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Sustainable control of mosquito by larval predating *Micronecta polhemus* Niser for the prevention of mosquito breeding in water retaining structures

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Abstract
Urban infrastructures with many receptacles at various sizes for water drainage and supply systems often become potential for water stagnation. This stagnant water can be infested with mosquitoes including the mosquito from *Aedes* genus that is known as the vector of dengue viruses. Many cases of dengue fever in tropical environment has been reported from time to time. The current method of mosquito control by fogging using chemicals is apparently not effective. This paper suggests an alternative method by bio-control using *Micronecta sp.* as a predator to mosquito larvae. The physical characteristics, its growth and predatory pattern of M*icronecta sp.* were investigated in this study. Field observation and experiments were conducted using earth jars to simulate the habitat. The jars were filled with rain water and left as stagnant water in open areas for a period of 3 months. The results indicate that mosquito could not bread in the stagnant water if the environment is established with *Micronecta sp.* This finding suggests that stagnant water in urban areas of tropical environment may be used for mosquito control by allowing exposure to sun radiation to establish a balance ecosystem.

Keywords: Rain water, stagnant, mosquito larvae, *micronecta* sp., biological control

Introduction

Mosquito in urban areas is one of the major health concerns because it is virus-transmitting vector [1]. Among mosquitoes, species of *Aedes* have become major worldwide concerns over the last decades. *Aedes* mosquitos can transmit several fatal diseases to human including dengue [2]. Based on recent reports, 390 million dengue infections happen annually and 3.9 billion people are at infection risk with this fastest spreading virus [3]. In Malaysia, around 101,357 dengue cases were reported in 2016 [4]. The common practice to control this vector is by fogging and larviciding. However, the statistic indicates that despite of regular fogging, the dengue cases are enhancing every year, suggesting that the chemical method for the control of mosquito is ineffective and not sustainable [5]. Therefore, an alternative biological control is needed to overcome this global problem of mosquito borne disease.

Breeding of mosquito in storm water drains depends on seasonal variation from summer to winter. High temperature and humid environment were shown to be more conducive for the breeding [11]. Mosquito-borne viruses proliferate seasonally in most parts of Asia. The seasonal patterns of viral transmission are associated with the abundance of both vector mosquitoes and vertebrate amplifying hosts [12]. The abundance of vector mosquitoes fluctuates with the amount of rainfall and irrigation [13]. Thus, the periods of greatest risk for virus transmitting vector abundances vary regionally from year to year. Global warming, pollutions, lack of public awareness, and inefficient mosquito management practices are among factors contributing to dengue resurgences.

Rapid industrial and economic growth during the past decades has caused massive infrastructural developments [14]. The development obviously provided many opportunities of wealth and wisdom to people. However, it has affected the ecosystem that may be linked to the global health concern. There was more than 80% of the dengue cases happen in urban areas [15]. *Aedes aegypti*, the mosquito vector, has proliferated in most areas of rapid and unplanned urbanization, water storage areas and areas with inadequate waste disposal services [16].

Stagnation of water in engineering structures is due to accumulation of silt or garbage which blocked the drains and may cause profuse breeding of mosquito. Drain that receives wastewater from commercial areas are likely to be polluted thus may become potential breeding for mosquito which leads to major health concerns [17]. Nevertheless, not all engineering structures that have water stagnation provide a breeding place for mosquito [18]. The findings suggested that in order to control mosquito breeding in engineering structures, the water need to meet the quality that can support natural predators of mosquito to survive.

Many indexes have been used to estimate the density of mosquito population in one particular area such as house index (HI), container index (CI), bruteau index (BI), and pupae index (PI) [3, 19]. These indices are based on the aquatic immature stages only. The larvae are collected from different water-holding containers infested with larvae and pupae. The Equations used are as follows:

$$HI = \frac{Nos\ of\ \ Infested\ \ Houses}{Nos\ \ \ of\ \ Inspected\ \ Houses} \times 100 \qquad \text{A}$$

$$CI = \frac{Nos\ of\ \ Infested\ \ Containers}{Nos\ \ \ of\ \ Inspected\ \ Containers} \times 100 \qquad \text{B}$$

$$BI = \frac{Nos\ of\ \ Infested\ \ Containers}{Nos\ \ \ of\ \ Inspected\ \ Houses} \times 100 \qquad \text{C}$$

$$PI = \frac{Nos\ of\ \ \ Pupae}{Nos\ of\ Inspected\ Houses} \times 100 \qquad \text{D}$$

These conventional indices are mainly practiced for the surveillance of immature stages of the mosquito for vector management programs. Other vector surveillances are based on man-landing / biting collection, resting collection and ovitrap method [20]. However, all the classical indices do not reflect the adult productivity and the environment of habitats. This method of surveillance also consumed labor works to make it efficient [21].

In the late 1940s, the modern synthetic insecticides were invented and applied to control mosquito as a replacement of primary means such as source reduction and inorganic chemicals and oil applications [22]. However, it also contributes to water pollution and kills other bio-organisms [10]. Therefore, the use of biological organisms to control pests is one of the most important weapons in the war against mosquito-borne disease. Biological control may become important tool and play a major role in mosquito management strategies in the future.

Biological control in mosquito management is defined as the introduction or manipulation of plants and animals or microorganisms in the habitats which can act as predators or pathogens that can eliminate the necessary steps for completion of the mosquitoes' life cycles [23]. The diversity of mosquito species and the wide range of aquatic habitats used by them dictate that the use of organisms for control must also be broad and wide ranging.

Several innovative methods using bio-control have been investigated. These include the use of bacteria *Wolbachia pipientis* [24], fish [25], tadpoles of *Polypedates cruciger* [26], water bugs [27], planarian [28], copepod [29], and copepod combined with plant extracts [30]. These bio-controls indicated certain degree of efficiency accordingly. Although the applications are restricted to geographical locations [31], the integrations of these methods could provide an environmentally friendly approach in controlling mosquito vectors [32].

This paper presents an ecological approach of mosquito control using an aquatic insect called *Micronecta polhemus* Nieser. This insect was chosen in this study for biological control of mosquito because of its larvivorous characteristics. *Micronecta* sp. is found distributed throughout the world and live in a healthy environment [33]. The objective of this research is to determine the biological features of Micronectidae to verify its ecosystem and to evaluate the effectiveness in natural ecological control of mosquito.

Materials and Methods

This research consists of two phases of experiments. The first phase involved an ecosystem study of a man-made container that had become populated and established with Micronectidae and the second experiment involved an application of such ecosystem for mosquito control.

A rain pan that was naturally established as an ecosystem of Micronectidae was examined in terms of its physico-chemical environment and the population density of the living organisms (Figure 1). The physical structures of the ecosystem and the energy system were measured. Water samples were collected for analyses on the pH, conductivity, turbidity, BOD, and COD. The observations on the ecosystem were carried out over a period of one year, covering both the biotic and abiotic environment of ecosystem.

Fig 1: Micronectidae habitat in evaporation tank

Three samples of 10 individual Micronectidae each were prepared in 100 mL of rain water in universal bottles. These samples were sent to Raffles Museum of Biodiversity Research, National University of Singapore, Singapore for taxonomy classification. The taxonomy identification was done within three days by the taxonomist in the research center. The physical structures and taxonomy of Micronectidae were made based on Nieser [33]. The samples were deposited in the museum samples collection for future reference.

An experiment was also conducted to study the growth pattern of Micronectidae in control field environment by using three populations of 30, 50, and 100 individual Micronectidae, respectively. They were counted individually and the change in total number was recorded. They were kept and observed in similar habitats made of 7×10×5 inch plastic containers filled with 2000 mL of the habitat water and was left in an open area under 12-h photoperiod. The growth was limited by the space and food supply as there was no additional food supply given except the algae in the water. Each population was observed and counted every alternate day for a total period of 40-days.

Another similar experiment was conducted in laboratory condition by using 64 Micronectidae. The lab has no direct exposure to light intensity from sun and the temperature was kept at 22ºC. They were kept and observed in 30×60×30 cm glass container filled with 7000 mL of water. The growth was limited by the light intensity and temperature. Each population was observed and counted every alternate day for a total period of 30-days.

Micronectidae collected from the field habitats were tested on their predation potential. Three population groups consisting of 30, 50, and 100 numbers of Micronectidae respectively were kept and observed under natural environment in artificial habitat made of 7×10×5 inch plastic containers filled with 2000 mL of the habitat water (Figure 3). Each population was given 50 mosquito larvae during the first day of the experiment. Observations on the predatory pattern were made every 24 h by counting the number of mosquito larvae left in the container. The temperature of the water in the containers ranged from 28 to 30°C with 12-h photoperiod. Each container was fully capped with 0.2 mm air-lined plastic cover to prevent any established mosquito from escaping.

The *Micronecta sp.* was also tested on its survivability under stressed conditions; high to low DO, high BOD and temperature.

Fig 2: Predation potential of Micronectidae (30, 50, and 100) and larvae (50)

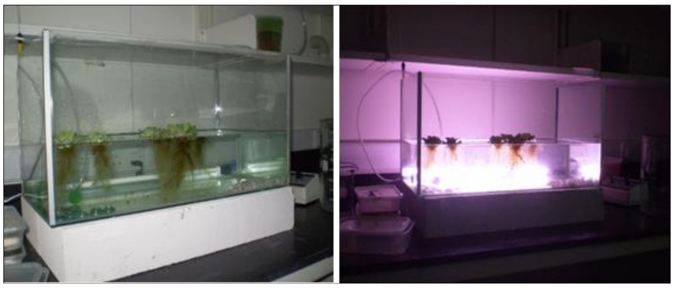

Fig 3: Growth pattern of 64 Micronectidae in laboratory aquarium

Results and Discussion

The biotic component of the habitat consists of producer (Algae), consumer (*Micronecta* sp.) and decomposer (bacteria).

There were no mosquito larvae found in the habitat. Result from the taxonomy study indicated that the species is *Micronecta polhemus* Nieser (Figure 4). This species was described by Nieser [33].

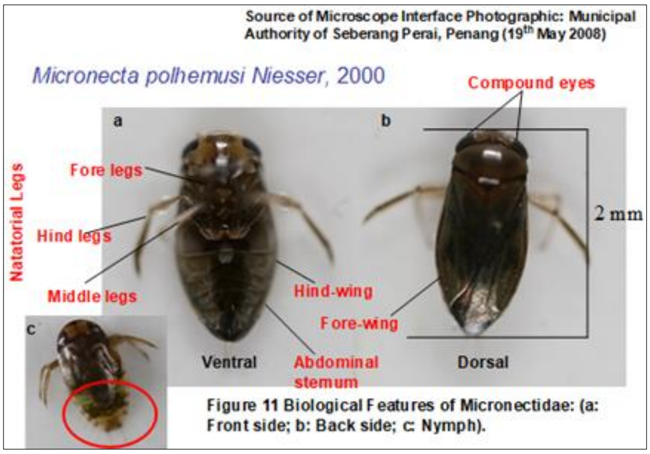

Fig 4: Biological features of Micronectidae (a: Front side; b: Back side; c: Nymph)

Algae are the main producer of the habitat. The amount of algae was found to be 93860 mg per liter of water in the evaporation pan. Some of the algae decompose to become organic detritus. Mosquito larvae may become the primary consumer in the stagnant water habitat. However, with the presence of the *Micronecta* sp. as the secondary consumer, the existence of mosquito larvae was hardly noticed. The presences were only represented by their skin after the larvae have been consumed by *Micronecta* sp. Although, the *Micronecta* sp. is the secondary consumer, it is the dominant consumer that feed on both algae and mosquito larvae (Figure 5).

Fig 5: *Micronecta* sp. as secondary consumer (a: Feeding on algae; b: Feeding on mosquito larvae)

The habitat of the *Micronecta* sp. in this study is stagnant rain water in an evaporation pan of a metrological station at Universiti Teknologi PETRONAS (UTP) under tropical environment with temperature between 28 and 30ºC. The dimension of the evaporation tank is 100 cm in diameter and 24 cm in height. Results on the water quality analysis of the habitat water are shown in table 1.

Table 1: Water quality of Micronectidae habitat

Water Quality Parameters	Results
Biochemical Oxygen Demand	12.5-15.4 mg/L
Chemical Oxygen Demand	25-31 mg O_2/L
Nitrogen Ammonia	0.55-0.56 mg/L NH_3-N
Dissolved Oxygen	6 mg/L
Temperature	30.0-31.5 ºC
Turbidity	5.5-6.65 NTU
Conductivity	7.85-7.9 µS/cm

The water quality of the habitat is comparable to Class I of Interim National Water Quality Standards of Malaysia [34]. It represents water body of excellent quality. The water bodies in this category meet the requirements for most aquatic life protection.

This habitat is located in an open area thus exposed to sunlight during the day with an average of light intensity level as shown in Figure 6.

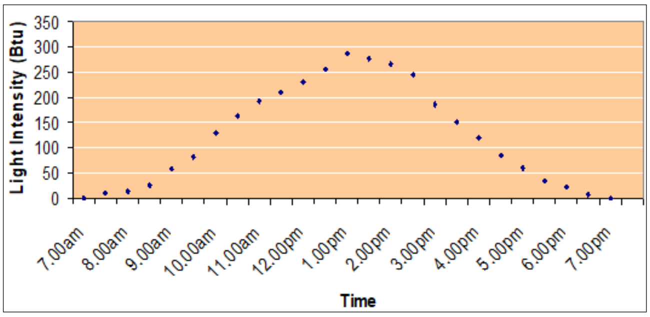

Fig 6: The average of light intensity of Micronectidae habitat at 12-h photoperiod for 3 days

The light intensity started to accumulate < 50 btu at 7.30 am and received the maximum peak ranged of 250 to 300 btu from 1 pm to 2.30 pm. It began to decrease after 2.30 pm and reached zero at about 7 pm.

The environment of the habitat is having high humidity. Figure 7 shows the humidity level of air at Micronectidae habitat from 7 pm to 5 am.

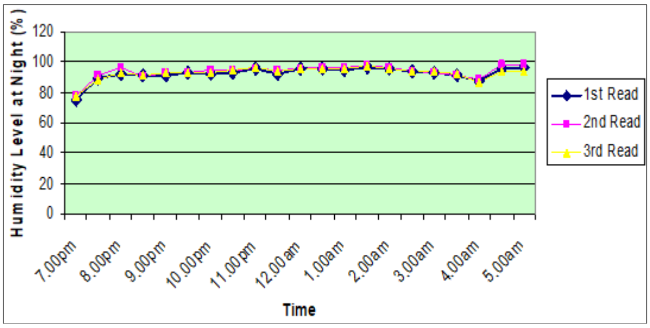

Fig 7: The humidity level of air at Micronectidae habitat from 7 pm to 5 am

The humidity level remained at almost constant level from 80% to 100%. Results of the study on the population growth of Micronectidae are shown in Figure 8.

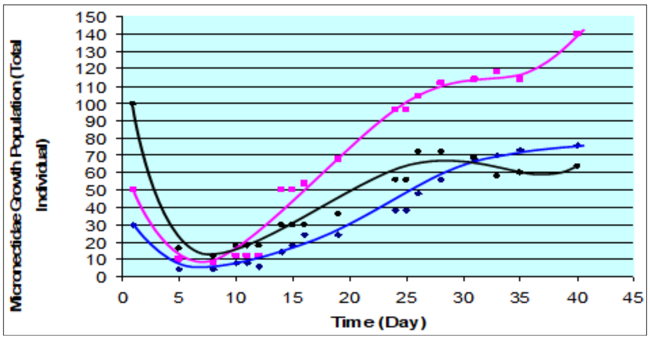

Fig 8: The growth pattern of 30, 50 and 100-Micronectidae under field environment

Studies on Micronectidae population growth pattern using containers as test-ecosystems with 2000 mL of water indicated that the population can reach the maximum number of around 120 after 40-days (Figure 8). Intra-specific competition was observed in the test-ecosystem that contained highest population of Micronectidae. This resulted in a slow growth pattern. Consequently, the population maintained only a total of 70-numbers of Micronectidae per 2000 mL of water. The same pattern was observed in the population that has limited number of 100 (Figure 8). However, the maximum population after 25 days were only around 70-Micronectidae. Apparently, Micronectidae population can reach to an optimum number within 25-days with a maximum population of about 120-Micronectidae in 4-litres water. The average from these 3-populations was found to be 87-Micronectidae in 4-litres of water.

The result of the population growth study of Micronectidae at low water temperature of 22ºC in lab environment for 30 days is shown in Figure 9.

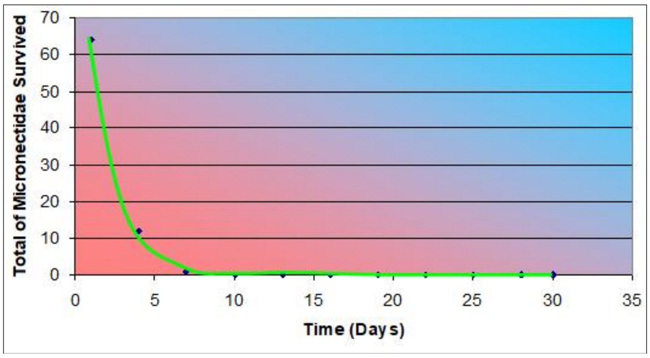

Fig 9: The growth pattern of 64-Micronectidae in lab environment

Micronecta sp. was unable to survive under a cool lab environment; the population dropped drastically and all the 64 individual *Micronecta* sp. died within 9 days. The condition was not exposed to direct solar radiation. Inherently, the food source of Micronectidae which is the algae were not able to grow.

Under stress condition of low dissolved oxygen (DO) and high biological oxygen demand (BOD) *Micronecta* sp. indicated more tolerance toward DO fluctuation (Figure 10).

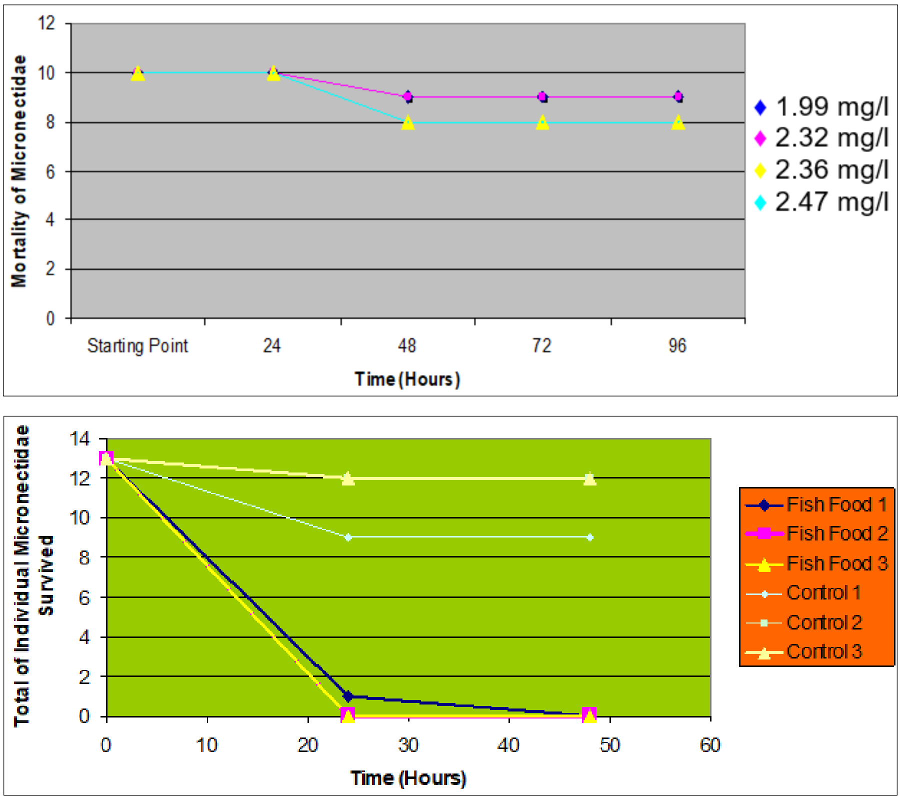

Fig 10: Number of *Micronecta* sp. survived under stress condition (a: Low DO; b: High BOD)

The predatory potential of Micronectidae on mosquito larvae from three different populations is presented in Table 2.

Table 2: Mosquito larvae and pupae left after consumption within 72-h period by different populations of Micronectidae (Test 1:30-Micronectidae; Test 2:50-Micronectidae; Test 3:100-Micronectidae)

Period (h)	Number of mosquito larvae and pupae left after consumption		
	Test 1	Test 2	Test 3
0 (Starting point)	50-larvae	50-larvae	50-larvae
24	8-larvae 14-pupae	0-larvae 10-pupae	0-larvae 4-pupae
48	4-larvae 10-pupae	0-larvae 6-pupae	0-larvae 2-pupae
72	0-larvae 0-pupae	0-larvae 0-pupae	0-larvae 0-pupae
Total	0-larvae 0-pupae	0-larvae 0-pupae	0-larvae 0-pupae

Results from the experiment on the predatory pattern of Micronectidae showed that they can consume both larvae and pupae (Table 2). In the first 24-h, the 30-Micronectidae was found to be able to consume 28 mosquito larvae (56% of the larvae), while the population of 50-Micronectidae predated 37 out of 50-mosquito larvae (74%) within 24-h and the population 3 which was 100-Micronectidae consumed 45 out of 50-mosquito larvae (90%). This type of food preference might be due to harder chitinous cuticle evolved on the pupae thorax. It was shown on the next 24-h, the total consumption for every population was increased to 72%, 88% and 96% respectively. Due to lack of food options, the remaining pupae were all consumed by Micronectidae after 72-h (Figure 10) and the total percentage of consumptions were 100%. Micronectidae species is an effective biological control because they are persistent and ubiquitous. In their natural habitat, they have a broad diet consuming detritus, benthic microorganism and filamentous algae Ulothrix sp. [35, 36]. They do not solely dependent on the quantity of mosquito larvae to survive allowing them to maintain large populations. Larval consumption at different prey densities were recorded. Overall, all group of total population of Micronectidae showed predatory behavior reaching total consumption at 72-h. As low as 30 Micronectidae were able to consumed more than half of mosquito larvae leading to high efficacy in removing larval population from aquatic sites. Thus, this Heteroptera provides a greater potential as biological control preventing the spread of disease-causing vectors like Dengue. The total number of larval consumed is shown in Figure 11.

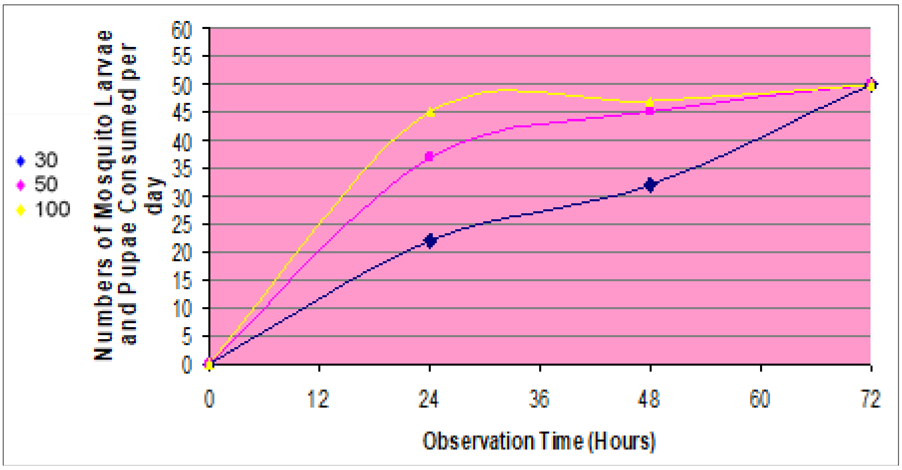

Fig 11: Total numbers of mosquito larvae consumed by Micronectidae populations over 72-h period

Conclusions

Stagnant water in open containers such as evaporator pan or engineering structures such as drains may become breeding places for mosquito. The current practice of controlling mosquito by fogging using chemical not only is ineffective but also polluting and destroying the balance ecosystem between mosquitoes and its predator.

Micronecta sp. was found as efficient predator for mosquito. A population of 30 *Micronecta* sp. can consume 50 mosquito larvae in 72 h. A stagnant water that is exposed to direct sunlight and is populated with *Micronecta* sp. was found to be free from mosquito larvae. However, this insect cannot survive in polluted water. The findings of this research suggest that mosquito breeding in engineering structures such as drains can be controlled by using *Micronecta polhemus* Nieser, a water insect that predate on mosquito larvae. This insect required clean water that is exposed to direct sunlight.

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