



Faculty of Resource Science and Technology

Population of Chironomidae (Insecta: Diptera) in UNIMAS Lake and Its Potential as a Culture Species in Laboratory Condition.

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This dissertation is submitted in partial fulfilment of requirement for the Degree of Bachelor Science with Honours in Aquatic Resource Science and Management

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DECLARATION

I hereby declare no portion of the work referred to this dissertation has been submitted in support of an application for another degree qualification of this or any other university or institution of higher learning.

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“In the name of Allah, the Most Gracious and the Most Compassionate”

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List of Abbreviations

Abbreviations	Descriptions
%	percentage
° / ' / ''	degree / minute/ second
°C	degree Celcius
±	plus minus
µm	micrometer
µg	microgram
a.m.	morning
cm	centimeter
<i>et al.</i>	and others
E	East
g	gram
g/sed.	gram per sediment
GPS	Global Positioning System
ind.	individual
L	litre
m	metre
ml	millilitre
mm	millimetre
N	North
NTU	Nephelometric Turbidity Unit
pH	potential of Hydrogen
p.m.	evening
PSU	Practical Salinity Unit
sp.	species
12 h D	Twelve hours dark
12 h L	Twelve hours light

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ABSTRACT

The study of chironomid populations and its relationship with water quality was conducted in UNIMAS Lake. The objectives of this study are to determine the density of chironomid larvae in UNIMAS Lake and its potential as culture species in the laboratory conditions. Sediments sampling were conducted by using Poinar Grab Sampler at four selected stations in the lake. The fish and prawn were caught for gut content analysis and live feed trial in the laboratory. Population of Chironomidae was nearly consistent within the UNIMAS Lake. Water parameters of pH, turbidity and chlorophyll *a* were significantly different between station with p -value = 0.000. Dissolved oxygen was found influenced the density of chironomid larvae in UNIMAS Lake. A number of fish species from UNIMAS Lake were discovered feed on bloodworm. Chironomidae has potential to be reared in laboratory condition with salinity and temperature as factors for prolongation of larvae stage.

Key words: Chironomid, population, UNIMAS Lake

ABSTRAK

Kajian populasi chironomid dan hubungannya dengan kualiti air dijalankan di Tasik UNIMAS. Objektif kajian ini adalah untuk menentukan ketumpatan larva chironomid di Tasik UNIMAS dan potensinya sebagai spesies ternakan dalam keadaan makmal. Sedimen pensampelan telah dijalankan dengan menggunakan Poinar Grab Sampler di empat stesen terpilih di tasik. Ikan dan udang ditangkap untuk dianalisis kandungan usus serta percubaan untuk memberinya makanan hidup di makmal. Populasi Chironomidae hampir konsisten dalam Tasik UNIMAS. Parameter kualiti air merangkumi pH, kekeruhan dan klorofil *a* nyata berbeza antara stesen dengan nilai $p = 0.000$. Oksigen terlarut didapati mempengaruhi ketumpatan larva chironomid di Tasik UNIMAS. Beberapa spesies ikan dari Tasik UNIMAS ditemui memakan chironomid. Chironomidae berpotensi untuk dipelihara di dalam keadaan makmal dengan kemasinan dan suhu sebagai faktor untuk pemanjangan peringkat larva.

Kata Kunci: Chironomid, populasi, Tasik UNIMAS

1.0 Introduction

Chironomidae belongs to Class Insecta and Order Diptera. It is a type of midges that non-biting and commonly found near water source areas. Larva of midge fly or also known as bloodworm is red in colour with segmented body.

According to Henriques-Oliveira *et al.* (2003), chironomids larvae are the most diverse and abundant groups of insects in aquatic environments. Chironomid larva has been used as indicators in lake ecosystems based on their behaviour that can tolerate with inadequate oxygen (Ward, 1992). They are among the high tolerance of aquatic insects towards water and air temperatures (Leonard, 2007) and play as prime role in aquatic food webs as well as linked the producers and secondary consumers (Henriques-Oliveira *et al.*, 2003).

Based on Williams and Feltmate (1992), immature stages of Chironomidae are crucial elements in food webs of aquatic ecosystems and it also contributes in the recycling of nutrients and decompositions. Bloodworms are very often used as bait either real bait or artificial bait that mimic its look. All stages of Chironomidae are important to the diets of aquatic invertebrates, fishes as well as birds (Williams & Feltmate, 1992). According to Sahragard and Rafatifard (2010), chironomid contains high quantity of protein and it is high digestibility, high capability of reproduction and lastly they act as growth promoter in fish diet.

To date, the population and methods on rearing chironomid larvae in Sarawak is still poorly studied. Therefore, this study is conducted to:

- a. determine the density of chironomid larvae in UNIMAS Lake
- b. investigate the relationship between chironomid populations and water parameters
- c. study the suitable culture methods of chironomid larvae and suitable parameters to prolong its larvae stage
- d. test the potential of chironomid larvae as live feed for selected fish species in UNIMAS Lake.

2.0 Literature Review

2.1 Life History and Biology of Chironomidae

According to McCafferty (1981), Dipteran has complete metamorphosis, which means their immature and adult form is completely varied. Life cycle of chironomid is shown in Figure 2.0.

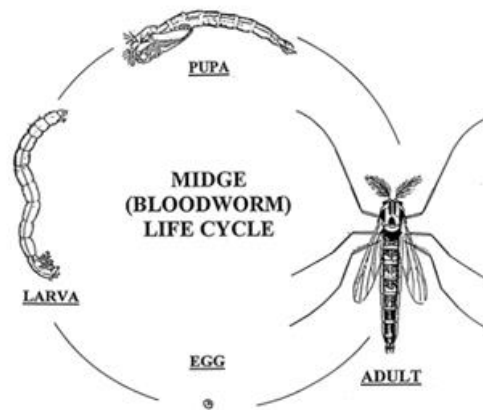


Figure 2.0: Life cycle of Chironomidae (Sources: www.gvsu.edu)

Adult midges (also known as imago) commonly are non-feeding, short-lived and they are attracted to light source (McCafferty, 1981). According to Williams and Feltmate (1992), adult female usually produce single batch of eggs that deposited in gelatinous mass. The eggs sometimes broadcast at water surface or on emergent vegetation (Williams & Feltmate, 1992). Oliver (1971) stated that family Chironomidae has four instars in their larval stage; however Styczynski (1963) reported as cited in Oliver (1971) that Tanypodinae has five larval instars. Normally, larvae built their own home called case from sediment particle lined by silk-like thread by their salivary gland. Only some predaceous species and subfamily Tanypodinae are free swimming larvae. Duration of

pupa is very short, within few hours or few days pupa ready to emerge as imago and then they will fly. Same as larval stage, Tanypodinae is free-swimming pupa whereas other subfamilies are sedentary either live in case or live free on the substrate (Oliver, 1971).

Some species in rock pools of Brazil may complete their life cycle in less than seven days whereas in other places such as Alaskan tundra pond may take long period up to 7 years to complete it (Leonard, 2007). According to Oliver (1971), in tropical countries, Chironomidae development is uninterrupted because their environment condition is uniform. *Chironomus strenzki* found in Amazon complete the life cycle within ten to twelve days only at 30 °C. Likewise, in temperate countries, larvae will diapauses due to unfavorable conditions during winter or summer (Oliver, 1971).

Generally, these larvae ingest five kinds of food: algae, detritus and associated microorganisms, macrophytes, wood debris, and invertebrates (Berg, 1995). According to Henriques-Oliveira *et al.* (2003), the most food item they found in the larval study is detritus. Berg explained that many factors, such as larval size, food quality and type of sediment might influence the larval feeding behavior (Henriques-Oliveira *et al.*, 2003).

2.2 Ecology and Distribution of Chironomidae

Ashe (1990) as cited in Al-Shami *et al.* (2006) stated that information about Chironomidae in oriental region is relatively low compared to the region of Afrotropical and Holarctic. Cranston (2004) also admitted that there is very slight information about Chironomidae in South East Asia region. In addition, the subfamilies that are known among Chironomidae in the oriental region are Chironominae, Orthocladiinae, Tanypodinae and Diamesinae. Javanese *Tanytus crus* was the first Chironomidae found in southern part of Asia (Cranston, 2004).

In Singapore, Al-Shami *et al.* (2006) stated that Karanukaran (1974) had studied about Chironomidae and contributed additional information about Chironomidae in the oriental region. Bishop (1973) as cited in Al-Shami *et al.* (2006) documented three subfamilies of Tanypodinae, Orthocladiinae and Chironominae that were found in Gombak River, Malaysia. A solo species of subfamily Diamesinae was reported in Mount Kinabalu, Sabah at the elevation of 3000 m above the sea (Cranston, 2004). Based on Siregar *et al.* (1999) as cited in Al-Shami *et al.* (2006), Chironomidae was major family recorded in Kerian River Basin located on the border of Kedah-Perak. Likewise, Che Salmah *et al.* (1999) reported Chironomidae was dominant family found in Kedah River Basin.

According to Wahizatul *et al.* (2011) studied the distribution of aquatic insect of freshwater stream in Hulu Terengganu with the relation of water quality. Chironomidae were found abundantly at all of the stations in the downstream. There were significant correlation between pH, total suspended solid (TSS) and width of the streams towards the abundance of aquatic insects. However, there were no significant correlations between other water quality parameters with aquatic insect populations (Wahizatul *et al.*, 2011).

2.3 Rearing History and Technique

Rearing insect is refers as culturing or raising aquatic insects from one life stage to another (McCafferty, 1981). In 1919, bloodworm has been found grow abundantly in a stream contaminated with milk wastes (Branch, 1923). Thus, Branch used this issue to cultivate midges outdoor and indoor to study chironomid life cycle.

In 1964, Biever had developed a new method on rearing non-biting midges since there was lack of suitable culturing method in laboratory. The technique was then published on the next year. For small rearing, Biever (1965) mentioned that two cylindrical plastic containers (each with 115 mm in height) were used with one of it is inverted placed on top of another to collect adult midges. A hole is made on top and recovered by 32 mesh plastic screens for ventilation. Fine sand (25 mm height) is placed in the lower container; 600 ml of water is added and aerated by air stone. Commercial dog food is given by Biever to the cultured species.

William *et al.* (1974) found new technique culturing and harvesting midge larvae. Suspended burlap was used in pools that fertilized with horse manure. Shaw and Mark (1980) used recycling farm manure which is chicken manure to rear Chironomid larvae. Instead of culturing midge larvae as live food for culture fish, this technique could also minimize stream pollution due to farm wastes in Hong Kong (Shaw & Mark, 1980).

In 1982, Bataccatalan and White (1982) had successfully culture non-biting midges after three years trial and errors. These researchers used carbon-filtered or conditioned tap water; artificial soft substrate made from shredded paper towel and feed the cultures with commercial fish pellet.

Rearing of chironomid larvae outdoor in Malaysia was done by Habib *et al.* (1997) in Universiti Putra Malaysia (UPM). They were using Palm oil mill effluent (POME) to cultivate chironomid larvae and compared the nutritional value grown in POME with larvae grown in algae. As the result, population of chironomid larvae in POME grew significantly higher than that in algal culture (Habib *et al.*, 1997).

2.4 Species Cultured and Potential

Biever (1965) had successfully found cultivating technique in laboratory condition for *Chironomus* sp. (species closely related to *Chironomus attenuates*), *Chironomus monochromus*, *Chironomus fulvipilus*, *Pentaneura pilosella*, *Tanytus grodhausi*, *Micropsectra nigripilus*). This rearing method had opened many other investigations in laboratory such as toxicological studies of chironomid larvae, studies on the nutritional requirements, life history, behavior, response and other uninvestigated previous study on biology of Chironomid (Biever, 1965). Armitage *et al.* (1995) stated that demand on mass-reared of Chironomid larvae is increasing for molecular studies. Habib *et al.* (1997) found that most species found in their culture is *Chironomus javanus* and *Xenochironomus australiansis* which the same abundance species found in Singapore.

According to Ferrington and Crisp in Sahragard and Rafatifard (2010), *Chironomous riparian* is one of the most crucial species used in fish cultivation. *Chironomus tentans* s is a hardy species and ideal in physiological and ecological studies and also can be used as toxicological test organism in laboratory (Bataccatalan & White, 1982). *C. tentans* also can be live food for some fresh water tropical fish such as gourami, Malacca hybrid tilapia, *Tilapia aurea* and *Tilapia zillii* (William *et al.* 1974). Armitage *et al.* (1995) also stated that *Tilapia* spp. could be used as biological control on midge population.

2.5 Factors Affecting Development of Bloodworm

Temperature, salinity, presence of food and photoperiod are factors that might affect Chironomid larval stage. However, the main factors that have been proved from previous research are mainly focused on temperature and salinity.

According to Frouz *et al.* (2002), temperature and development rate of *Chironomus crassicaudatus* have a bell-shaped curve in their graph. Ideal temperature for this species to grow and develop is at 20°C. Below and beyond that temperature the development and growth rate is decreased (Frouz *et al.*, 2002). Based on research carried out by Sahragard and Rafatifard (2010), temperature is highly affected larval development time and developmental rate of *C. riparius* and the favorable condition for larval development was at 22-26 °C.

Some researchers have studied impact of salt intrusion into river towards the growth of freshwater invertebrates. Study done by Clark *et al.* (2004) explained that delay in larval stage of *Aedes aegypti* is due to decrease of feeding rate to avoid ingestion of ion during feeding activity or maybe due to increase of energy used for osmoregulation (Clark *et al.*, 2004). Hassell *et al.* (2006) concluded that increase in salinity will delay the development time of chironomid and resulting in prolongation of chironomid life cycle.

3.0 Materials and Methods

3.1 Study Area

This study was conducted mainly at west campus UNIMAS Lake (Figure 3.0). It is the largest lake in UNIMAS and the north and south parts of the lake is links by the bridge.

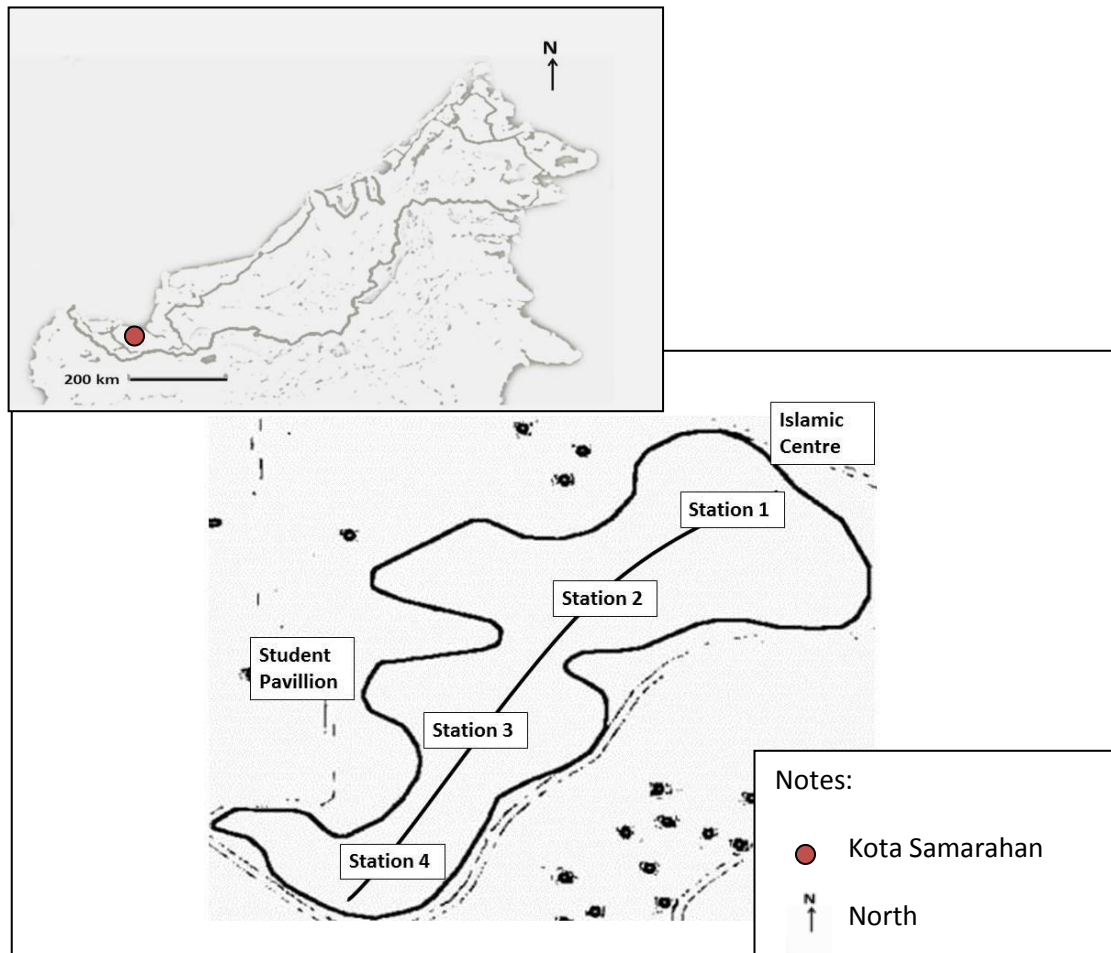


Figure 3.0: The location of sampling stations in UNIMAS Lake

Many recreational activities are performed on this lake such as kayaking, boating and aqua biking. A number of fish species that found in this lake are *Tilapia* sp., Carp, Sultan, Goby as well as Guppy fishes. Freshwater shrimp, *Macrobrachium rosenbergii* was also found in this lake.

3.2 Field Works

3.2.1 Chironomids Density Study

Chironomid samples were collected at four stations in UNIMAS Lake as shown in Figure 2. The location of sampling stations was measured using GPS (Garmin, 62s) and depth of the stations was recorded using depth finder (Speedtech) as shown in Table 3.0.

Triplicate of sediments samples were collected at each station using Poinar Grab Sampler (Wildco). The sediments were then sieved using 500um sieve and retained materials (chironomid larvae) was preserved in 5 % formalin and later stained with Rose bengal in laboratory. Sediment samples were also taken in each station for measuring the chlorophyll *a* and Total Organic Matter (TOM) using modified-syringe corer. Two cm sediment was taken for chlorophyll analysis and another two cm of sediment was taken for TOM analysis. All sediments samples were brought back to laboratory for further analyses.

The selected of physico-chemical parameters of water were measured *in situ*. Rain was heavily poured during the measurement of water quality in station 1, whereas in other station, it was raining lightly. The dissolved oxygen (DO) was measured using DO meter (Eutech Instrument, Cyberscan DO 11), temperature and pH were measured using pH probe meter (Martini, Mi 415), turbidity was measured using turbidity meter (Martini, Mi 415) and salinity were measured using refractometer (Atago, Manual). All water samples were taken at the subsurface using vertical Van Dorn water sampler. Subsurface water samples were also taken for chlorophyll *a* analysis.

Table 3.0: The location of sampling stations in UNIMAS Lake.

No.	Station	GPS reading	Depth (m)
1.	Near Islamic Centre	N 01° 28' 09.7" E 110° 25' 52.9"	4.0
2.	Near Gazebo	N 01° 28' 09.7" E 110° 25' 52.9"	3.6
3.	Near Student Pavilion	N 01° 28' 09.7" E 110° 25' 52.9"	2.6
4.	Near Amphitheatre	N 01° 28' 09.7" E 110° 25' 52.9"	4.2

3.2.2 Fish Sampling

Cast net was used for catching fishes in UNIMAS Lake. Collected fish samples for gut content study were injected with 5% formalin into the stomach to stop the digestion process. The gut was placed in labelled plastic bag and brought back to laboratory for further analyses. The cast net was also used to catch live fish for testing the potential of chironomid larvae as live feed for selected fish species in UNIMAS Lake. All the live fishes were brought back to laboratory and placed in aerated aquarium. Table 3.1 presented the GPS reading for each station.

Table 3.1: The location of fish sampling stations in UNIMAS Lake

No.	Station	GPS reading
1.	Near Islamic Centre	N 01° 28' 09.7"
		E 110° 25' 52.9"
2.	Near Recreational Park	N 01° 28' 09.7"
		E 110° 25' 52.9"
3.	Near Student Administrative Building	N 01° 28' 09.7"
		E 110° 25' 52.9"

3.2.3 Chironomid Larvae Sampling for Culture Study

Chironomid larva in UNIMAS Lake had been collected randomly using Ekman Grab Sampler (Wildco). Samples were sieved by using 500 µm sieve. Retained organisms in 500 µm sieve were washed out into a plastic container. The container labelled as 'stock' together with date and time collected. Five to six grab of sediment had been taken at different locations of the lake. The specimens brought to laboratory for further culturing experiments.

3.3 Laboratory Works

3.3.1 Chlorophyll *a* analysis

500 mL water samples from each station were filtered using filter pump system in semi-darken room. Whatman filter papers with the pore size of 47 mm then were ground using mortar pestle in 10 mL of 90% acetone. The solutions then were place in centrifuge tube, and were centrifuged for 10 minutes at 3000 rpm using centrifuge machine (Nuve, NF 615). Supernatant were analyzed using spectrophotometer (Hach, DR 2800). Wavelength readings were recorded for chlorophyll *a* calculation using following formula:

$$C_a (\mu\text{g m}^{-3}) = 11.58 E_{664} - 1.54 E_{647} - 0.08 E_{630};$$

Where

C_a = chlorophyll *a* in $\mu\text{g/m}^3$

E = absorbance of respective wavelength.

To change the unit to mg L^{-1} , the following formula was used:

$$C (\text{mg/L}) = C_a (v) / V1;$$

Where

C = chlorophyll *a* in mg/L

v = volume of 90% aqueous acetone (mL) used

V = volume of filtered water sample

l = path length (cm) of the cuvette used in the spectrophotometer