SOME ASPECTS OF ECOLOGY AND GENETICS OF CHIRONOMIDAE (DIPTERA) IN RICE FIELD AND THE EFFECT OF SELECTED HERBICIDES ON ITS POPULATION

By

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LIST OF ABBREVIATION AND SYMBOLS

ai	=	Active Ingredient
BMAES	=	Bukit Merah Agricultural Experimental Station
bp	=	Base Pair
cm	=	Centimetre
COI	=	Cytochrome oxidase subunit I
df	=	Degree of Freedom
DNA	=	Deoxyribonucleic Acid
dNTPs	=	deoxynucleotide triphosphates
E	=	Emergence
ETOH	=	Ethanol
F	=	Fallow Phase
Fe	=	Fertilizer
G	=	Germination Phase
H	=	Herbicide
I	=	Insecticide
L	=	Lime
LC	=	Lethal Concentration
M	=	Mature Phase
mg ml^{-1}	=	Milligram per milliliter
$MgCl_2$	=	Magnesium Chloride
mM	=	MiliMolar
NS	=	Not Significant
P	=	Plough Phase
PCR	=	Polymerase Chain Reaction
PH	=	Preharvesting Phase
r	=	Correlation Coefficient
rpm	=	Revolution Per Minute
S1	=	First Season
S2	=	Second Season
SPSS	=	Statistical Package for Social Science
T	=	Tiller Phase

t	=	Ton
Taq	=	*Thermus aquaticus*
TBE	=	Tris Borate EDTA
TOM	=	Total Organic Matter
TSS	=	Total Suspended Solids
YS	=	Young Seedling Phase
µg	=	Microgram
µL	=	Microlitre
µm	=	Micrometer

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1. Al-Shami S., Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2006). Influence of physico-chemical variables on the abundance of Chironomidae (Diptera) in rice agroecosystem. Proceedings of KMITL International Conference on Science and Applied Science 8-10 March 2006, Bangkok, Thailand.

2. Al-Shami S., Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2006). Influence of physico-chemical variables on the abundance of Chironomidae (Diptera) in rice agroecosystem. *KMITL Science Journal* **6** (1) (in press).

3. Al-Shami S., Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2006). Toxicity of Two Herbicides 2,4-D Dimethylamine and Bensulfuron methyl to *Chironomus kiiensis* (Chironomidae Diptera). Proceedings of 3rd Life Science Postgraduate Conference. Universiti Sains Malaysia, Penang, 24-27 May 2006.

4. Al-Shami S., Siti Azizah M. N., Che Salmah M. R. and Abu Hassan A. (2006). Molecular Identification of Rice Field Chironomids (Chironomidae: Diptera). Proceedings of 5th IMT-GT UNINET Conference and International Seminar. Medan, Indonesia 22-23 June 2006.

5. Al-Shami S., Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2006). Toxicity of Two Herbicides 2,4-D Dimethylamine and Bensulfuron Methyl to *Chironomus kiiensis* (Chironomidae Diptera). Proceedings of ISEB/ESEB/JSEB 2006 International Conference of Environmental Biotechnology. Leipzig, Germany 9-13 July 2006.

6. Al-Shami S., Che Salmah M. R., Siti Azizah M. N. and Abu Hassan A. (2006). Distribution and Abundance of Chironomidae (Diptera) in Tropical Rice Agroecosystem. 16th International Chironomid Symposium. Funchal, Madeira, Portugal. 25-28 July 2006.

SEBAHAGIAN ASPEK EKOLOGI DAN GENETIKS CHIRONOMIDAE (DIPTERA) DI SAWAH PADI DAN KESAN BEBERAPA HERBISID YANG DIPILIH TERHADAP POPULASINYA

ABSTRAK

Kajian ekologi dan molekul dijalankan terhadap serangga akuatik Chironomidae (Diptera) yang mendiami kawasan sawah padi di Stesen Kaiian Pertanian Bukit Merah (BMAES), Seberang Perai, Pulau Pinang, Malaysia. Penyiasatan terhadap taburan dan kelimpahan chironomid (Chironomidae: Diptera) menunjukkan bahawa taburan populasi larva chironomid meningkat dan menurun mengikut perubahan dinamik ekosistem sawah padi. Kepadatan tertinggi dalam musim pertama ialah 294.39m^{-2} dan 306.37m^{-2} dalam musim kedua. Penyampelan larva setiap dua minggu selama dua musim penanaman 2004/2005 menunjukkan bahawa *Chironomus kiiensis* adalah spesies yang paling dominan ditemui. Manakala empat lagi spesies, *Polypedilum trigonus*, *Tanytarsus formosanus*, *Tanypus punctipennis* and *Clinotanypus* sp didapati dalam jumlah yang rendah walaupun ditemui pada sepanjang masa sawah padi dipenuhi air.

Jumlah purata populasi larva ialah 120.95 m^{-2} dan berubah secara signifikan pada sepanjang penyampelan dan mengikut musim (Kruskal-Wallis, X^2 = 174.29, df=16, P=0.000 dan ujian Mann-Whitney = 19984, P = 0.000 masing-masing). Lebih banyak larva dijumpai semasa fasa pembajakan ketika ketiadaan pokok padi di sawah. Populasi larva Chironomidae meningkat dua minggu selepas aplikasi herbisid. Racun serangga dan baja, termasuk juga kemunculan dewasa berkemungkinan menyebabkan penurunan bilangan populasi di sepanjang musim. Ampat parameter fizik-kimia: paras air, ketinggian

pokok padi, konduktiviti, kandungan dan nitrat-nitrogen mempengaruhi taburan kepadatan larva pada $P < 0.05$ dengan lemah. Paras pH, keterlarutan oksigen, fosfat, suhu, jumlah bahan organik dan jumlah pepejal terampai menunjukkan tiada hubungan sinifikan dengan populasi larva chironomid di dalam sawah.

Kesan dua jenis herbisid 2,4-D Dimethylamine dan Bensulfuron methyl terhadap peringkat larva instar ke *C. kiiensis* turut dikaji. Kematian larva yang dirawat dengan 2,4-D Dimethylamine di perhatikan selama 24 jam dan LC_{50} nya ialah 2638 mg ai L^{-1}. Namun demikaian, ketoksikan Bensulfuron methyl hanya dilihat selepas 72 jam dan LC_{50} ialah 1.29 mg ai L^{-1}. Tiga peratus pupa berjaya menjadi dewasa setelah didedahkan dalam kepekatan yang tertinggi (500 mg ai L^{-1}) berbanding 60% kejayaan kemunculan di dalam kawalan. Oleh itu 2,4-D Dimethylamine merupakan herbisid yang selamat dan bertindak pantas membunuh larva *C. kiinesis* dalam tempuh 24 jam. Bensulfuron methyl bertindak perlahan tetapi dapat menurunkan jumlah kemunculan dewasa. Kebanyakan larva mati semasa peringkat pupa.

Kajian terhadap perhubungan melekul dijalankan kepada *C. kiiensis, P. trigonus, T. formosanus, T. punctipennis* and *Clinotanypus* sp berdasarkan kepada kepingan 710 bp mtokondria sitokrome oksidase subunit I (COI). Parsimoni Maksimum (MP) menghasilkan pokok filogeni yang dibina mengikut hipotesis yang berdasarkan kepada kaitan morfologi. Semua ahli dari setiap spesies membentuk kumpulan monofiletik. Subfamili Chironominae membentuk takson 'sister' kepada Tanypodinae *P. trigonus* yang menjadi kumpulan dasar. Kaedah DNA didapati berguna untuk mengenalpasti peringkat hidup chironomid yang tidak jelas dan menawarkan pendekatan yang boleh dipercayai dan pantas untuk pencaman berterusan spesies-spesies yang dikenali.

SOME ASPECTS OF ECOLOGY AND GENETICS OF CHIRONOMIDAE (DIPTERA) IN RICE FIELD AND THE EFFECT OF SELECTED HERBICIDES ON ITS POPULATION

ABSTRACT

An ecological and molecular study was conducted on the aquatic insect Chironomidae (Diptera) inhabiting the rice field in Bukit Merah Agricultural Research Station (BMAES), Seberang Perai, Pulau Pinang Malaysia. Investigation on the distribution and abundance of chironomid larvae (Chironomidae: Diptera) showed that the larval population fluctuated following the dynamic changes in rice field ecosystem. The maximum density in the first season was 294.39 m^{-2} and 306.37 m^{-2} in the second season. Biweekly sampling of larvae over two rice growing seasons of 2004/2005 revealed that *Chironomus kinesis* was the most abundant species while four other species *Polypedilum trigonus, Tanytarsus formosanus, Tanypus punctipennis* and *Clinotanypus* sp were found in low numbers although they occurred during all rice wet phases.

The mean larval population was 120.95 larvae m^{-2} and varied significantly among sampling occasions and seasons (Kruskal-Wallis, χ^2= 174.29, df = 16, P= 0.000 and Mann-Whitney test = 19984, P = 0.000 respectively). More larvae were found in the plough phase in the absence of rice plants in the field. The population was observed to increase approximately two weeks after herbicide applications. Insecticides and fertilizers as well as the onset of emergence of adults from larvae to adults probably accounted for reduction of population numbers throughout the seasons. Four physico-chemical variables; water level, height of rice plants, conductivity of water and

nitrate-nitrogen weakly influenced the larval density ($P < 0.05$). The pH, dissolved oxygen, temperature, total organic matter, total suspended solids and phosphate showed no significant interaction with the population of chironomid larvae in this rice field.

The effect of two herbicides 2,4-D Dimethylamine and Bensulfuron methyl on the fourth instar larvae of *Chironomus kiiensis* was investigated. Mortality of the larvae treated with 2,4-D Dimethylamine was observed after 24 h and the LC_{50} was 2638 mg ai L^{-1} which was a hundred times higher than the normal applied dose of 0.753 mg ai L^{-1}. However, the direct toxicity of Bensulfuron methyl was only observed after 72 h and the LC_{50} was 1.29 mg ai L^{-1}. Three percent of the pupae successfully emerged to adult in the highest concentration (500 mg ai L^{-1}) of Bensulfuron methyl compared with 60% in the control. Thus 2,4-D Dimethylamine was a fast acting herbicide, killing the larvae of *C. kiiensis* within 24 h. Bensulfuron methyl was slow acting but it reduced adult emergence, most larval individuals died during the pupal stage.

A preliminary study on the genetic relationship was conducted on *C. kiiensis*, *P. trigonus*, *T. formosanus*, *T. punctipennis* and *Clinotanypus* sp based on a 710 bp fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene. The Maximum Parsimony (MP) analysis produced a phylogenetic tree which was in general agreement with the hypothesis based on morphological traits. All members of each species form monophyletic groups. The Chironomidae subfamilies form a sister taxon to the Tanypodinae with *P. trigonus* as the basal group. The DNA method has proven useful in identifying chironomid during certain life stages and offers a reliable and rapid approach for routine identifications of ambiguous species or individuals.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Chironomidae is considered a species rich family of flies, with almost 15000 species have been described worldwide (Cranston, 1995a). Adults are known as non-biting midges or blind mosquitoes while the larvae as bloodworms due to the presence of the red blood pigment, haemoglobin, in their bodies. Chironomid adults are similar in their morphology to mosquitoes (Culicidae) and biting midges (Ceratopogonidae). However, unlike nasty mosquitoes or biting midges, adult chironomids do not feed; neither suck the blood nor transmit any diseases (Epler, 2001).

Generally, Chironomidae larvae are inhabitants of organically enriched places such as in flowing water of streams and rivers or standing water of lakes and pools as well as temporary rain-pools. They consume organic matter, phytoplankton (Real *et al.*, 2000) and green algae (Cranston, 1995a). They are successful as early colonists of aquatic habitat (Watton and Armitage, 1995) even in the arctic zone (Oliver and Dillon, 1997). They are very important in the colonisation dynamics of ecosystem (Boothroyd, 1995). Larvae of Chironomidae have become known as a favourite fish food amongst the aquarist community in many countries (Cranston, 1995a). As an important component of aquatic insect community, Chironomidae have been proven useful as biological indicators because of their sensitivity to chemical changes in aquatic ecosystems (Dudley and Blair, 1992). Therefore, they are often included in most ecological and toxicological studies (Boothroyd, 1995).

Like other dipteran, Chironomidae have a holometabolous life cycle. They have four distinct life stages; eggs, larvae (four instars), pupae, and adult (imago). The duration of the life cycle is influenced by the species and environmental conditions, for instance warm water and high quality of food correlate with short life cycles.

Aquatic invertebrates respond to variations in their physical habitat as well as physical and chemical variations in water (Hellawell, 1986). In rice agroecosystem, chironomids are subjected to changes in physico-chemical conditions such as temperature, dissolved oxygen, pH and conductivity, water levels and inputs of nutrients (Lim, 1990).

The quality and quantity of food control the abundance patterns of Chironomidae. The species composition changes following changes in the particle regimes, especially the organic matter coating and the amount of detritus deposited on the substratum (Galdean *et al.*, 2000; Hirabayashi *et al.*, 2004). Addition of fertilizers has been reported to cause an increase of nitrate-nitrogen and phosphate contents that would affect the distribution of some chironomid subfamilies in the ecosystem (Ali, 1989). Herbicides were found to have a negative effect on benthic insect community including *Chironomus tetanus* based on a laboratory experiment (Dewey, 1986).

Rice fields cover almost 70 million hectares in Southeast Asia. In Malaysia, rice is the third most important economic crop covering an area of about 209300 ha (Karim *et al.*, 2004). Rice fields are considered a very important aquatic ecosystem not only as commercial resources but also as a biotic resource especially as wetland habitats (Lim, 1990). Human interaction with aquatic community in Southeast Asian rice fields is clearly evident, and directly affects the biodiversity of the aquatic species (Heckman, 1979).

Cultivation of rice has led to some major changes in this swampy ecosystem (Lim, 1990). Tropical rice fields are diverse in insect fauna, which serve as an important food source for fishes (Ali and Ahmad, 1988; Che Salmah and Abu Hassan, 2002). Many aquatic insects especially chironomids, dragonflies and mosquitoes colonise rice fields, and their population dynamics are closely related to rice plant growth, cultivation practices, and seasonal climatic changes. The succession of the insect community structure in rice field follows the patterns of water availability and phases of rice growth (Che Salmah *et al.*, 1998). Tropical rice field is a dynamic ecosystem that is continuously subjected to applications of chemicals such as fertilisers and pesticides.

In Malaysia, herbicides are used intensively (Moody, 1996; Naylor, 1996). The effect of herbicides on Chironomidae and other aquatic community can be demonstrated as direct or indirect (Robson and Barrett, 1996) depending mainly on the type of the herbicides used (Hatakeyama and Shiraishi, 1991).

Besides its positive contribution to the aquatic communities and environment, some studies revealed negative aspects of Chironomidae. Stevens (1995) recorded that chironomid larvae caused significant damage to rice plants in Australia. The adults can occur in such large numbers that they become a nuisance or even cause inhalant allergies (Tabaru *et al.*, 1987; Land *et al.*, 2000).

Chironomidae have been recorded in rice fields throughout the world including in many countries such as India, Australia, and the USA (Martin and

Porter, 1977; Chaudhuri and Chattopadhyay, 1990; Stevens, 1995; Stevens *et al.*, 2000, Stevens and Warren 2003). Within this family, four genera, *Tanytarsus*, *Paralauterbomiella*, *Chironomus,* and *Cricotopus* are commonly found in rice agroecosystem (Berg, 1995). However, in the Peninsular Malaysia, very little is known about distribution, taxonomy, biology, and ecology of Chironomidae in rice fields. Nevertheless, some pioneering studies on rice field Chironomidae have been conducted in several rice fields in Kedah and Selangor states (Lim, 1990; Che Salmah and Abu Hassan, 2002).

Studies in Chironomidae have been shunned typically by many benthologists because of perceived difficulties in specimen preparation, identification, morphology, and literature. Many studies listed Chironomidae only at the family level because identification at the generic or species level requires a lot of background literature which is not readily available (Epler, 2001). Carew *et al.*, (2003) mentioned that the acquisition of genus or species-level information for macroinvertebrates is time consuming. Taxonomic problems, particularly with chironomid larvae, have hindered intensive ecological study (Bishop, 1973; Blinov *et al.*, 1997; Guryev *et al.*, 2001; Carew *et al.*, 2003). Moreover, their small size, superficial similarity, and high diversity require slide mounting and microscopic examination for positive species identification (Wrubleski, 1987). For instance, the two diverse genera belonging to the subfamily Chironominae, *Polypedilum* and *Tanytarsus*, are problematical to identify as larvae (Cranston and Dimitridis, 2004). Similarly, taxonomical identification of *Chironomus* sp larvae in lentic environment has only been made at the genus or 'larval type' level because of difficulties in species identification (Real *et al.*, 2000). Despite useful cytological studies on the chironomids chromosomes that support the morphological identification, combination of the data from the two methods (morphology and cytology) are yet insufficient (Wuelker, 2003).

DNA variation has the potential to distinguish closely related individuals or species. The analysis of DNA has been used in entomological studies to solve the taxonomical ambiguities especially with Chironomidae. Molecular methods provide an effective tool for Chironomidae identification that give the basis of obtaining species-specific DNA profile (Guryev *et al.*, 2001; Martin *et al.*, 2002; Spies *et al.*, 2002; Carew *et al.*, 2003; Papoucheva *et al.*, 2003; Sharley *et al.*, 2004).

1.2 Objectives

Chironomidae are found in abundance in rice fields and their population fluctuates following rice phases and management practices. They are exposed to pesticides when the chemicals are used to control rice pests. Due to their ecological importance in the rice field, this study was undertaken with the following objectives:

1. To investigate the composition, distribution and seasonal abundance of the Chironomidae larvae in the rice fields of Bukit Merah Agricultural Experimental Station (BMAES), Pulau Pinang.
2. To study the relationship between their distribution, composition and abundance with the environmental variables at the study sites.
3. To run the bioassay analysis of Chironomidae larvae for gradient concentration of selected herbicides.

4. To conduct a preliminary molecular study for the identification and phylogenetics of the Chironomidae based on the mitochondrial DNA sequence of the partial fragment of cytochrome oxidase subunit I (COI) gene.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Chironomidae is a worldwide dipteran family. They inhabit a wide variety of freshwater lakes and streams (Cranston, 1995a) and form a major constituent of the freshwater benthos (McLachlan, 1971). Moreover, they are considered one of the most ubiquitous, diverse, and ecologically important groups of the aquatic macroinvertebrates (Ali and Lobinske, 2002). They colonise all types of water bodies (river, ponds, etc) as well as temporary water bodies. They are successful as early colonists of aquatic habitat (Watton and Armitage, 1995) even in arctic zone (Oliver and Dillon, 1997). They are also found in temporary water in plants (Phytotelmata) (Prat and Capitulo, 1995). Furthermore, they are reported in unexpected terrestrial habitats; some Orthocladiinae larvae were found in leaf litter (Ashe, 1990). Chironomids have also invaded the sea. They have been found along coastlines worldwide and have been recorded to occur at least 30 m down in the ocean. They have a large ability tó tolerate high osmotic levels in brackish and shoreline saline pools (Cranston, 1995a; Epler, 2001). Some species of Chironomidae can live in glaciated areas of the highest mountains, including at elevation of up to 5600m in the Himalayas. The *Sergentia* sp can live in water of over 1000m deep and are still active at -16 °C (Cranston, 1995a). In addition, Walker *et al.* (2003) recorded many genera of Chironomidae such as *Monodiamesa* and *Paratanytarsus* from Yukon Territory near to the Arctic Ocean.

2.2 Life Cycle and Morphology of Chironomidae

Chironomidae is a holometabolous insect having four distinct life stages; eggs, larvae (four instars), pupae, and adult.

2.2.1 Egg

Usually Chironomidae lays eggs in a gelatinous mass except in the subfamily Telmatogetoninae where the eggs are laid singly. The eggs mass form an elongated ribbon or more compact cylindrical to tear-shape globule. The eggs inside the gelatinous matrix are placed randomly, arranged helically or linearly. Eggs of the Chironominae subfamily are predominantly arranged helically and Tanypodinae eggs are arranged more irregularly in a more or less globular matrix. However, Orthoclad and diamesine eggs tend to be arranged more linearly in a ribbon-like mass (Cranston, 1995b).

The number of eggs in the gelatinous matrix varies among species (Koehler and Ali, 1980). The egg mass floats and hatches after an incubation period, which depends on the temperature and varies among the species. In a study conducted in Japan, it was recorded that the eggs of *C. plumosus* hatched after 3 days at 24 °C and 14 days at 9 °C (Hirabayashi *et al.*, 2004). After hatching, the larvae remained in the egg mass for 1-2 days (Shida and Xinhua, 1994).

2.2.2 Larvae

The chironomid larvae have four instar stages, with unconfirmed report of a fifth instar stage in Tanypodinae (Cranston, 1995b). The duration of larval stage may last from about two weeks to several years depending on the taxon and environmental circumstances (Shida and Xinhua, 1994). Some chironomid larvae build their cases by binding particles from the environment with salivary secretions. These tubes may also be composed of silk-like threads. Most larval tubes have an opening at each end to allow the larvae to feed from one end (Berg, 1995). Larvae of the Chironominae may live in tubes or tunnels having only one open end. The larvae spend most of their time undulating rapidly within the tube to circulate water. They extract oxygen and food from water. Spiracles are predominantly absent (apneustic) and gas exchange may occur through gills or general body surface (Ward, 1992). The southern African/Australian amphipneustic genus *Archaeochlus* is an exception with anterior and posterior spiracles present (Cranston, 1999). However, some tubeless larvae crawl, float, or swim in the water.

The larvae feed on suspended matter in the water and organic matter in the mud (Koehler and Ali, 1980). However, the larvae of other chironomid subfamilies, such as Tanypodinae and Orthocladiinae, are reported as omnivorous and their food consists mainly of small aquatic invertebrates, algae, and small particles of organic detritus (Berg, 1995; Shida and Xinhua, 1994).

Chironomidae larvae are typical nematocerous dipterans. The body is divided into nearly three segments with somewhat broad thoracic segments, followed by nine narrower abdominal segments. The only appendages of the thorax are the interior parapods, which are paired, fleshy, unsegmented, bearing claws and are placed ventrolaterally on the first thoracic segment. Very similar posterior parapods are found ventrolaterally on the terminal abdominal segment. Parapods are found in all chironomid larvae and may be partially or completely fused. Pre-anally paired procerci with an apical tuft of setae are usually prominent. *Chironomus* and some relatives often have hemolymph-filled abdominal tubules associated with respiration, therefore termed tracheal gills (Cranston, 1995b).

All Chironomidae larvae are prognathous, with the mouth parts directed anteriorly. However, terrestrial Orthocladiinae larvae may closely resemble some ceratopogonid larvae (Cranston, 1995b). The chironomid larvae have a well-developed, exposed, complete, non-retractile head capsule (Shida and Xinhua, 1994). This head capsule consists of fully sclerotized cranium that comprises a dorsal apotome and a pair of lateral genae. Multi-segmented antennae are placed dorsally on the upper genae. The antennae are retractile into the head in all Tanypodinae. The eyes of Chironomidae are typically simple. Chironominae larvae have double eye spots in contrast to the single spot in the Orthocladiinae and most other subfamilies (Cranston, 1995b).

Mandibles are articulated, paired, and operated in an oblique plane between the labrum and the maxilla. The mandible is toothed with variable number of inner teeth. Maxillae lay dorsolateral to the mentum and comprise a base of variable breadth bearing a ventrolateral maxillary palp, dorsomedial galea, and postermedial lacinia. In some Chironominae, the apicomedially lacinia bears long setae directed behind the mentum (Cranston, 1995b).

2.2.3 Pupae

Chironomid pupae are comma-shaped. The duration of chironomid pupal stage is very short when compared with the duration of larval stage. The durations range from a few hours up to several days for the long-lived Podonominae. When the larvae change to pupae, they leave their mud tube and swim to the water surface to emerge. During that time they suspend themselves between the bottom and surface of water (Cranston, 1995b).

2.2.4 Adult

Due to their morphological similarity Chironomidae adult are often mistaken for mosquitoes. Unlike the nasty mosquitoes, chironomid adults do not feed as reflected by the unusual condition of reduced mouthparts, except in the females of southern hemisphere Podonominae *Archaeochlus* that has functional mandibles (Cranston, 1995b). The adults have short life spans just sufficient to achieve reproduction (Shida and Xinhua, 1994).

Chironomidae adults swarm near to their aquatic environment, sometimes in huge numbers (Cranston, 1995b). When the swarming and mating is completed, the chironomid females lay their egg masses on the water surface. The egg masses are then attached to natural or artificial substrates (Charles *et al.*, 2004).

2.3 Importance of Chironomidae

Chironomids are very important in the colonisation dynamics of ecosystems (Boothroyd, 1995). Their ecological function includes release of nutrients from bottom sediment into overlying waters and uptake of oxygen. The burrowing or mining activity of many chironomid larvae into fallen litter tends to increase the rate of microbial decomposition of these materials (Wrubleski, 1987). Chironomid larvae have been used extensively in genetic research because they possess giant chromosomes (Epler, 2001). These chromosomes have many useful characters such as different chromosome number, different combination of chromosome arms, number and position of nuclear organisers, amount of heterochromatin, presence of puffs and Balbiani rings and banding pattern (Wuelker, 2003).

Chironomidae are good indicators of toxicants because they are easy to culture, sensitive to many pollutants and have a short life cycle (Charles *et al.*, 2004). They have proven to be useful indicators of a wide variety of environmental impacts on freshwaters. This may include the effects of eutrophication, acidification, toxic metals and chemicals, and physical disturbances (Brooks and Birks, 2001). Thus, they are very important in ecological and ecotoxicological studies. For example *C. riparius* have been utilised successfully in the assessment for the toxicity of 2,4,5 trichlorphenol (Ristola, 2000) and metal elements (Groenedijk, 1999).

In addition, Chironomidae are now widely recognised as powerful biological proxies for inferring past climatic change. They are considered the best indictors for pale climatic changes (Brooks and Birks 2001; Walker *et al.*, 2003). Additionally, the remains of larval Chironomidae (mostly as head capsules) recovered from lake sediment cores have proven to be an extremely valuable tool in reconstructing past temperatures, climate, and other environmental variables (Francis *et al.*, 2003).

As one of the most important component of food web in the aquatic ecosystem, larvae of Chironomidae have been known as a favourite fish food amongst the aquarist community in many countries. At least 19 fish groups (families) including bream, bullhead, char, grayling, gudgeon, minnow, perch, pope, tench, *Tilapia*, brown and rainbow trout, walleye, white and mormyrids consume chironomid larvae (Cranston, 1995a). Consequently, commercial rearing of chironomids has been attempted in several countries such as Hong Kong and Thailand for producing large quantities of these delicacies for tropical fishes (Cranston, 1995a). In Singapore, the seasonal occurrence of the chironomid larvae (bloodworms) accompanies the flourishing time of the aquarium fish industry. A sustainable cultural method to produce larvae under control conditions in Singapore has been developed on the common local species *C. javanus* (Karunakaran, 1974).

In addition to their important role as a favourite food for fishes, chironomid larvae, pupae, and adults form an integrated part of the food web in the aquatic ecosystem. They are preys for other invertebrates (Prichard, 1964) and many vertebrates such as water fowl (Wrubleski, 1987), other aquatic birds (Charles *et al.*, 2004) amphibians (Epler, 2001) and even humans in central and east African lakes (Cranston, 1995a).

However, despite their many positive aspects, some studies have revealed that the adult chironomids pose a variety of nuisance and economic problems. At night, the adults are attracted to light, swarming around indoor and outdoor fixtures. Adults of some small species can pass through screen on doors and windows (Land *et al.*, 2000) and thus enhance nuisance and economic problems indoors, such as staining laundry, walls, ceilings, draperies and other furnishings as well as causing discomfort for the residents (Land *et al.*, 2000; Ali and Lobinske, 2002). The nuisance midge in Japan, *C. yoshimatsui*, is distributed all over Japan, and its population can reach 53000-93000 m^{-2} in urban areas (Tabaru *et al.*, 1987). Other nuisance species include *P. nubifer* and *C. alternans* in Australia (Land *et al.*, 2000), and *C. salinaris* in Italy (Majori *et al.*, 1986).

Aldunate *et al.* (1999) recorded that some chironomids are responsible for type I hypersensitivity reactions which cause rhinoconjunctivitis, bronchial asthma, urticaria and angioedema, and for type IV reactions that give rise to contact dermatitis. Although Chironomidae adults do not transmit any disease; some studies have revealed that the isolation of *Vibrio cholerae* from chironomid egg masses has been reported. The bacteria feed on the gelatinous matrix which may serve as the sole source of carbon for *V. cholerae*, thereby providing a natural reservoir for the organism (Broza *et al.*, 2003).

Another negative aspect of chironomid midges is that they are found throughout the world's rice growing areas, and have been recorded as pests in several countries. The larvae can cause extensive plant losses during crop establishment if not effectively controlled. Chironomid larvae, particularly those of the rice bloodworm, *C. tepperi*, either penetrate the seed and feed on the endosperm, or more typically feed on the roots of young rice plants, retarding growth and making the seedlings vulnerable to wind damage (Stevens, 1995; Stevens *et al.*, 2000).

2.4 Classification of Chironomidae

Chironomidae family belong to the order: Diptera, suborder: Nematocera, Infraorder: Culicomorpha and superfamily: Chironomoidea (Fauna Europaea Web Service, 2004). According to Cranston (1995c), Chironomidae family has been classified into 10 subfamilies:

2.4.1 Tanypodinae

Skuse established this subfamily in 1889 (Spies, 2005). Most of the genera of this subfamily have a worldwide distribution (Johannsen, 1970). Larval feeding apparatus differ strongly from other Chironomidae, with strong development of premental elements such as a ligula and paired paraligulae. Tanypodinae are predominantly predators in late instars, though often feeding on diatoms (Cranston, 1999) or other chironomid (Johannsen, 1970) in early instars.

2.4.2 Chironominae

Macquart established this subfamily in 1838 (Spies, 2005; Cranston, 1995c). Monophly of the subfamily is evident, based on the larvae synapomorphy of striated ventromental plate and cardo. Although these features are strongly reduced in *Hattisius* sp and *Stenochironomus* sp, some elements remain detectable. Chironominae are aquatic, and have an extraordinary range of biology. There is a preponderance of eurythermic (tolerating a wide range of temperatures) taxa compared to other subfamilies (Cranston, 1999). Species of Chironominae subfamily are always predominant in warm standing waters. The apparently universal presence of haemoglobin in the larvae allows uptake of oxygen from warm and benthic waters of poor oxygen contents (Cranston and Dimitridis, 2004).

2.4.3 Telmatogetoninae

This subfamily was established by Wirth in 1949 (Spies, 2005; Cranston, 1995c). It has a worldwide distribution and is a small group of predominantly marine intertidal species (Cranston, 1995c).

2.4.4 Chilenomyiinae

It is the most recently erected subfamily of Chironomidae by Brundin in 1983 (Spies, 2005). It comes from the recognition of the Chilean species, *Chilenomyia paradoxa* (Spies, 2005; Cranston, 1995c).

2.4.5 Aphroteniinae

Brundin established this subfamily in 1966 (Spies, 2005; Cranston, 1995c). It consists of three species distributed exclusively in the southern hemisphere (Cranston, 1995c).

2.4.6 Podonominae

Thienemann in 1937 established this subfamily (Spies, 2005; Cranston, 1995c). They are distributed in the northern hemisphere (Cranston, 1995c).

2.4.7 Buchonomyiinae

This subfamily is known based upon a single genus *Buchonomyia*, with three included species: *B. thienemanni*, from the western Palaearctic and Iran, *B. burmanica* from Burma (Brundin and Saether, 1978) an unnamed species from Costa Rica (Cranston, 1995c).

2.4.8 Diamesinae

Kieffer in 1922 established this subfamily (Spies, 2005; Cranston, 1995c). Diamesinae is a predominantly cool-adapted subfamily with its greatest diversity in the northern hemisphere (Cranston, 1999).

2.4.9 Prodiamesinae

Saether in 1976 established this subfamily (Spies, 2005; Cranston, 1995c). It consists of three anomalous genera. These genera were previously treated either as Diamesinae or as Orthocladiinae: *Prodiamesa*, *Odontomesa,* and *Monodiamesa* (Cranston, 1995c).

2.4.10 Orthocladiinae

Kieffer in 1911 established this subfamily (Spies, 2005; Cranston, 1995c). It is probably the least understood subfamily of Chironomidae. It is quite rich in species and genera. Orthocladiinae tend to inhabit cool stenothermy environment (tolerating a narrow range of temperatures). Thus, the cold, oxygenated lakes have much higher orthoclad diversity than warm, oxygen-depleted ones (Cranston and Dimitridis, 2004). They are very diverse and include species that live on stream or river banks (riparian areas), terrestrial, or phytotelm (living in small water bodies in parts of terrestrial plants) (Cranston, 1999).

2.5 Chironomidae in the Oriental Region

Knowledge of Chironomidae fauna in the oriental region is very lacking although extensive information is available in the Holarctic region (Nearctic region and Palaearctic region) and Afrotropical region (Ashe, 1990). Among Chironomidae subfamilies, only Tanypodinae, Orthocladiinae, Chironominae, and Diamesinae are known from the oriental region (Cranston, 2004).

Ashe (1990) stated that the information of the regional Chironomidae fauna derived principally from the collections made during the famous German Limnological Sunda Expedition (Deutschen Limnologischen Sunda- Expedition) led by the co-founder of limnology, August Thienemann, together with F. Ruttner and H. J. Feueborn. They travelled in 1928-1929 to Sumatra, Java, and Bali. Since the Sunda Expedition, only a few additional regional species have been described. Dutta *et al.* (1996) recorded five genera of *Harnischia* complex in the Duars in the Himalayas of West Bengal and four species namely *Cryptochironomus curryi*, *C. ramus*, *Demicryptochironomus vulneratus* and *Harnischia curtilamellata*. These species were recorded for the first time from India in addition to four species; *Cryptochironomus acuminatus*, *C. gracilis*, *Harnischia minuta,* and *Paracladopelma diutinistyla*, which were described as new species. Chaudhuri *et al.* (1992) recorded three new species of *Tanytarsus* from India; *T. fuscimarginalis*, *T. monstrosus,* and *T. vinculus*. In addition, Hazra and Chaudhuri (2000) reported a new species of chironomid midge *Macropelopia amlituberculata* and a Palaearctic species *M. nebulosa*, from the hilly areas of Darjeeling and Sikkim in India. Kaul (1970) described *Adiamesa khoksarensis* and *Prodiamesa rahlus* as new species from Northeast Himalaya in India.

Perhaps, the most important work on Chironomidae is the study of Bhattacharyay *et al.* (1991) who surveyed the Indian Orthocladiinae in Darjeeling (India). During their three years of investigation, they described many species belonging to the genera *Cricotopus*, *Eukiefferiella*, *Orthocladius,* and *Rheocricotopus*.

Cranston (2003) added more information about the wide distribution of the oriental genus *Shangomyia* ranging from South India, Burma and Hainan and Thailand and Borneo. In the Changbai Mountains in Northeast China, Wang and Halvorsen (2002) reported a new species, *Eukiefferiella changbaiensis* in a snowy environment at an attitude of 2100 m above sea level. Wang and Saether (2002) described the male imago of *Paralimnophyes jii* from Southern China at an attitude of 1000 m above sea level. This was given an amended generic diagnosis and described the genus as belonging to Orthocladiinae.

Shida and Xinhua (1994) gave a brief summary of Chironomidae in China including their habitats, trophic relationships, and number of taxa (number of known species). They reported that the known taxa of Chironomidae in China was almost 1000; Podonominae (12 taxa), Tanypodinae (140 taxa), Diamesinae (40 taxa), Prodiamesinae (8 taxa) Orthocladiinae (400 taxa), and Chironominae (330 taxa).

2.5.1 Chironomidae in South East Asia

Ekrem (2002) mentioned during his revision of the genus *Tanytarsus* in South and East Asia subregions, that many of the recently described species are reported only in Japan or India although they probably have a wider distributional range. However, in the southeastern part of Asia very little is known about Chironomidae. Cranston (2004) documented that the first known Chironomidae species in South East Asia was the Javanese *Tanypus crux*.

Karunakaran (1974) studied Chironomidae in Singapore and contributed more information about Chironomidae in this region. He reported many species from the subfamily Chironominae such as *Chironomus apucatus*, *C. crassiforceps*, *C. costatus*, *C. incertus*, *C. stupidus*, *C. tumidus*, *Polypedilum anticus*, *P. convexum*, *Tanytarsus* sp, and *Tanypus kraatzi* from the subfamily Tanypodinae.

However, the most valuable information comes from the study of Ashe (1990). A huge number of specimens were obtained during the 1985 Royal Entomological Society "Project Wallace" expedition to Sulawesi, Indonesia. The output of this study was the reporting of around 31 genera of Chironomidae from Sulawesi. Five years later, Murray (1995) reported *Conchapelopia insolens* as a new species of subfamily Tanypodinae, from Sulawesi. Ashe and Connor (1995) reported a new species of *Subletta* from northern Sulawesi, Indonesia.

2.5.2 Chironomidae in Malaysia

Bishop (1973) recorded three subfamilies i.e. Chironominae, Tanypodinae, and Orthocladiinae from a small river, Sungai Gombak in Peninsular Malaysia. In addition, a single species of Diamesinae was recorded from high elevation (above 3000 m) on Mount Kinabalu in Sabah, Malaysia (Cranston, 2004). Bishop (1973) pointed out that the larvae of the complex *Cryptochironomus* was the most conspicuous and showed succession of various types. Siregar *et al.* (1999) studied the distribution of aquatic insects in five streams in the Kerian River Basin along Kedah- Perak border. They recorded that Chironomidae was one of the most dominant families in all streams. Similarly, Che Salmah *et al.* (1999) recorded that Chironomidae made up most of the collection during the sampling of the aquatic insects In Kedah River Basin.

2.6 Rice Fields as a Habitat for Chironomidae

The rice field is a unique man-made environment with high diversity of aquatic organisms. Routine agricultural practices such as ploughing, draining, fertilizers and pesticides application affect the diversity and abundance of aquatic community. The nature of the rice field ecosystem influences the changing abundance of its resident aquatic community (Che Salmah *et al.*, 1998). Aquatic organisms in the rice field lose their habitats during field drainage. Thus, the harvest time and drained periods that persist through the off-crop season entail a difficult period for these organisms to survive. Evidently, survival of Diptera, especially Chironomidae, decreased immediately after drainage (Yamazaki *et al.*, 2001a).

Although rice fields have been recognized as habitat for chironomids, there are only a few studies dealing with this habitat (Chaudhuri and Chattopadhyay, 1990). Chironomidae larvae have been recorded in rice fields throughout the world in many countries (Stevens, 1995). The larvae of some species have been recorded as pests of rice in temperate rice growing countries (Stevens, 1995). The species of *C. tepperi* is known to cause extensive rice damage in New South Wales Australia, and their density can reach 13000 m^{-2}. At such high densities, more than 85% of seedlings are destroyed (Stevens *et al.*, 2002; Stevens and Warren, 2003). They either attack the seed or feed on the roots and shoots of young seedlings (Helliwell and Stevens, 2000). The most commonly reported genera in rice fields are *Chironomus* sp (Martin and

Porter, 1977; Stevens, 1995; Stevens *et al.*, 2000; Stevens and Warren 2003; Stevens *et al.*, 2006), *Polypedilum*, *Procladius* (Stevens and Warren, 2003), *Cricotopus*, *Paralauterborniella* and *Tanytarsus* (Berg, 1995; Stevens *et al.*, 2006).

Chattopadhyay *et al.* (1991) reported that *C. sasmoensis* is most widespread in rice fields of India. Furthermore, Chaudhuri and Chattopadhyay (1990) recorded fifty three species of chironomids for the first time from rice fields of West Bengal, India. Out of these numbers, 24 of them including five new species were new to India. The author built up a valuable key for all stages of the recorded species.

However, in the Peninsular Malaysia, very little is known about the distribution, taxonomy, biology, and ecology of Chironomidae in rice fields. Nevertheless, preliminary studies on rice field Chironomidae have been carried out in several rice fields in Kedah and Selangor states (Lim, 1990; Che Salmah and Abu Hassan, 2002). Meanwhile, Chironomidae taxonomic problems are encountered by most entomologists and because of their small size, morphological similarity, high diversity, and lack of literature, morphological identification of chironomid is very difficult, and requires a lot of experience (Epler, 2001).

2.7 Factors Influencing the Abundance of Chironomidae Population

Invertebrate responds quantitatively not only to the availability of trophic resources but to variations in their physical habitat and to physical and chemical variations of the water. The environmental factors, which control distribution of organisms in freshwater are current speed, the stability of water depth, light and temperature regimes and water quality (Hellawell, 1986).

Each habitat is unique and presents its own opportunities for exploitation by suitably adapted organisms. There is a wealth of evidence from field studies and from laboratory investigations that many organisms show anatomical and physiological responses to environmental conditions (Wahizatul Afzan, 2004).

In permanent water ecosystems, the chironomid distributions appear to be driven principally by in-lake variables such as lake depth, pH, dissolved oxygen, trophic status, and substrate-type (Brooks and Birks, 2001). Che Salmah and Abu Hassan (2002) recorded that the abundance of aquatic insects especially Chironomidae in the rice field was very dependent on the availability of water. Moreover, Chironomidae like other benthic fauna shows aggregated distribution in all environments presumably because of microenvironmental variations. This suggests that the interactions of environmental factors causing aggregation of any benthic species are in a fine balance, so any successful attempt to delineate these factors in one sampling site may not have any applicability to the aggregation produced in other areas (Paterson and Fernando, 1971).

Chironomidae live in a wide range of environmental conditions. These include in habitats with extreme fluctuation of water level, temperature and dissolved oxygen associated with life in shallow water (Cranston, 1995a; Epler, 2001). There are also terrestrial and semi-terrestrial chironomids within Orthocladiinae, which are able to tolerate dry condition without arresting growth or development, which include many genera such as *Limnophyes* sp, *Paraphaenocladius* sp, and *Pseudosmittia* sp, *Smittia* sp (Wrubleski, 1987).

Tolerance levels to environmental conditions within this taxon vary considerably. For example, *Tanytarsus dissimilis* cannot complete their development below pH of 5.5. However, *Chironomus riparius* lives successfully in acidic tundra ponds of pH 3-5 (Wrubleski, 1987).

In aquatic ecosystems with optimum physical and chemical environment, biotic interactions with other macroinvertebrates, such as competition and predation can explain species abundance and composition (Real *et al.*, 2000). Paterson and Fernando (1971) found there is efficacy of the interaction between the species of chironomid as illustrated by the negative association *Chironomus digitatus* and *Glyptotendipes paripes*.

2.7.1 Temperature

Many hydrobiologists consider the temperature regime to be the most important physical factor controlling the ecology of aquatic communities. All organisms associated with fresh water (except birds and mammals) are poikilothermic, that is they are highly dependent on ambient temperature (Hellawell, 1986).

Although Siregar *et al.* (1999) reported that the optimum range of water temperature for aquatic life is 27-30 °C, water temperature in the tropics varies from as low as 24 °C in the morning or during cloudy or rainy days and up to 40 °C on hot sunny days (Ali and Ahmad, 1988). In the rice fields the water temperature is affected directly by the height of the rice plants. Ali and Ahmad (1988) reported during their study on water quality in rice fields and sump ponds that the shaded sump ponds had lower mean biweekly temperature than the shallower and more exposed fields. They recorded that the highest temperature