed that the energy input in the river, organic matter transport and storage, also the interaction of macroinvertebrate functional feeding groups along the river systems are regulated largely by the fluvial geomorphic process. It has been predicted that insects' communities that consumed allocthonous food source in head water river have shifted to autocthonous food source in the mid-order rivers and consequently increase the species richness along the river order (Arscott *et al*., 2005).

In addition, the aquatic assemblages in rivers are also related to the changes in environmental factors (Miserendino and Pizzalon, 2003) and habitat stability (Death, 1996). The physicochemical of water characteristics including water temperature, dissolved oxygen, pH and total suspended solids (Dudgeon, 1999) also contributed to the variation of insects' abundance and diversity in certain microhabitat. Most of freshwater ecologists considered water temperature and dissolved oxygen play important roles in benthic distribution in aquatic environment (Singh and Sharma, 2014). The oxygen concentration is higher in cooler water due to high solubility (Bispo *et al*., 2006). As described by Lewis (2008), rivers at higher altitude usually low in temperature and high oxygen in water favored the assemblages of insects compared to lowland rivers. Several sensitive case insect especially mayflies showed high sensitivity to low oxygen concentration.

Suspended solid concentration in water flow also need to be considered in the study of aquatic insect ecology. Although the upland rivers are believed to be less exposed to the suspended solid loads, the ecological process in rivers such as photosynthesis are vulnerable to the sediment concentration in water (Lewis, 2008). Walling and Web (1992) studied several factors that contributed to the presence of suspended solids in a water catchment such as precipitation, vegetation, soil types and human activities. Anthropogenic disturbance associated with decreasing of pH level in water reduced the species richness (Petrin *et al*., 2008). The nutrients input such as phosphorus, nitrogen and carbon in tropical rivers also related to the changes in water pH (Lewis, 2008). Instead of physicochemical factors solely affecting the aquatic assemblages, the biotic interaction such as insects' predation and competition also need to be considered (Creed, 2006).

## 2.2 Habitat heterogeneity and aquatic insect composition

The stability of microhabitat also contributed to aquatic insect allocation in rivers. Most of Asian upland rivers including Malaysian freshwater rivers composed of stony bed substrate and covered with canopy vegetation. However, the topography of river at upland which is steeper and less covered with vegetation cause the river vulnerable to spate-prone (Jacobsen, 2008). Spate in river consequently cause aquatic insect to drift from their microhabitat. This kind of disturbance affected the composition and distribution of aquatic insects in rivers.

Several studies on the interaction of fluvial geomorphology factors with insects' fauna have been documented such as composition of substrate types (Gurtz and Wallace, 1984), physical features of substrate types (Downes and Jordan, 1993), flow refugia availability (Gjerlov *et al*., 2003), disturbance (Leopold, 1994) and food source availability (Sanson *et al*., 1995). Meanwhile, Silveira *et al*. (2006) has suggested the main factor influencing the microdistribution of macroinvertebrate in river is substrate size while other factors such as water current, water chemistry and food availability are also important. The substrate size and water velocity are intercorrelated. The basic interaction was different substrate types in riverbed could produce different rate of water current (Jowett *et al*., 1991). The interaction of these two factors has been documented to be a major factor in determining distribution of taxa in riverbed (Minshall and Minshall, 1977; Statzner *et al*., 1988; Rempel *et al*., 2000; Buss *et. al*., 2004). Substrate surface types are important for insect colonization. Boyero (2003) reported that river macroinvertebrate in Penalara Natural Park, Central Spain prefer to colonize on cobble stones and are found abundant in rough substrate types. Parallel to his finding, other studies also discovered the preference of insect over various substrates such as non-stony substrate complexity of wood (O'Connor, 1991) and macrophytes (Jeffries, 1993). The accumulation of insect over rougher and complex substrate is related to the availability of food. A rough substrate surface would enhance a development of epilithic layer (Sanson *et al*., 1995) and favors community of insect functional feeding group such as scrapers (Boyero, 2003).

Besides substrate surface preference, some other studies have considered substrate complexity like the presence of pits and grooves which serve as refugia from predators and heavy flow (Downes and Jordan, 1993; Way *et al*., 1995). Hart (1978) also stated that substrate with more complex shapes were inhabited by many taxa of macroinvertebrate rather than simpler building structure of substrate. As reported by Jowett and Richardson (2010), many macroinvertebrates such as mayflies, stoneflies, cased caddisflies and insects from order Diptera prefer to colonize moderate to large size substrates such as cobble and boulder while beetle community prefer smaller substrate sizes such as gravel and small cobble. The preferences of aquatic fauna over substrate microhabitat gradually changes with their life-stage (Minshall, 1984). The hydrological study and substrate stability of upland river need further research in order to further understand the aquatic insect preferences over habitat heterogeneity.

In Malaysia, several studies from freshwater researchers have focused on the selection of substrate types on macroinvertebrate communities (Suhaila and Che Salmah, 2010; Nur Adibah, 2011; Che Salmah *et al*., 2013; Cob *et al*., 2014) and the assessment of substrate thus far relates substrate size measurement and percentage of substrate embeddedness. Che Salmah *et al*., (2013) appointed that substrate qualities as the determinant factor that controls the diversity pattern of aquatic insects in uphill rivers. The finding showed several orders of aquatic insects including Odonata, Plecoptera, Ephemeroptera and Trichoptera preferred suitable size substrate as a better habitat together with other factors such as high canopy cover, fast water flow and high dissolved oxygen in river. Another study on Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages on stony substrate riverbed by Suhaila and Che Salmah (2010) has shown that these insects especially *Phanoperla* sp. (Plecoptera) and *Macrostemum* sp. (Trichoptera) increase in abundance along with substrate embeddedness. Aquatic fauna also have high preference for other type of microhabitat heterogeneity in spite of microhabitat stony substrate stability alone. Cob *et al*. (2013) found that conch snails (Gastropoda: Strombidae) accumulated most on the mixture of sea grasses and it could be possibly explained by the association with feeding strategies and inter-specific interactions.

Further influence of embedded substrate surface on distribution of macroinvertebrate taxa was described by Nur Adibah (2011) using a modified substrate sizes classification of Substrate Codes by Krstolic *et al*. (2006). She discovered a high diversity of aquatic macroinvertebrate in Class 2 (large cobble; 12.9 cm) substrate embeddedness of which 25-49% of the surface area was within sand bed. According to Death and Winterbourn (1995), substrate embeddedness is closely related to the habitat stability. Moderately embedded substrate are more stable and can support a diverse community of macroinvertebrates than the loosely, slightly embedded substrate (Melo and Froehlich, 2001).

### 2.2.1 Application of gravelometer and pebble counts technique

Studies on particle size distribution usually used a visual estimation technique and comprised of substrate codes classification (Kondolf and Li, 1992). However, there are some weaknesses with the visual estimates of surface bed material. First, the estimation of bed material would rely on the judgment of an observer whereby the observer need to look at a particular assigned small area of riverbed and do the estimation in a percentage value (Platts *et al*., 1983; Bain *et al*., 1985). Thus, in this study, a sampling coarse riverbed material method applied is modified from the pebble count technique (Wolman, 1954) using a gravel template called a gravelometer (Stream Systems Technology Center, 1996) for pebble counting in gravel-bed rivers to produce more precise riverbed materials assessment.

The pebble count method (Wolman, 1954) needs approximately 100 stones which are randomly collected in an establish area in a river. Then, intermediate axes on each stone were measured and recorded according to stony size classes following the Wentworth Scale (Wentworth, 1922). Based on the classification sizes, a cumulative size distribution curve was produced and a median grain size class can be determined directly from the graph. The pebble selection must be random in which the investigator must not try to look at the riverbed while picking the stones. Moreover, to be consistent, use a specific finger as a specific point when touch the substrate. This method can be applied in various types of desired reach areas such as pool and riffle, riffle alone or pool alone

(Wolman, 1954). The pebble count technique has been applied by many earlier studies due to its convenient to perform along a river gradient (Brush, 1961; Hey and Thorne, 1983; Mosley and Tinsdale, 1985).

However, this study has employed different method from standard practice by Wolman (1954). Instead of measuring all three mutually perpendicular axes of a stone (a-axis, the largest dimension; b-axis, the intermediate; c-axis, the smallest axis), a gravel template has been used to simplifies the technique. In fact, a determination of particle size frequency from a b-axis alone could lead to systematic bias especially in sieving method. The method usually produced a measurement slightly smaller than the particle size. To overcome this problem, a gravelometer has been created and was proven that the operator bias is minimized (Stream Systems Technology Center, 1996). The gravelometer usage has been applied in broad ranges of study especially in river restoration (Parkyn *et al*., 2010), freshwater ecology study (Sponseller and Benfield, 2001; Scott *et al*., 2008; Peterson, 2010), biological assessment (Joy and Death, 2003), hydraulic modeling (Clark *et al*., 2008) and sediment assessment (Clapcott *et al*., 2011). Thus, the application of gravelometer in research is suitable especially in freshwater ecological study in Malaysia.

## 2.3 Seasonal changes and water characteristics influence on aquatic insect distribution

Freshwater rivers which are located in upland usually encounter water flow fluctuation due to its natural condition such as rainfall or drought. Understanding the rainfall variability would improve knowledge on water balance dynamics on various scales including water resources management and ecosystem (Wong *et al*., 2009). However, lack of study in peninsular Malaysia concerning rainfall variation was due to limited numbers of weather stations and encountered problem with missing data (Moten, 1993). Generally, tropical rivers including Malaysia are mainly stable in thermal condition but seasonality is considered as a resultant from hydrological changes and some climatic aspects (Dudgeon, 1999). Jacobsen *et al*. (2008) classified tropical rivers into aseasonality in equatorial headwater rivers to a truly marked seasonality, wet and dry season within monsoonal regions. Latitude, local topography, wind patterns and continentality are the factors influencing the variation of precipitation and altitude in different regions (Beniston, 2006). Precipitation level is lesser at the higher than in lower altitudes (Jacobsen, 2008), but heavy rainfall in upland is most likely to cause high velocity of water and often affect the population of benthic organisms (Death and Winterbourn, 1995; Townsend *et al*., 1997).

Based on the River Continuum Concept (RCC) introduced by Vannote *et al*. (1980), there is a consequence topic focusing on a prevalence of equilibrium and non-equilibrium states of a community structure. As discussed by Minshall *et al*. (1985), in equilibrium state, most of insects are controlled by biotic factors such as predation and competition while in non-equilibrium condition, community are influenced by abiotic factors such as spates. Dudgeon (1999) and Death (2008) discovered in tropical river, the community structure is assumed to be in a non-equilibrium state because the hydrological variability is still an important factor contributing to decreases in macroinvertebrate abundances. Various studies have found decrease in macroinvertebrate communities during wet season and this has generally been attributed to the scouring effects of increased flow (Flecker and Feifarek, 1994; Bispo *et al*., 2001).

The fluvial geomorphic process also has potential to change the river landscape (Stanford *et al*., 2005) by producing high water discharge at a time. Water discharge was defined as a volume of water flowing through a cross section of a river channel per unit time (Gore, 2006). It may produce a river power which able to dislocated suspended sediment, bed material, particulate organic matter and other nutrients in river. The allocation of river material has substantial influence on the distribution of living organism in rivers by altering physical habitat conditions such as dislocation of the substrates and the availability of energy, as measured by primary production or the state of particulate organic matter (Vannote *et al*., 1980; Vannote and Minshall, 1984; Statzner *et al*., 1988).

The hydrological changes in river water column also will lead to the colonization of insects at microhabitat. The period of insects' colonization ranges from few days to several years depending on the environment and impact of perturbation (Bispo *et al*., 2006). Several studies have found a rapid colonization of insects after experiencing habitat disturbance (Flecker and Feifarek, 1994). According to Flecker and Feifarek (1994), the abundance of insects in microhabitat has positive correlation with the days of last perturbation and peak of precipitation. It is indicated that, a longer days elapsed since last perturbation, more times for insects to reconstruct their population. Bispo *et al*.

(2006) also reported rainy season might cause entry of organic discharge into river that enhanced insect abundance during that season. In fact, the positive correlation of insects' density with rainy season was related to the improvement of water quality due to dilution of organic matter concentration. However, other studies show unremarkable influence of rainfall over aquatic insect. Melo and Froehlich (2001) stated that insect population was probably adapted to the predictable drought condition. Poff (1992) also agreed that seasonal spates are predictable and insects are potentially resistant over disturbances.

Many temporal studies have been performed especially on aquatic insects in tropical freshwater rivers (Owen and Owen, 1974; Wolda, 1978, 1979; Denliger, 1980; Wolda and Flowers, 1985). However, in peninsular Malaysia, only several researchers have worked on the seasonal effects in abundance and diversity of aquatic insects (Bishop, 1973; Bright, 1982; Jongkar, 2000; Suhaila *et al*., 2014). A study conducted by Suhaila *et al*. (2014) in rivers of Gunung Jerai Forest Reserve, Malaysia has found a significant influence of seasonal changes on the aquatic insect distribution especially the Ephemeroptera, Plecoptera and Trichoptera (EPT) orders. The insect abundance and seasonal relationship was different for all insect orders. The seasonal variation caused by rainfall changed the environmental characteristics as well as the number of insect population in the upland rivers. Meanwhile, a study on diversity and habitat preference in rivers of Rio de Janeiro, Brazil (Baptista *et al*., 2001) revealed that richness and diversity of aquatic insects were the highest during the dry season. This was explained by the presence of habitat stability and availability during the dry season. Baptista *et al*. (2001) have found high colonization of insects on leaf litters was favored with the presence of food and good shelter during that period.

However, seasonal changes caused by rainfall also influence the physicochemical variation in water. Ramirez *et al*. (2006) found a negative relationship among rainfall, water level and pH in the water. It is indicated that high water level during rainy season caused runoff of materials from floodplains and contirubuted to decrease in pH level. During wet season, many allochthonous inputs from riparian vegetation enter the water column and produce suspended humic acids that decrease the pH level in water (Clark *et al*., 2003). In contrast, increase in water turbidity during rainy season caused reduction in dissolved oxygen (Ishaq and Khan, 2013). The deficiency of oxygen level in water influenced the diversity of macroinvertebrate (Ward, 1992) because Sharma *et al*. (2009) had found that the diversity of macroinvertebrate was positively correlated with dissolved oxygen.

In addition, seasonal variation caused by precipitation level also may affect the insect growth by altering the hatching period and mortality rates (Jacobsen *et al*., 2008). Reduced flow due to drought may result in extreme temperature which is harmful to the aquatic fauna (Singh and Sharma, 2014). Temperature is the most important factor that influence the life cycle of aquatic insects (Resh *et al*., 1988). Bispo *et al*. (2006) stated that the increase in temperature reduced the oxygen solubility. Thus, the distribution of some groups of insect is restricted to certain microhabitat of cooler environment (Dominguez and Ballesteros, 1992). Several studies have showed diversity of invertebrates decreases as water temperature increases (Stanford and Ward, 1982; Sharma *et al*., 2004; Palit *et al*., 2013). Pinto and Uieda (2007) discovered that seasonal changes also influence food availability in forest rivers by focusing on forest canopy. The importance of forest canopy is for controlling water discharge, retaining excessive draining during heavy rainfall and keeping water flow during dry season, and also providing shade in order to keep the water temperature stable (Pinto and Uieda, 2007). Consequently, it also controls the distribution of aquatic fauna in river.

### 2. 4 Measuring river performance using ecological indices

The ecological indices is a basic approach to quantify changes in ecosystem in order to have better understanding in biological pollution impacts on aquatic fauna (Nur Adibah, 2011). Generally, the ecological indices are applied to diversity, richness and evenness of community structure while biological indices are used to measure indicator organisms.  However, only ecological indices were employed in this study to assess quality of environment on aquatic insect communities. According to Magurran (2004), different ecological indices yield different function, reliability and effectiveness. In fact, each of them has potential advantages and limitations.

### 2.4.1 Shannon-Wiener Index (H’) (Shannon and Weaver, 1949)

Shannon- Wiener Index (H’) is the most common index used in aquatic ecology studies compared to other diversity indices (Magurran, 2004). The assumption of this index is, the value of H’ increase according to the increase of number and distribution of taxa within a community (Mandaville, 2002).

The equation for the Shannon’s function which uses natural logarithms (ln) is;

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

(Shannon and Weaver, 1949)

Where; H’ = Shannon-Wiener Index

N = Total individuals of population sampled

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

Table 2.1: Index value indicator from the calculation of Shannon-Wiener Index (Ludwig and Reynolds, 1988)

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

### 2.4.2 Margalef's ($R_1$) and Menhinick's Richness Index ($R_2$) (Ludwig and Reynolds, 1988)

Margalef's ($R_1$) and Menhinick's Richness ($R_2$) indices are used to indicate the number of species in a sample per unit area. The equation for Margalef Index ($R_1$) is;

$$\text{Margalef Index } (R_1) = S\text{-}1/\ \ln (N)$$

(Margalef, 1958)

While the equation for Menhinick's Index ($R_2$) is;

$$\text{Menhinick Index } (R_2) = S/\sqrt{N}$$

(Menhinick, 1964)

Where; $R_1$ = Margalef Index

$R_2$ = Menhinick Index

S = Total of genera/species

N= Total of individuals sampled

### 2.4.3 Pielou's Evenness Index (J') (Pielou, 1966; Ludwig and Reynolds, 1988)

Species evenness is a measure of biodiversity which quantifies how similar of aquatic insect distribution between sites throughout sampling occasion. When there are similar proportions of all subspecies, the evenness index is equal to 1. But, when the abundances are dissimilar, in which the community of insects are made up of common and rare species, the value of this index will be low. The evenness of a community can be represented by Pielou's evenness index (J');

Pielou's evenness index (J') = H'/ln S

Where; H' = Diversity index

S = Total number of species

**2.4.4 Beta Diversity Whittaker's Index ($\beta_w$) (Whittaker, 1960)**

Beta Diversity Whittaker's Index ($\beta_w$) is used to measure biodiversity by comparing the species diversity of aquatic insects between habitats or along environmental gradients. It involves a comparison of unique taxa in each habitat because it also represents the rate of change in taxa composition among communities or across habitat. Beta diversity ($\beta_w$) can be calculated using the following equation;

$$\beta_w = \frac{S}{\alpha} - 1 \quad \text{(Whittaker, 1960)}$$

Where; S = total number of species recorded in the system. If for example the samples are collected from 2 sites, then S = a + b + c

Where; a = number of species shared between two sites

b = number of species found at habitat 1

c = number of species found at habitat 2

$\alpha$ = average sample diversity. In case the samples are collected from 2 sites, then $\alpha = \frac{(2a + b + c)}{2}$ (Magurran, 2004; Southwood and Henderson, 2000)

Therefore the equation also can be presented as;

$$\beta_w = \frac{(b+c)}{(2a+b+c)}$$ (Wilson and Shimda, 1984)

The value of beta diversity ranges from 0 (complete similarity) to 1(no overlapping species composition).