STUDY ON BASAL FOOD TROPHIC IN TWO DIFFERENT FRESHWATER ECOSYSTEM

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2016

STUDY ON BASAL FOOD TROPHIC IN TWO DIFFERENT FRESHWATER ECOSYSTEM

By

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Thesis submitted in fulfilment of the requirements

for the degree of

Master of Science

September 2016

ACKNOWLEDGEMENTS

Alhamdulillah, praise be to Allah, after an intensive period of four semesters, today is the day. Writing this note of thanks is the finishing touch of my thesis. It has been a period of extreme learning for me, not only in the scientific arena, but also on a personal level. Struggling between taekwondo, lifesaving and RMNVR trainings and writing this thesis has had a huge impact on me. I would like to reflect on the people who have supported and helped me so much throughout this period.

Foremost, I would like to express my sincere gratitude to my supervisor, Dr. Suhaila Ab. Hamid for the continuous guidance of my MSc. study and research, for her patience, motivation, enthusiasm and immense knowledge. Her support have helped me not only in all the time of research and writing of this thesis, in fact in my co-curricular activities as well. I could not have imagined having a better supervisor and mentor for my study.

My sincere thanks to the dean of School of Biological Sciences, USM for providing all facilities needed for this study. And this study was financially supported by the USM Research University Grant – RUI (1001/PBIOLOGI/811247) awarded to my supervisor.

I would also like to acknowledge Prof. Che Salmah Md Rawi and Prof. Abu Hasan Ahmad for reviewing my thesis and I am gratefully indebted to their very valuable comments on this thesis. My sincere thanks also goes to Pn. Ainol Syakira Mohd. Rodhi, science officer from Doping Control Centre (DCC) for her kind and expert assistance in analysing the samples for stable isotope analysis.

I thank my fellow Aquatic Entomology lab mates: Kak Dhiya, Miera and Fakrul; my squads: Fattah, Akmar, Bashiroh and Syafiah for stimulating discussions, for the sleepless nights during drift samplings and for the fun we had throughout this journey. Also, I appreciate my lab seniors: Dr. Kak Huda and Dr. Abang Zul for enlightening me especially in the statistical analyses. To lab assistant: Mr. Mohd. Hafizul Hasmadi a.k.a Pokjo and to school drivers, thank you.

To my friends, taekwondo teammates, scuba diving buddies, lifesaving teammates: we were not only able to support each other by deliberating over our problems and findings, but also happily by talking about things other than just our studies, thank you. My heartiest appreciation, my gratefulness goes to my family members for their encouragements throughout my years of study.

Finally, to my late granny, thank you for all your prayers, for raising me, for taking care of me, for the strength and for who I am today. I miss you. Al-Fatihah.

Thank you very much everyone!
Terima kasih, 谢谢，ありがとうございました、감사합니다.

TABLE OF CONTENTS

CHAPTER 2: LITERATURE REVIEW

CHAPTER 3: COMPOSITION OF AQUATIC COMMUNITIES IN RELATION TO ENVIRONMENTAL PARAMETERS AND WATER QUALITY STATUS OF RIVERS IN BUKIT MERAH

CHAPTER 4: DIEL PERIODICITY OF AQUATIC INSECTS DRIFT

CHAPTER 5: FOOD SOURCES AND TROPHIC STRUCTURE DETERMINATION IN FRESHWATER ECOSYSTEMS USING STABLE ISOTOPE ANALYSIS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATIONS

AN	Ammoniacal-nitrogen
AWQI	Acceptability Water Quality Index
BOD	Biological Oxygen Demand
C	Carbon
CCA	Canonical Correspondence Analysis
CF	Collector-filterer
CG	Collector-gatherer
cm	Centimetre
COD	Chemical Oxygen Demand
CPOM	Coarse Particulate Organic Matter
DCC	Doping Control Centre
DO	Dissolved Oxygen
DOE	Department of Environment
EA-IRMS	Elemental Analyser-Isotopic Ratio Mass Spectrometry
FFG	Functional Feeding Group
FPOM	Fine Particulate Organic Matter
Ha	Hectares
hr	Hour
INWQS	Interim National Water Quality Standards
km	kilometre
L	Litre
m	Metre
mg	Milligram

mm	Millimetre
N	Nitrogen
NH_3-N	Ammoniacal-nitrogen
O	Oxygen
P	Predator
pH	Hydrogenic potential
RCC	River Continuum Concept
RM	Reference Material
SC	Scraper
SCA	Stomach Content Analysis
SE	Standard Error
SH	Shredder
SIA	Stable Isotope Analysis
SPSS	Statistical Package for the Social Sciences
SWQI	Source Water Quality Index
TDS	Total Dissolve Solids
TI	Total Inertia
TSS	Total Suspended Solids
TVE	Total Variance Explained
USM	Universiti Sains Malaysia
VPDB	Vienna PeeDee Belemnite
WQI	Water Quality Index
^{o}C	Temperature in degree Celsius
δ	An isotopic signature for stable isotope ratio measurements
$\delta^{13}C$	Stable isotope of carbon signatures

$\delta^{15}N$	Stable isotope of nitrogen signatures
%	Percent
µg	Microgram
‰	Parts per thousand (per mil)

List of abbreviated family names in Canonical Correspondence Analysis (CCA) ordination biplot

Abbreviation	**Family names**	**Abbreviation**	**Family names**
Aesh	Aeshnidae	Hydropsy	Hydropsychidae
Amphip	Amphipterygidae	Iso	Isonychidae
Aphel	Aphelocheiridae	Lepido	Lepidostomatidae
Ath	Athericidae	Leptoce	Leptoceridae
Bae	Baetidae	Leptophl	Leptophlebiidae
Belo	Belostomatidae	Lib	Libellulidae
Cae	Caenidae	Nau	Naucoridae
Cala	Calamoceratidae	Neoephe	Neoephemeridae
Calop	Calopterygidae	Nep	Nepidae
Cer	Ceratopogonidae	Per	Perlidae
Chi	Chironomidae	Philo	Philopotamidae
Chlorocy	Chlorocyphidae	Plei	Pleidae
Coena	Coenagrionidae	Polycentro	Polycentropodidae
Cordu	Corduliidae	Potaman	Potamanthidae
Dyt	Dysticidae	Pse	Psephenidae
Elm	Elmidae	Serico	Sericostomatidae
Euph	Euphaeidae	Sim	Simuliidea
Ger	Gerrridae	Steno	Stenopsychidae
Gom	Gomphidae	Tab	Tabanidae
Gyr	Gyrinidae	Tip	Tipulidae
Helo	Helotrephidae	Trico	Tricorythidae
Hep	Heptageniidae	Vel	Veliidae
Hydrophi	Hydrophilidae		

KAJIAN ARAS TROFIK MAKANAN DALAM DUA EKOSISTEM AIR TAWAR

ABSTRAK

Kajian ini menyiasat komposisi serangga akuatik, aktiviti hanyutan dan jaringan makanan mereka di kawasan terpilih di negeri Perak, utara Malaysia. Empat sungai di Bukit Merah telah dipilih untuk mengkaji hubungan komposisi serangga akuatik dengan ciri-ciri sungai. Satu ribu lapan puluh tujuh (1087) individu daripada tujuh order dan 27 famili telah dipungut daripada semua sungai kajian. Koleksi serangga akuatik yang terbanyak telah diperolehi di Sungai Ara dengan 580 individu. Selain menggunakan komposisi serangga akuatik, ciri-ciri fizikokimia air juga telah digunakan untuk memantau kesihatan sungai secara meluas. Semua sungai kajian di Bukit Merah diklasifikasikan sebagai bersih (Kelas II) berdasarkan Indeks Kualiti Air (IKA). Fizikokimia sungai iaitu, kedalaman, ammonia-nitrogen, jumlah pepejal terampai, keperluan oksigen kimia dan halaju mempengaruhi taburan dan kepelbagaian fauna akuatik. Untuk lebih memahami kepelbagaian dan kaedah penyebaran serangga akuatik, corak hanyutan dan keberkalaan diel serangga akuatik telah dikaji. Serangga akuatik yang hanyut telah dipungut pada setiap selang enam jam dalam tempoh 24 jam menggunakan pensampel drif. Tiga ribu seratus empat puluh tujuh (3147) individu yang diwakili oleh sembilan order, 49 famili dan 81 genera serangga akuatik yang hanyut telah dikumpulkan. Terdapat perbezaan yang signifikan dalam hanyutan serangga akuatik antara empat selang masa (ujian Kruskal-Wallis, $\chi^2 = 25.68$, $P = 0.00$, df = 3). Hanyutan terbanyak berlaku pada waktu malam, di mana kira-kira 40% lebih tinggi daripada sampel siang, menandakan keberkalaan diel. Di samping itu, malam tidak berbulan mempamerkan kadar hanyutan lebih tinggi berbanding malam yang terang dan berbeza dengan signifikan (ujian Mann-Whitney U, ($Z = -2.093$, $P < 0.05$). Serangga akuatik yang hanyut memaparkan corak *alternans* dengan kelimpahan tertinggi pada selang masa 0200 – 0800 j (1357 individu dengan 63 genera). Mangsa hanyut dengan lebih aktif pada waktu malam (70.42 %) berbanding siang (29.58 %). Hanyutan yang paling dominan adalah Ephemeroptera, Trichoptera dan Coleoptera. Secara keseluruhan, nisbah mangsa-pemangsa dalam kajian ini adalah kira-kira 8: 1. Pendekatan isotop stabil digunakan untuk memahami jaringan makanan akuatik dan hubungan trofik dua ekosistem air tawar yang berbeza (sungai dan sawah padi). Penanda karbon dan nitrogen serangga akuatik di dalam sungai adalah di antara – 15.03 ke – 33.08 ‰ untuk $\delta^{13}C$ dan 2.59 hingga 8.11 ‰ untuk $\delta^{15}N$. Di sawah padi, penanda tersebut adalah dari – 23.59 ke – 30.58 ‰ untuk $\delta^{13}C$ dan 2.58 hingga 7.75 ‰ untuk $\delta^{15}N$. Sampel berbeza dengan signifikan antara semua kawasan kajian dan aras trofik yang ditentukan oleh ANOVA sehala dengan ujian Tukey post hoc ($F_{(6, 62)} = 2.69$, $P = 0.022$) untuk $\delta^{15}N$ dan ($F_{(6, 62)} = 15.35$, $P = 0.000$) untuk $\delta^{13}C$. Sehubungan dengan itu, dengan kesemua nilai $\delta^{13}C$ dan $\delta^{15}N$ yang direkodkan, ia boleh disimpulkan bahawa terdapat empat aras trofik yang wujud dalam ekosistem air tawar.

STUDY ON BASAL FOOD TROPHIC IN TWO DIFFERENT FRESHWATER ECOSYSTEM

ABSTRACT

Present study investigated the composition of aquatic insects, their drift activities and their food webs in selected sites in Perak, northern state of Malaysia. Four rivers in Bukit Merah were chosen to study the relationship of aquatic insect composition with the characteristics of the river. One thousand and eighty-seven (1087) individuals from seven orders and 27 families were collected from all study rivers. The most abundant aquatic insect collection was obtained at Ara River with 580 individuals. Besides using composition of aquatic insects, the physicochemical characteristics of the water also has been used to monitor health of rivers world widely. All study rivers in Bukit Merah were classified as clean (Class II) based on the Water Quality Index (WQI). The physicochemistry of the rivers i.e., depth, ammoniacal-nitrogen, TSS, COD and velocity affected the distribution and diversity of the aquatic fauna. To further understand aquatic insects' diversity and their method of dispersal, drift pattern and diel periodicity of aquatic insects was investigated. Drifting aquatic insects were collected at every six hourly intervals within 24-hour period using a drift sampler. Three thousand one hundred and forty-seven (3147) individuals represented by nine orders, 49 families and 81 genera of drifting aquatic insects were collected. There was a significant difference in aquatic insects' drift between four time intervals (Kruskal-Wallis test, $\chi^2 = 25.68$, $P = 0.00$, df = 3). Greatest drift abundance occurred during night time, where approximately 40 % higher than in the daylight samples, signifying diel periodicity. In addition, moonless nights exhibited more drift rate as compared to bright, full-moonlit nights and differed significantly (Mann-Whitney U test, ($Z = -2.093$, $P < 0.05$). Drifting aquatic insects displayed alternans pattern with the greatest abundance at 0200 – 0800 h interval (1357 individuals with 63 genera). Preys drifted more actively at nighttime (70.42 %) as compared to daytime (29.58 %). The most dominant drifters were Ephemeroptera, Trichoptera and Coleoptera. Overall, the prey-predator ratio in this study was about 8: 1. Stable isotope approach was used to comprehend the aquatic food webs and trophic relationships of two different freshwater ecosystems (rivers and paddy fields). The aquatic insects' carbon and nitrogen signatures in the rivers ranged from – 15.03 to – 33.08 ‰ for $\delta^{13}C$ and 2.59 to 8.11 ‰ for $\delta^{15}N$. In paddy fields, the signatures ranged from – 23.59 to 30.58 ‰ for $\delta^{13}C$ and 2.58 to 7.75 ‰ for $\delta^{15}N$. The samples varied significantly between all study sites and trophic levels as determined by one-way ANOVA with Tukey post hoc test ($F_{(6, 62)} = 2.69$, $P = 0.022$) for $\delta^{15}N$ and ($F_{(6, 62)} = 15.35$, $P = 0.000$) for $\delta^{13}C$. Correspondingly, with all the $\delta^{13}C$ and $\delta^{15}N$ values recorded, it can be deduced that there are four trophic levels existed in the freshwater ecosystems.

CHAPTER 1

GENERAL INTRODUCTION

Invertebrates are animals that do not possess an internal skeleton of cartilage or bone (Reese Voshell, 2002). While macroinvertebrates refer to invertebrates that exceed 0.5 mm and are large enough to be seen with naked eyes. Freshwater macroinvertebrates include members of Insecta (insects), Hirudinea (leeches), Hydrachnida (water mites), Oligochaeta (segmented worms), Rotifera (rotifers), Branchiopoda (clam shrimps), Nematomorpha (horse hair worms), Crustacea (shrimps and crabs), Mollusca (bivalves and gastropods), Nemertea (ribbon worms), Nematoda (roundworms), and Turbellarians (flatworms) (Yule and Yong, 2004).

The composition of aquatic macroinvertebrates in headwater stream differ with downstream reaches. The River Continuum Concept (RCC) introduced by Vannote *et al*. (1980) makes predictions about downstream changes of aquatic fauna are based on the macroinvertebrates functional feeding group representation in response to changes of food availability (Dudgeon, 1999) and microhabitats (Meyer *et al*., 2007). Hence, it causes a variation in the food webs according to the organic carbon sources and types of invertebrate population that occupy the habitat. Stream communities are grouped according to channel size into headwaters, medium-sized streams and large rivers. Headwater streams are influenced greatly by riparian vegetation, which reduces aquatic primary production by shading and contributes large amount of leaf litters (Dudgeon, 1999). Shredders are co-dominant with collectors in this stream.

According to Dudgeon (1999), as stream width increases and canopy shading decreases, aquatic primary production rises and leaf litter inputs reduced. Aquatic insects inhabiting in this microhabitat also depend on fine organic particles imported from upstream. Scraper biomass is maximised yet collectors are numerous too. In addition, large rivers obtain more fine particulate organic matter (FPOM) from upstream, and thus, collector-gatherers and collector-filterers dominate the aquatic insect assemblages downstream. Shading effect is no longer significant here however, aquatic primary production will be limited by water depth and turbidity.

Other than microhabitat availability and physicochemical characteristics of water, the drift of aquatic insect larvae also contributes to their diversity and distribution pattern. Drift is also one of the prey-predator activity that contributes to food web construction in aquatic ecosystems. Mechanisms causing drift are thought to include dislodgement by current, pollution, changes in food supply and predation (Brittain and Eikeland, 1988). However, it is obvious that drift has a behavioural component (Muller, 1974; Wiley and Kohler, 1980). Nocturnal drift is genetically encoded as this behaviour is retained by some species in a predator free environment (Elliot, 1968) and light intensity is the main factor responsible for drift patterns (Bishop, 1969). Many studies also have proved greater numbers of macroinvertebrates in the drift during hours of darkness, most probably due to visual-oriented predator avoidance or associated with diel changes in foraging movements that results in accidental dislodgement (Elliot, 1968). The presence of predators influenced the drift density of preys significantly (Lancaster, 1990).

Therefore, it is obvious that riparian and aquatic ecosystem are interdependent and was described by VanDongen *et al*. (2011) as intricate and complex continuum of linked habitat. A network of predatory interactions by Charles Darwin referred to as an “entangled bank”, that is now known as the “food web”. Food web structure may have several meanings that can basically refer to the number of trophic levels in a food chain, or contrariwise, can symbolise the degree of complexity in a food web network (Vander Zanden *et al*., 2006).

As food webs are complex and trophic interactions are greatly variable in space and time, the interaction between these two habitats (riparian and aquatic) can be computed by measuring the rate of energy transfer between trophic levels using stable isotope techniques (Gladyshev, 2009). Stable isotopes are becoming a standard analytical tool in studying food web ecology (Gladyshev, 2009) besides solving problems in plant and animal physiology, biogeochemistry, migration patterns and diet composition (Fry, 2006). Stable isotope analysis had been applied in present study to determine the basal food sources of the aquatic faunas in several freshwater ecosystems. The main sources of fixed energy that drive stream food webs are from organic carbon that can be found from allochthonous riparian sources (e.g., leaf litters) or autochthonous sources (primary producers e.g., algae). Most streams depend on both allochthonous and autochthonous energy, although the relative importance of each sources varies with elevation, stream size and some other factors. For example, terrestrial carbon is more important in forested headwater streams, while autochthonous carbon is more important in open-canopied, medium-sized rivers. Therefore, stable isotopes of carbon ($\delta^{13}C$) can be used as an indicator for the ultimate source carbon in food webs, while nitrogen isotopes ($\delta^{15}N$) have been used to infer trophic position of consumers in complex food webs as there is 3 % to 4 % $\delta^{15}N$ enrichment from prey to predator (Vander Zanden and Rasmussen, 2001).

Using stable isotope approach as a tool in comprehending food web structure is neglected in Malaysia due to lack of proper facilities. Recently, there were several studies that have been conducted on trophic structure determination in mangroves (Newell *et al.*, 1995; Zulkifli *et al.*, 2012, 2014) and in forested tropical streams (Dhiya Shafiqah, 2014) using stable isotope approach. However, those studies were less meaningful due to lack of specific taxa of the organisms living in the differently polluted rivers and paddy fields, as different species of consumer consumes different type of food. For that reason, this study attempted to complement and complete the information gap and can serve as baseline data for future reference, studies and also for future conservation efforts.

Hence, this research emphasised on the significance of aquatic insect in freshwater ecosystems, focusing on the following objectives:

1) To investigate the abundance, diversity and richness of aquatic insect larvae in relation to water quality in four of the Bukit Merah rivers.
2) To investigate the diel periodicity and effect of moonlight on the drift of aquatic insect, as well as the prey-predator relationship among drifted aquatic insects.
3) To identify the food sources of aquatic insects in the freshwater ecosystems specifically at less disturbed, disturbed rivers and paddy fields and to determine the trophic structure of the food web by using stable isotope analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Aquatic insect in freshwater ecosystem

Aquatic macroinvertebrates play a vital role in the ecosystem of which, they not only serve as food for larger aquatic organisms such as fish, as useful as they are, they also involved in nutrients and organic matter decomposition and breakdown (Juma, 1998). Insects provide a critical linkage in energy flow from microbial to vertebrate populations in aquatic ecosystems and between aquatic and terrestrial ecosystems (Nakano and Murakami, 2001). Hershey *et al.* (2010) noted that food web studies have been used to comprehend this linkage and thus integrate organic matter processing with community interaction. Aquatic insects can be agreed as conduits for energy flow in aquatic food webs. In most aquatic ecosystems, three or four trophic levels are existing, including primary producers and detritus; primary consumers (shredders, collectors and scrapers); secondary consumers (invertebrate predators) and tertiary consumers (vertebrate predators). Combination factors of altering resource and invertebrate abundance and distribution will reflect the food web structure in any particular aquatic ecosystem. Study of food web relationships in lotic and lentic systems and linkages between stream and riparian ecosystems have been started using stable isotope analysis in recent years.

Moreover, aquatic insects are often used as river health assessments whereby they could be good indicators for biological monitoring of the water quality. This application as biomonitoring tool has been widely reported in tropical and temperate region streams (Yule and Yong, 2004; Rosenberg *et al*., 2008). Several other freshwater organisms such as bacteria, algae, vascular plants and fish have been used in biological monitoring, however, aquatic macroinvertebrates including molluscs, crustaceans, annelids and insects are often recommended for biomonitoring programmes because of their diversity, easy of collection, sensitive and intolerant to pollution, habitat changes and severe natural events and ease of identification to levels needed for bioassessments (Hershey *et al.*, 2010). For instance, a recent assessment noted that 49 of 50 U.S. states use macroinvertebrates in their water quality monitoring programmes, with about two-thirds of the programmes use fish and only one-third use algae (Hershey *et al.*, 2010). Measuring their abundance and diversity offer more ecologically meaningful measure of pollutions than do direct chemical analyses as they are considered sensitive indicators of environmental perturbations. Nevertheless, the application of macroinvertebrates in biomonitoring programmes in Malaysia is barely documented as compared to neighbouring countries including China, Japan, South Korea, Mongolia, Russia and Thailand as revised by Morse *et al.* (2007).

In addition, aquatic insects and other aquatic organisms found in paddy field ecosystems also play an essential role as the biological control agents of vectors and pests. They can be of both agricultural significance and public health, and are acknowledged elements of Integrated Pest Management (IPM) (Halwart, 2006). However, the diversity of aquatic species in paddy fields is usually invisible, both for people looking at the paddy field itself and in terms of aquatic production in national statistics. For example, fish that are specialised to feed on mosquito (Diptera) larvae or on snail may control vectors of malaria and schistosomiasis (Halwart, 2006). Other than that, aquatic insects also occupy key positions at every level of the food webs in paddy fields. Fish that inhabit the paddy fields also feed on weeds and other aquatic insects thereby reducing pest problems and maintaining balance in an ecosystem (Way and Heong, 1994). In Costa Rica, benthic macroinvertebrates have been used as water quality indicator in conventional rice plantations (Castillo *et al*., 1997). Macroinvertebrates biodiversity, especially aquatic insects, can be affected by direct exposure to pesticides and insecticides and thus, decreasing species richness and faunal composition after each application. According to Castillo (2000), higher temperatures could increase pesticide solubility in water and increase pesticide uptake by the aquatic insects. At higher temperatures, concentration of dissolve oxygen decreases, resulting in more challenging water conditions for the survival of macroinvertebrates. Besides that, the abundance of primary consumer increases with nitrogen-based fertilisers due to increase in photosynthetic aquatic biomass (Roger *et al*., 1995).

Furthermore, some of these aquatic insects are important as disease vectors and can cause biting nuisance to human. For example, *Aedes aegypti* (Diptera: Culicidae) is a vector of outrageous viral diseases such as dengue and dengue hemorrhagic fever. The outbreak of this kind of diseases are getting worse with human alteration on hydrological systems and expands of irrigation channels (Dudgeon *et al*., 2006). In tropical region, water-borne diseases contributed to almost 80 % of all illness (Dudgeon *et al*., 2006), 46.5 million cases of malaria, 5.8 million cases of lymphatic filariasis and

1.7 million cases of schistosomiasis. Water-borne disease is caused by pathogenic microorganisms that are transmitted in contaminated fresh water. According to the World Health Organization (2016), various forms of waterborne disease probably are the most prominent examples and affect mostly children in developing countries.

Some aquatic insects also play significant roles as bio-control agents. Other than mosquito larvae, simuliid (blackfly) larvae and chironomid (non-biting midges) larvae are the most predated prey in the freshwater habitat (Bay, 1974). The odonates and aquatic coleopterans, especially Notonectidae and Dysticidae, have been observed to ingest mosquito larvae as a part of their natural food consumption (Kumar and Hwang, 2006). Besides that, odonate naiads also prey upon simuliid larvae (Peterson and Davies, 1960). In addition, *Toxorhynchites* (Diptera: Culicidae), a mosquito genus with cannibalistic larvae, has attracted much attention as *Aedes aegypti* and other *Aedes* (Diptera: Culicidae) mosquito larvae biological control agents. In fact, *Chaoborus crystallinus* (Diptera: Chaoboridae) is a potential bio-control agent against various woodland mosquitoes in Poland (Skierska, 1969); and *Mochlonyx culiciformis* is also a predator of *Aedes communis* (Chodorowski, 1968). Other than that, Washino (1969) reported, hemipteran species of *Belostoma flumineum* and *Abedus indentatus* were good mosquito predators, while *Laccotrephes* sp. predated on *Aedes vittatus* larvae. Also, trichopteran families of Hydropsychidae and Rhyacophilidae were regarded as important simuliid predators in South Africa (Chutter, 1968) and Utah (Peterson, 1960).

2.2 Aquatic food web

Food webs are the central in shaping ecology. The organisms that consist in food webs live in a spatially diverse world where habitats differ greatly in productivity, abundance of resources and behaviour of consumer and also demography (Polis *et al.*, 1997). The food webs in freshwater ecosystems are made up not only from aquatic organisms but also compose of food sources from the riparian and terrestrial environments. In fact, the basic constituents of food webs comprise of nutrients, detritus, organisms and also aquatic producers, all cross spatial boundaries.

Basal food sources for the aquatic insects in most streams originates from riparian forests as allochthonous source and growing algae in rocks as autochthonous source. Nevertheless, forest canopy covers the streams and the amount of sunlight that reaches the substrate decreases and algae which serve as one of the food sources might not grow abundant on rocks. Therefore, fallen leaves, fallen branches and woody debris that derived from riparian forest and smaller aquatic insects would be the main constituents of organic matter in the streams. The input of riparian detritus is crucial for the conservation or restoration of stream food webs (Wallace *et al.*, 1997; Whiles and Wallace, 1997). Fallen leaves and branches initially pile on the substrate as coarse particulate organic matter (CPOM), which is then enriched by microorganisms in the streams (Yoshiomura, 2012). According to Yoshiomura (2012), riparian-derived CPOM has high nitrogen content which makes it advantageous food for shredders. Shredders break the enriched litter into pieces while feeding. In this way, CPOM is then converted into fine particulate organic matter (FPOM) and by battering the CPOM on rocks also results in FPOM formation. Other than functioning as food for collectors, grazers and filterers, FPOM is also used by some trichopterans as material for their larvae cases.

General studies of freshwater ecosystems have arranged aquatic insects as primary and secondary consumer in the trophic levels (Cummins, 1973), more precisely, herbivores and carnivores. The functional feeding groups and feeding mechanisms of the aquatic insects are associated with their foraging strategies and the morphology of their mouthparts (Lancaster and Downes, 2013). Five general classification system for aquatic insect trophic guilds are shredders, collector-gatherers, collector-filterers, scrapers and predators. The organisation of the basis of common types of feeding mechanisms engaged with a broad types of food categories, to be exact, shredders – vascular plant tissues; collector-gatherers and collector-filterers – suspended detrital particles; scarpers – attached algae; predators – live prey (Table 2.1). Some collectors, shredders, grazers and filterers are consumed by predators and then might be preyed by fishes and other vertebrates. Woody debris in streams also serve as food for the collectors, besides providing habitat for aquatic insects and fishes (Fausch and Northcote, 1992). For example, trichopterans *Anisocentropus* sp. (Calamoceratidae) and *Lepidostoma* sp. (Lepidostomatidae) use shredded dead plants and debris to construct portable cases as their ‘home’.

Table 2.1. General classification system for functional feeding groups of aquatic insects

General classification based on feeding mechanism	General particle size of food (µm)	Sub-division based on feeding mechanisms	Sub-division based on food consumption	Examples of taxa
Shredders	$> 10^3$	Chewers and miners	Herbivores: Living vascular plant tissue	Trichoptera (Phryganeidae) Lepidoptera
		Chewers and miners	Detritivores: Decomposing vascular plant tissue	Trichoptera (Limnaephilidae) Diptera (Tipulidae)
Collectors	$< 10^3$	Filter (suspension) feeders	Herbivore-detritivores: Living algal cells, decomposing organic matter	Ephemeroptera (Siphlonuridae) Trichoptera (Hydropsychidae, Stenopsychidae) Lepidoptera Diptera (Simuliidae, Chironomidae)
		Deposit (surface) feeders	Detritivores: Decomposing organic matter	Ephemeroptera (Baetidae, Caenidae) Coleoptera (Elmidae)
Scrapers	$< 10^3$	Mineral and organic scrapers	Herbivores: Algae and associated material	Ephemeroptera (Heptageniidae) Coleoptera (Psephenidae)
Predators	$> 10^3$	Swallowers	Carnivores: Whole animals	Odonata Plecoptera Trichoptera (Rhyacophilidae) Coleoptera (Dytiscidae, Gyrinidae)
		Piercers	Carnivores: Cell and tissue fluids	Hemiptera (Belostomatidae, Nepidae)

Adapted from Morse *et al*. (1994).

2.3 Stable isotope analysis

There are two approaches that can be conducted in order to study food web structure in a particular environment, specifically, stomach content analysis (SCA) and stable isotope analysis (SIA). Stable isotope analysis was chosen for this study, over stomach content analysis regardless of the status of the animal's stomach, the isotope tracers in the tissues will portray an understanding of its trophic position and food source (Michener, 2007) and likewise, the stomach content in an organism may only exhibit the food consumed shortly just before the organism is captured. Carbon and nitrogen stable isotopes are normally used for dietary study and to study energy sources and food web structure in ecosystem and also, to study the effects of anthropogenic stress on aquatic ecosystem (Bergfur *et al.*, 2009) and thus, provide insight into foraging ecology. There is an increasing recognition that stable isotopes of naturally occurring elements of carbon, nitrogen, sulphur, oxygen and hydrogen, are a powerful tool to trace these flows (Peterson and Fry, 1987; Schindler and Lubetkin, 2004; Fry, 2006; West *et al.*, 2006). In food web and organic matter flow studies, the enrichments of the stable isotopes ^{13}C and ^{15}N across trophic levels is a commonly used tool in aquatic and terrestrial ecosystems (Jacob *et al.*, 2005).

Stable isotopes of carbon and nitrogen provide a powerful tool to quantify the importance of nutrients and energy to freshwater food webs (Hershey *et al.*, 2010). Stable isotopes of carbon ($\delta^{13}C$) can be used to infer the consumer’s food source because various organic matter sources for consumer often have different relative abundances of the two stable isotopes of carbon, ^{13}C, ^{12}C. The relative abundances of these isotopes only change slightly as the organic matter is processed by various consumers, the difference between ratios ^{13}C:^{12}C in a consumer and a standard can be used to determine the food sources of consumer. While ^{15}N has been used in trophic and enrichment studies and to serve as a tracer of consumer food sources as well as nitrogen distribution through the system (Hershey *et al.*, 2010). Therefore, consumers in a food web can generally be assigned to their trophic level even if their precise food source is unknown. On average, $\delta^{13}C$ and $\delta^{15}N$ increase by 0.5 to 1 and 2.8 to 3.4 ‰, respectively, from one trophic level to the next (Minagawa and Wada, 1984; Peterson and Fry, 1987; Michener and Schell, 1994). In many macroinvertebrates, carbon is contained in both inorganic and organic forms (Mateo *et al.*, 2008). Inorganic carbon from carbonates, largely present in shells and exoskeletons, is highly enriched with in ^{13}C with respect to the soft tissue carbon has $\delta^{13}C$ values around 0 ‰ (Klump and Arthur, 1999). Although the percentage of carbon in carbonates is only 12 %, which is about three times lower than in animal organic tissues, depending on the carbonate abundance and owing to its high ^{13}C content, its weight the $\delta^{13}C$ value of the bulk sample can be substantial.

Previous studies have shown that $\delta^{15}N$ changes with not only increasing nutrient concentrations but also with changes in land-use. The increment of $\delta^{15}N$ in coarse particulate organic matter (CPOM) and periphyton most likely originates from increased nitrogen runoff from agricultural land use (Osmond *et al.*, 1995). $\delta^{15}N$ increases along nutrient and agricultural gradients (Harrington *et al.*, 1998; Vander Zanden *et al.*, 2005; Udy *et al.*, 2006) and increased $\delta^{15}N$ has even been used to track fertilisers from nearby landscapes in aquatic environment (Exner and Spalding, 1994; Penncock *et al.*, 1996). As stream ecosystems often depend on nutrient inputs of terrestrial source, it is not astonished that both land use and nutrients have been argued to be the best predictors of $\delta^{15}N$ in aquatic biota (Vander Zanden *et al*., 2005; Udy *et al*., 2006).

Other than ecology, food webs and trophic relationships study of aquatic insects in freshwater ecosystems, stable isotope techniques was also applied in the diet determination of Neotropic Cormorant (*Phalacrocorax brasilianus*) marine bird communities along the northern coast of South America (Munoz-Gil *et al*., 2013). Besides ecological studies in aquatic ecosystems, stable isotope analysis were also widespread in natural sciences that include numerous applications in the biological, environmental and earth sciences.

In archaeology, the reconstruction of paleodiet used carbon and nitrogen isotope to reconstruct diet, while oxygen isotopes are used to identify geographic origin and paleoenvironment. Strontinum and lead isotopes are used to discover population movements, seasonal mobility and migration (Carlson, 1996). Moreover, characterization of artifacts in the Bronze Age Mediterranean era, lead isotope is used to analyse archaeological material sourcing (Budd *et al*., 1996). Archaeological materials such as metals, glass and lead-based pigments have been sourced using isotopic techniques (Shortland, 2006).

In forensic sciences, analysis of hair strands could possibly identify recent geographic histories of an individual by analysing sulphur and oxygen isotope variations of the hair strands as those variations are different all over the world. Hair growth is basically a reflect of diet consumed, especially drinking water intake, whereby stable isotopic ratios of drinking water could reveal location and the geology that the water percolates through (White, 2004). For example, it is promising to identify whether a terrorist suspect had recently been to a particular location based on hair analysis. This hair strand analysis is a non-invasive method which is becoming more recognised in cases that even DNA test or other traditional method could not solve.

Likewise in palaeoclimatology (the study of changes in climate based on the scale of the entire Earth’s history), the ratio of ^{18}O to ^{16}O in ice and deep sea cores can be used as a proxy for reconstructing climate change. During colder periods of history of Earth, such as during the ice age, ^{16}O is favourably evaporated from the colder oceans, leaving heavier and more sluggish ^{18}O behind. Single-celled protists Foraminifera combine dissolved oxygen in the water with carbon and calcium to build their shells and thus incorporate with temperature-dependent ^{18}O to ^{16}O ratio. When these organisms die, they settle down on the sea bed, preserving a long and important record of global climate change through much of the Quaternary. Studies of past fluctuations in the biodiversity and environment often reveal on the current situation, precisely the impact of climate on mass extinctions and biotic recovery (Shaney and Benton, 2008).

Other than that, stable isotopes are also applied into an aspect of geology that are based upon natural variations in various elements. Variations in isotopic abundance reflect information about the origin and ages of rocks, air or water bodies, or processes of mixing between them. It branches into more complicated field of studies, to be exact, stable isotope geochemistry, radiogenic isotope geochemistry, noble gas isotopes, uranium-series isotopes and anthropogenic isotopes.

To establish an isotopic signature for a material, the ratios of the stable isotopes of a number of elements such as $^{15}N/^{14}N$ and $^{13}C/^{12}C$ need to be measured. The isotopic abundances of these elements were fixed on a global scale. To make the resulting figures more manageable, the "small delta notation" (δ) is adopted. δ–values are commonly multiplied by 1000 so that they are reported in parts per thousand (‰ or per mil). Carbon ($\delta^{13}C$) changes relatively little as trophic level change and is used to identify the ultimate source of carbon, or the primary energy source for an ecosystem or group of organisms (Fry and Sherr, 1984). On average, $\delta^{13}C$ only increased by 0.5 to 1 ‰ from one trophic level to the next (Michener and Schell, 1994; Minagawa and Wada, 1984; Peterson and Fry, 1987). Nitrogen ($\delta^{15}N$) on the other hand, becomes enriched when transferred throughout a food web by means of feeding and predation (Peterson and Fry, 1987). This means nitrogen can be used to better identify trophic position and give more information on dynamics between trophic changes, since the $\delta^{15}N$ of a consumer is typically enriched by 3.4 ‰ relative to its diet (De Niro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987).

On the contrary, there are certain limitations need to be considered upon estimating the trophic position (Vander Zanden *et al*., 1997). One of the limitations is the trophic position estimation required assumptions of the trophic position of prey organisms. For example, Vander Zanden *et al*. (1997) estimated that the trophic position of zooplankton prey (2.5) contrasts with Sprules and Bowerman (1988) who reported that North American food webs have a modal food chain length varying from one to eight trophic levels, averaging between three and five trophic levels. Nevertheless, when estimating the trophic position for more than one nitrogen sources, then it must be calculated using the following equation (Post, 2002):

$$\text{Trophic position} = \lambda + \frac{(\delta^{15}\text{Nsc}-[\delta^{15}\text{Nbase}_1 \text{ X } (\alpha + \delta^{15}\text{Nbase}_2) \text{ X } (1-\alpha)])}{\Delta_n}$$

Where, α is the proportion of nitrogen in the consumer that ultimately derived from the base of food web one. Three assumptions are generally made: the trophic fraction of δ^{15}N is 3.4 ‰, the trophic fractionation of δ^{13}C is near 0 ‰ and carbon and nitrogen move through the food web with a similar stoichiometry. When the movement of nitrogen and carbon through the food web is similar, α can be estimated using carbon isotopes such that: $\alpha = \frac{(\delta^{13}\text{Csc}-\delta^{13}\text{Cbase}_2)}{(\delta^{13}\text{Cbase}_1-\delta^{13}\text{Cbase}_2)}$.

2.4 Aquatic insect drift

There are many activities involved in constructing a complete food web in a particular ecosystem. One of those activities is the movement of aquatic insects, or specifically, aquatic insects drift. Drift of aquatic organisms was first observed in early twentieth century. Amongst the earliest studies, Needham (1928) initially discovered that invertebrate drift occurred when terrestrial insects fell into the river and serve as food source for fish. However, in collecting benthos in streams near Ithaca, New York, he found large number of drifting aquatic insects present in his samples. Several other studies were completed in the next two decades, drawing a conclusion that a continuous drift of invertebrates is considered as natural feature of a stream (Ide, 1942; Dendy, 1944). Muller (1954) also reported large quantities of drifting invertebrates and made observations on the relationships among drift, bottom organisms and food consumed by fish. Subsequent investigations by Waters in 1962 also helped to initiate more interest in aquatic insect drift on his discovery of diel periodicity in drift.

Drifting aquatic insects spend very short time in the water column. There are several causes that can explain drift. The factors that affect drift are normally thought to be catastrophic, which could alter a certain habitat to be unsuitable to be lived in and might results in drifting. Factors for “catastrophic drift” for instance, flooding or drought (Anderson and Lehmkuhl, 1967), pesticides (Davies and Cook, 1993) and poor water quality (Brittain and Eikeland, 1988) such as low dissolved oxygen, low pH, high temperature, might make life unsuitable which will result in drift. Drift preference in the day or nighttime, or even other consistent period of the day that resulted from behaviour of certain species is termed as “behavioural drift”. Dispersal (Allan, 1995), piscine predator avoidance (Flecker, 1992) and invertebrate predator avoidance (Peckarsky, 1979; 1980) could be important causes that influence behavioural drift. Whereas, the continuous movement of all species in low number, taking place at almost all time is called “constant drift”, which might have resulted from accidental dislodgement and loosing contact from the substrate and hence, entering the drift. Nevertheless, it is not always possible to differentiate among these three types as they might overlap and also interact each other.

Most drifting aquatic insects exhibit diel periodicity in their drifting activities. A recurrent temporal pattern in a 24-hour period defines a diel periodicity (Waters, 1972). Usually there appeared a dramatic increase at full darkness and then a sharp return to daytime at dawn. According to Waters (1972), *Baetis* (Ephemeroptera: Baetidae) mayfly and *Gammarus* (Amphipoda: Gammaridae) shrimp, and mostly all aquatic insects are night-active, with greater drift rate in darkness while most trichopterans are day-active periodicities, showing higher drift occurrence during the daytime. Apparently, the benefit of drifting in the dark is that being able to forage with maximum protection against potential predators in darkness (Chaston, 1969a). Day-active movement on the other hand, probably resulted from a direct metabolism activity relationship related to water temperature (Waters, 1968). Waters (1972) noted that there are also possibilities that during case-building activity, some pre-pupation or pre-emergence activity could also result in drift periodicities. Hence, foraging and predator avoidance are not the only factors in periodic behaviour.

Despite that, the relationship of drifting macroinvertebrates and fish feeding has been of considerable interest to stream ecologist and stream managers. Early studies of drift had investigated the association of aquatic insects as available fish food (Needham, 1928; Ide, 1942). An investigation by White (1967) found a significant feeding of brook trout on the diurnal-drifting *Brachycentrus americanus*, was observed during daytime. Other than feeding, drift also functions as transportation for the young salmonids fry as they seem to depend heavily on drift to move about, as the fish grow, larger salmonids rely greater dependence on bottom foraging (Elliot, 1970). It has been observed that maximum fish feeding occurred during greatest drift periodicities. Mason (1969) discovered that juvenile coho salmon rely mainly on drift and feed mostly at night. Brown trout also appeared to feed well at night, consuming the drifting aquatic insects (Elliot, 1967; 1970). Consequently, it can be deduced that stream fishes are opportunist and often utilising stream invertebrate drift as their source of food. However, full dependence on drifting insect for feeding would not be sufficient and above all, bottom foraging (in older fish) strengthen their food chain in trophic ecology (Waters, 1972).

2.5 Water Quality Index (WQI)

A number of indices have been established to summarise water quality data in an easily understandable and expressible format for general use, such as political decision-makers, non-technical water managers and general public. A Water Quality Index (WQI) is a degree of water quality value of an aggregate set of measured water parameters. It is a simplified form of extensive amount of unitless numbers ranging from 1 to 100, with a higher number indicates of better water quality. Six water chemical parameters of dissolved oxygen (DO), five-day biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), ammoniacal-nitrate (AN), suspended solids (SS) and pH are used in WQI. Calculations are performed on the values of their sub-indices, instead of the actual data themselves (Appendix 1).

Once the sub-indices have been calculated, the WQI can be computed using the following equation of DOE (2009):

$$WQI = 0.22 \times SI\ DO + 0.19 \times SI\ BOD + 0.16 \times SI\ COD + 0.15 \times SI\ AN + 0.16 \times SI\ SS + 0.12 \times SI\ pH$$

Once the WQI value is acquired, the water quality status, or degree of pollution can be defined (Appendix 2). There are 5 water quality classes according to the WQI ranges. The description of water quality classes is explained in Appendix 3.

However, there are several limitations that may deter the index to deliver water quality information accurately (Hallock, 2002). Besides being general in nature (imprecise), the pre-identified sets of water quality at one location might obtain good WQI score than other locations from the same river, and yet may be impaired by the parameters not included in this index. Indeed, the water quality parameters used in WQI are all physicochemical based parameters, without including fecal coliform or coli-based indicators and skin contact relevance, nor considering heavy metals and carcinogens. Moreover, the aggregation of data may conceal short-term water quality problems which may related to temporal variations of the river. A satisfactory WQI at a particular location does not reflect the overall water quality was always satisfactory. In fact, a good score ought to indicate that poor water quality was not chronic during the period included in the index.

2.6 Inland waters categorisation

According to UK Maritime and Coastguard Agency (2014), inland waters include all water that is not categorized as sea, for example, canals, rivers, lakes and some estuarial waters. Inland waters are categorised as A, B, C or D, which are defined and listed in UK Merchant Shipping Notice (MSN) 1776. Inland waters are classified as one of four categories below (Table 2.2).

Table 2.2. Inland waters classification

River category	Description
A	Narrow rivers and canals where the depth of water is generally less than 1.5 metres
B	Wider rivers and canals where the depth of the water is generally 1.5 metres or more and where the significant wave height could not be expected to exceed 0.6 metres at any time
C	Tidal rivers, estuaries and large, deep lakes and lochs where the significant wave height could not be expected to exceed 1.2 metres at any time
D	Tidal rivers and estuaries where the significant wave height could not be expected to exceed 2 metres at any time

Adapted from UK Maritime and Coastguard Agency (2014).

CHAPTER 3

COMPOSITION OF AQUATIC INSECTS IN RELATION TO ENVIRONMENTAL PARAMETERS AND WATER QUALITY STATUS OF RIVERS IN BUKIT MERAH CATCHMENT AREA

3.1 Introduction

The distribution of aquatic macroinvertebrate assemblages in disturbed streams is influenced by many factors, such as, habitat degradation (Hilsenhoff, 1977; 1982) and organic pollution (Whitehurst, 1991). Land use is an important determinant in the structure of benthic macroinvertebrate communities including aquatic insects, as benthic macroinvertebrates are often used as biological indicators of the aquatic environments. According to Wallace and Webster (1996), aquatic macroinvertebrates are good indicators of watershed health because most of them spend most of their life cycles in water. Their assemblages could be used for classification of degree of pollution in aquatic ecosystem by determining the sensitivity and tolerance of each species (Rosenberg *et al*., 2008).

Aquatic macroinvertebrates are sensitive towards changes in their microhabitat. They behave towards their environmental conditions by showing different distribution pattern based on their morphology and behaviour towards their environmental conditions. This is because aquatic macroinvrtebrates are sensitive to habitat characteristic and substrates (Che Salmah *et al*., 2005; Subramaniam and

Sivaramakrishnan, 2005), water temperature (Triplehorn and Johnson, 2005), pH, oxygen content and riparian vegetation (Subramaniam *et al.*, 2005). As aquatic macroinvertebrates are crucial components in aquatic food web, the changes of physicochemical parameters of water would alter the macroinvertebrate assemblages and thus, the food web will be impaired.

Since rivers in Bukit Merah catchment area have developed into many land uses, the water quality might change too. In Malaysia, Water Quality Index (WQI) has been used to measure and interpret level of water quality, which is used extensively by government departments such as Department of Irrigation and Drainage and Department of Environment. This index will in turn, transform the complex water quality data into a simple information that could describe the water quality status of the rivers. Calculations of WQI values are made based on the values of each of the water parameters' sub-indices. From the WQI value calculated, it can be classified into five river categories (DOE, 2009). Previously, only one study has been conducted in Kerian district, Perak at Bogak River, Serdang River and Kerian River by Nurul Huda (2011). There are numerous activities that have been conducted along the rivers within Bukit Merah area, for example, sand mining, oil palm plantations, flood gates, recreational and human settlements. All of these human disturbances usually contribute to many adverse effects such as low diversity and lower productivity of macroinvertebrate community. Therefore, there is high possibility that these study areas have different composition of aquatic insects and water quality status that needs to be investigated. Aquatic insect assemblages could be used as biological indicator for water quality assessment of the rivers that were impacted by human activities. Their population can be used to classify