CHAPTER 4

ANALYSIS OF DATA AND DISCUSSION OF RESULTS

4.1 The Niche of *MpN2000*

MpN2000 habitat in this study is in an aquatic ecosystem in man-made containers of stagnant rain water under tropical environment with temperature between 28ºC to 30ºC. There were two types of water retaining containers that established the population of *MpN2000*; Evaporation tank (120 cm in diameter and 45 cm in height) in meteorological station, UTP and earth jars (20 cm in diameter and 30 cm in height). Results from the niche study are presented in abiotic and biotic environment.

4.1.1 Results for Abiotic Component

The abiotic component identification was done on the energy (sun light intensity), water quality and humidity level as follows:

4.1.1.1 The sunlight intensity results

Sunlight plays important role in photosynthesis process for plants. Micronectidae niche has a huge dense of algae population in the water body and has a direct exposure to sunlight. Figure 4.1 shows the reading of light intensity level of *MpN2000* niche for averages of 3 days over 12-hour photoperiod per day. The light intensity started to accumulate from less than 50 Btu/m^2 at 7.30 am and increased until the maximum peak around 250 to 300 Btu/m^2 from 1 pm to 2.30 pm. It began to decrease after 2.30 pm and reached zero at 7 pm everyday.

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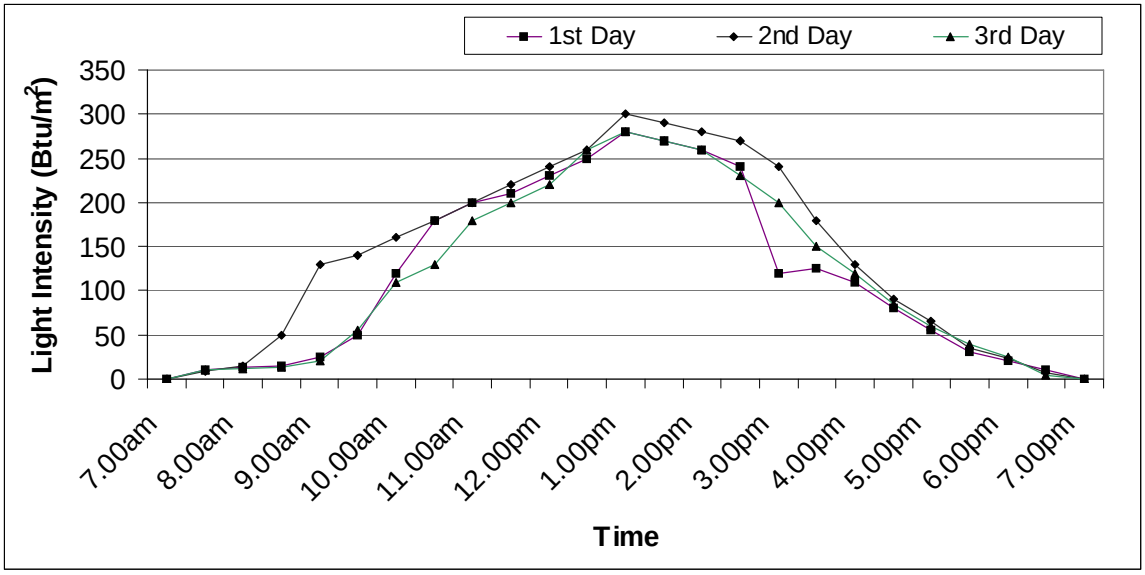

Figure 4.1 The light intensity condition (Btu/m^2) over three days period of *MpN2000* niche

From the two niches that were observed in this study, two received sunlight for at least 6 hour period during noon and one of the habitats was exposed to sunlight for the whole day. Both lighting conditions were found to be suitable for *MpN2000* habitat.

4.1.1.2 Water quality

Water quality is based on the Water Quality Index (WQI). It consists of five parameters namely, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrogen Ammonia, Suspended Solids (SS) and pH. The WQI is a 100 point scale that summarizes results from these five different measurements base on mean averages. The mean averages are used to combine values between parameters so that the field measurement values could be converted to index values (0 to 100). As for general purpose, water quality data collected from field sampling was compared with the Interim National Water Quality Standards for Malaysia (NWQS) to determine their WQI status as clean, slightly polluted or polluted (Appendix 1).

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Results of water quality analysis on BOD, COD, Nitrogen Ammonia, Total Phosphorous, Potassium, Dissolved Oxygen (DO), Temperature, Turbidity and Conductivity are presented in Table 4.1.

Table 4.1 Water quality of *MpN2000* habitat and DOE Standards

Water Quality Parameters	Results	DOE, Standards on Water Quality	
		Class I	Class IIA/IIB
Biochemical Oxygen Demand	12.5-15.4 mg/L	>1 mg/L	>0.2 mg/L
Chemical Oxygen Demand	25-31 mg O_2/L	>10 mg/L	>25 mg/L
Nitrogen Ammonia	0.23 mg/L NH_3-N	>0.1 mg/L	>0.3 mg/L
Dissolved Oxygen	6 mg/L	>7 mg/L	5-7 mg/L
Temperature	30.0-31.5 ºC	NL	NL
pH	6.5-7.5	6.5-8.5	6.5-9
Turbidity	5.5-6.65 NTU	>5 NTU	>50 NTU
Conductivity	280 µS/cm	>1000 µmhos/cm	>1000 µmhos/cm
Total Phosphorous	0.79-0.82 mg/L	NL	>0.2 mg/L

* NL (Natural Level): Included in the DOE, Interim Standards on Water Quality as Natural Level.

The quality of water from *MpN2000* habitat is comparable to Class I and Class IIA/IIB of National Water Quality Standards (DOE, 2005) represents water body of excellent quality. The water bodies in this category meet the requirements for most aquatic life protection.

4.1.1.3 Humidity level

Results of the humidity measurements of air at *MpN2000* niche located at evaporation pan, meteorological station, UTP from 7 pm to 5 am is shown in Figure 4.2. The humidity level remained at constantly high level from 80% to 100% throughout the observation period.

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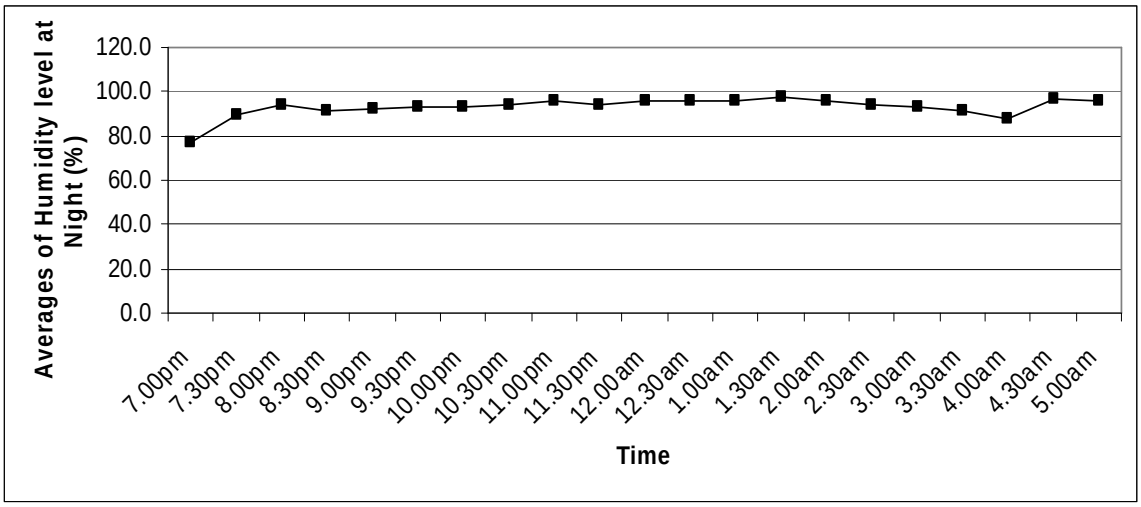

Figure 4.2 The mean relative humidity of air at evaporation pan, meteorological station UTP from 7 pm to 5 am

4.1.2 Results for Biotic Component

The abiotic components in *MpN2000* niche consist predominantly of producer (algae), consumer (*MpN2000*) and decomposer (bacteria). Mosquito larvae may be present from time to time as occasional consumer that feed on detrital particles of organic materials. The studies also include taxonomy classification, its growth pattern and its predation potential towards mosquito larvae. Emphasis is given in this study to *MpN2000* particularly as predator of mosquito larvae. Results for biotic environment are shown as follows:

4.1.2.1 Producer

The primary producer in the habitat is algae which depend on the sun light as the source of energy. Results from the analysis based on the gravimetric methods indicated that the water contents mostly inorganic structures such as diatoms (eukaryotic algae). The ash free dry weight showed that it has 93860 mg/L of algae and 4949 mg/L of other forms (organic structures such as silt and organic detritus). It contains 95% of algae and 5% of clay.

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4.1.2.2 Primary consumer

Mosquito larvae may become the primary consumer in a stagnant water habitat. However, with the presence of *MpN2000* as the secondary consumer, the presence of mosquito larvae hardly noticed. Its presence was only represented by its skin after being consumed by *MpN2000*.

4.1.2.3 Secondary consumer

Although *MpN2000* is the secondary consumer, it is the dominant consumer that feed both on algae and mosquito larvae. Results from the sampling of *MpN2000* for its habitat indicated an average population number of 30 *MpN2000* / L.

Results from the taxonomic study showed that the species is *Micronecta polhemusi* Nieser, 2000 (Nieser, 2002.b) as shown in Figure 4.3. This species also known as "pigmy water boatmen" due to its smaller size compared to Corixidea (water boatment).

Figure 4.3 *Micronecta polhemusi Niesser* 2000

a. The population dynamics of MpN2000

Results of the growth population of *MpN2000* over 40 days of observation are shown in Figures 4.4, 4.5 and 4.6.

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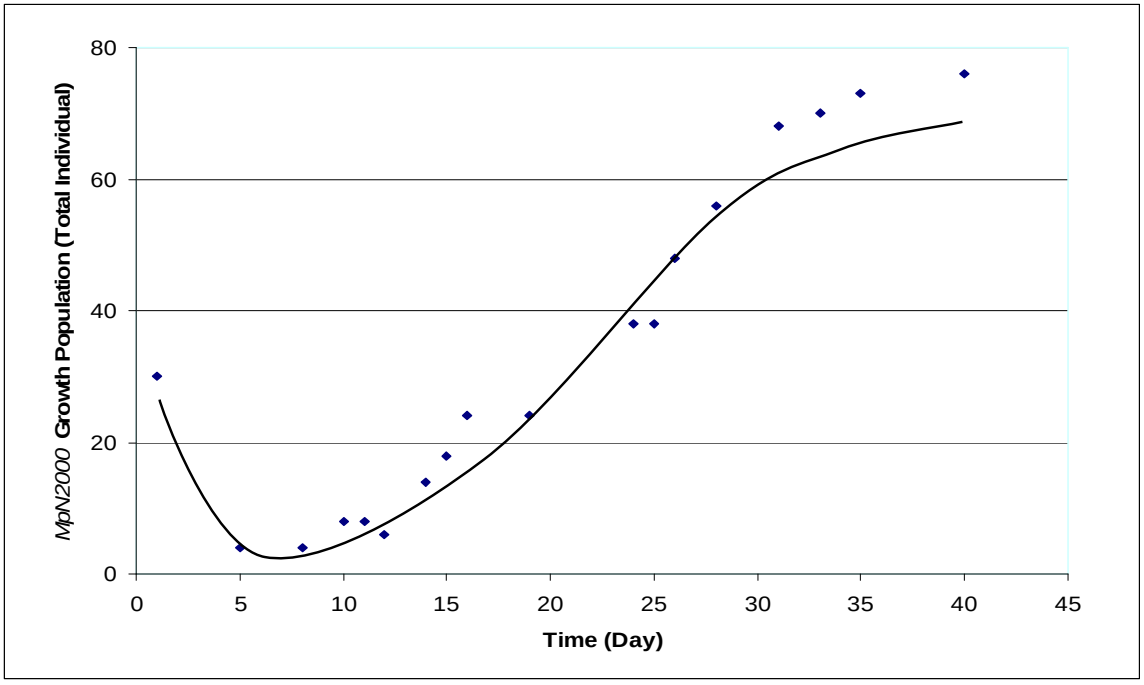

Figure 4.4 The growth population of 30 *MpN2000* in container

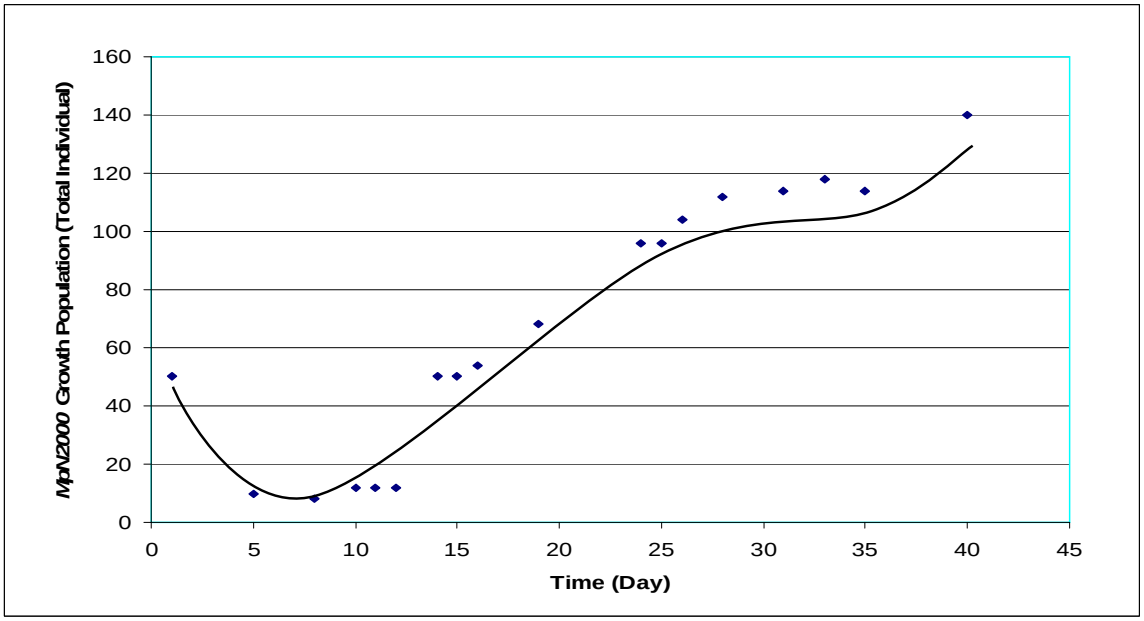

Figure 4.5 The growth population of 50 *MpN2000* in container

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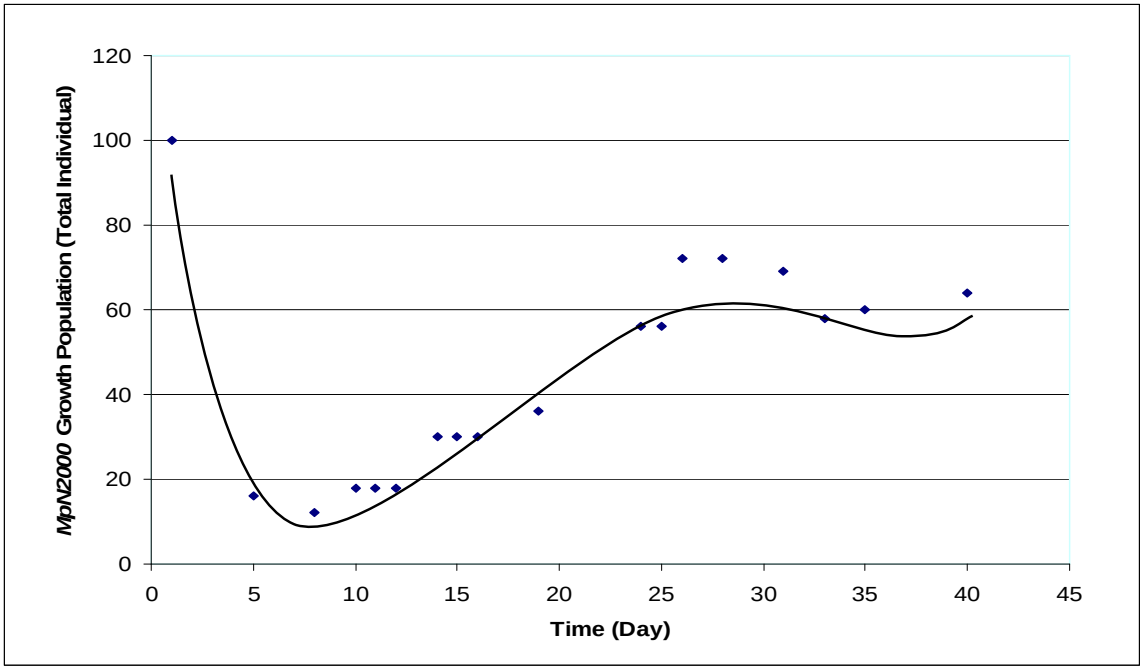

Figure 4.6 The growth population of 100 *MpN2000* in container

Studies on *MpN2000* growth population pattern using containers as test-ecosystems with 2000 ml of water indicated that the population can reach the maximum number of around 140 after 40-days (Figure 4.5). Intra-specific competition was observed in the test-ecosystem that contained highest population of *MpN2000*. This resulted in a slow growth population. Consequently, the population can grow only a total of 70-numbers of *MpN2000* per 2000 ml of water. The growth population 30 *MpN2000* indicated a lag phase where it was decreased from 30 to 5-*MpN2000* within 5-days (Figure 4.4). This was probably due to adaptation stage. After 5-days, the population increased exponentially to around 78-numbers within 20-days. Subsequently, the population growth became slow probably due to limited food sources. The growth rate, r for 30-*MpN2000* was 0.07 per day per *MpN2000*.

Similar pattern was observed for population that started with 50 *MpN2000* (Figure 4.5). Initial drop in population started from 50 to 10-*MpN2000* within 5-days. After 5-days, the population began to increase until it reached of around 100-*MpN2000* within 20-days. The growth after 25-days became slower probably due to intra-specific

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competition. The growth rate, r for 50-*MpN2000* was 0.08 per day per *MpN2000*. The same pattern was observed in population that started with 100-*MpN2000* (Figure 4.6). However, the maximum population after 25 days were only around 70-*MpN2000*. The growth rate, r for 100-*MpN2000* was 0.10 per day per *MpN2000*. Apparently, *MpN2000* population can reach to an optimum number within 25-days with a maximum population of 140-*MpN2000*. The average from these 3-populations was found to be 94-*MpN2000* in 4-litres of water. The averages growth rate, *r* for population of *MpN2000* based on exponential growth model equation was 0.08 per day per *MpN2000.*

The result of the population growth study of *MpN2000* in laboratory for 30 days under air conditioned environment is shown in Figure 4.7.

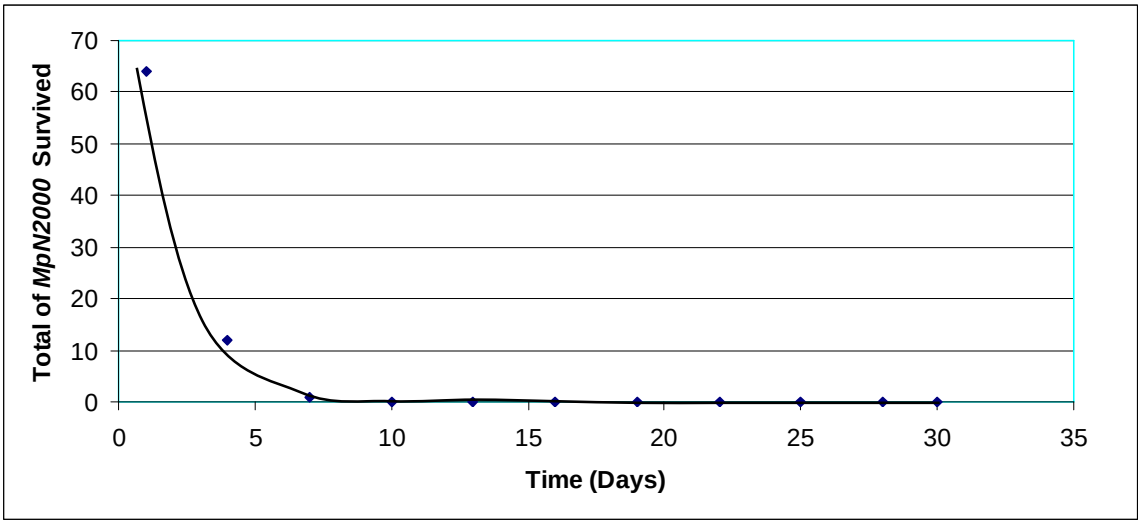

Figure 4.7 The growth pattern of 64-*MpN2000* in laboratory environment

The 64 individual *MpN2000* were not able to survive in laboratory environment. The temperature ranged from 24 ºC to 27 ºC and was not exposed to direct solar radiation. Inherently, the food source of *MpN2000*, algae was absence. Without the presence of sun light, algae were not able to undergo photosynthesis. Consequently, *MpN2000* population dropped within 5 days and no population growth of *MpN2000* was observed for a month period.

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b. The predation potential of MpN2000

The predatory potential of *MpN2000* on mosquito larvae from three different populations is presented in Table 4.2.

Table 4.2 Mosquito larvae and pupae left after consumption within 72-hours. Container 1 contained 30 *MpN2000*, Container 2 contained 50 *MpN2000* and Container 3 contained 100 *MpN2000*

Period (Hours)	Number of mosquito larvae and pupae left after consumption		
	Container 1	Container 2	Container 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 *MpN2000* showed that in the existent of algae in the habitat water, *MpN2000* can consume the given larvae and pupae within 3 days period. The food preference by *MpN2000* on algae or larvae as the food sources was not an issue as the experiment was conducted in the water habitat collected from evaporation pan, UTP. The evaporation pan has established with a dense of algae mass and huge population of *MpN2000*. The water habitat itself contained of 93860 mg/L of algae mass. The actual algae density in the 2000 mL of water habitat used for this experiment was 187720 mg. Therefore, the algae density in the water habitat reflected the natural niche of *MpN2000*. Under this condition, the 50 larvae were totally consumed by *MpN2000* within 3 days period.

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The presence of algae and larvae in this experiment also reflected the food web of *MpN2000* in natural environment as indicated in Figure 3.11 in Chapter 3. Hemiptera (Suborder: Heteroptera) like *MpN2000* have pretarsal structures (claws), that is important in maintaining a purchase on smooth, exposed surfaces of plants or in holding preys. Heteroptera includes a minority of predatory and ectoparasitic species but most species of the larger, more diverse family families are principally phytophagous (feed on plants) (Daly *et al.*, 1998). *Micronecta spp* can be classified in the minority group of Heteropteran that have omnivorous type of food preference.

Heteropteran have evolved special holdfast organs and have highly specialized piercing tube for delivering salivary secretions and taking up the haemolymph of the prey (Daly *et al.*, 1998). The predation of *MpN2000* on mosquito larvae was also reflected the same way. *MpN2000* was found piercing the prey and sucking the haemolymph of mosquito larvae.

Plant fluids do not provide a nutritionally complete diet and most Heteropteran support symbiotic bacteria that may occur freely in the alimentary canal or may be housed in special gut diverticula. Diverticula is a fluid filled structure of the body particularly referred to the Heteropteran gut or alimentary canal. The alimentary canal is modified for uptake of liquid food. Salivary glands are universally present. In predatory species, saliva is highly toxic and paralytic, enabling relatively large prey to be quickly subdued (Daly *et al.*, 1998). It can be observed on the voracious predatory behavior of *MpN2000* on mosquito larvae in the experiment.

Suphapathom *et al.*, (2002) reported the presence of *Micronecta* species approximately 2-3.5mm in size in water containers in the rural areas. The research stated that *Micronecta* sp is not only consumed *Aedes aegypti* larvae but also other weaker organisms by sucking the haemolymph of the prey until it is dead. The study showed that the food preference of *Micronecta* sp was on larval/aquatic stage of insects particularly *Aedes aegypti* larvae. However, the details of this predator, the species classification and its effectiveness were not yet determine.

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The averaged of total consumptions on mosquito larvae were 76% and 85% larvae for the first and second 24-hours. *MpN2000* most likely prefer to feed on larvae rather than pupae. This type of food preference might be due to harder chitinous cuticle evolved on the pupae thorax. It was shown by the averaged total consumption of pupae on the second 24-hours. A total of 10 from 28 pupae were consumed within 48 hours (Table 4.2). Due to lacking of food options, the remaining pupae were all consumed by *MpN2000* after 72-hours. As for comparison to planarians, these predators were found avoiding first instar of mosquito larvae because of their fast movement and prefer more on fourth instar larvae and pupae (Suprakash and Aditya, 2003).

In the first 24-hours, the 30-*MpN2000* was found to be able to consume 28 mosquito larvae (56% of the larvae). While the population of 50-*MpN2000* predated 37 out of 50-mosquito larvae (74%) within 24-hours and the population 3 which was 100-*MpN2000* consumed 45 out of 50-mosquito larvae (90%). On the next 24-hours, the total consumption for every population was increased to 72%, 88% and 96% respectively. After 72-hours, the total consumptions were 100% consumed by these predators (Figure 4.8).

The total number of larval consumed by the *MpN2000* is shown in Figure 4.8.

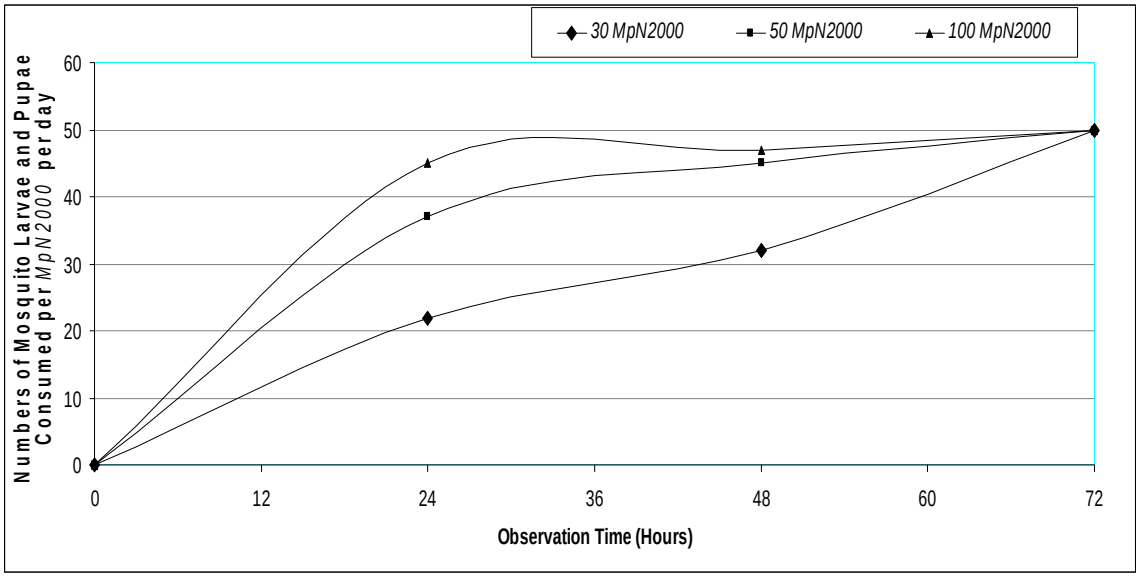

Figure 4.8 Numbers of mosquito larvae consumed by *MpN2000*

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The rate of larval consumption by the *MpN2000* is shown in Figure 4.9.

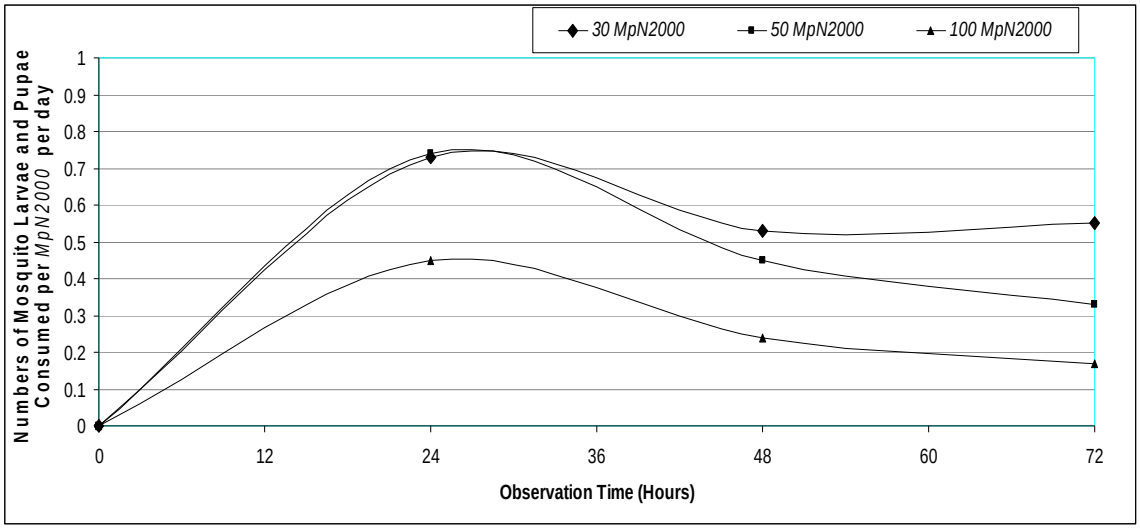

Figure 4.9 Predation rates of three *MpN2000* populations on mosquito larvae and pupae

The average rate of consumption within 24 hours for the first group of population was 0.93 mosquito larvae per *MpN2000* per day, while the second and third groups were 0.80 and 0.46 mosquito larvae per *MpN2000* per day, respectively. It appears that the rate of consumption was higher for the group with smaller population. On the second 24 hours, the average rate consumptions for 30, 50 and 100 *MpN2000* were 0.14, 0.04 and 0.24 mosquito larvae per *MpN2000* per day accordingly. However, after 72 hours, all the 50 mosquito larvae that were introduced into each population were consumed by the *MpN2000* with the rate consumptions of 0.16, 0.04 and 0.01 mosquito larvae per *MpN2000* per day accordingly. The average daily rates of consumptions were 0.41, 0.29 and 0.24 mosquito larvae per *MpN2000* per day, respectively. Overall, this rate was 0.31 mosquito larvae per *MpN2000* per day. (Figure 4.9).

The predation potential of dragonfly nymph depends on the temperature and its body weight. The more body weight it has, the more mosquito larvae consumed (Pandian *et al.*, 1979). The predation potential of spiders towards mosquito larvae was 40.7% per day and different species of spiders tested prey readily upon mosquito larvae at different rate of consumption (Breene *et al.*, 1988). Predation potential of the dytiscid beetle, *Rhantus*

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sikkimensis 18.67 and 35.33 larvae/day and the mosquito larvae of *Toxorhynchites splendens* 7.67 and 11.33 larvae/day towards mosquito larvae of *Culex* sp (Gautam *et al.*, 2006). Unfortunately, the exact numbers of predators tested was not mentioned in the report and it does not reflect the actual potential for the predatory rate of these predators. Meanwhile, the predation potential of *MpN2000* was solely depends on its population densities as indicated by the higher percentages of consumption on mosquito larvae in the first 24-hours for 100 *MpN2000*. A total of 90% of mosquito larvae were consumed by 100 *MpN2000* during the first 24-hours. The rate of consumption was higher for the smallest population densities, 30 *MpN2000*. It was 0.93 mosquito larvae per *MpN2000* per day

4.1.2.4 Decomposer

The results for total coliform and *E.coli* count were made base on Most Probable Number (MPN) count.The results for total coliform and *E.coli* count were 1011.2 MPN and 9.8/2000 MPN accordingly as shown in Table 4.3.

Table 4.3 The results for total coliform and *E.coli* count for *MpN2000* habitat water

Decomposer	Results	DOE, Standards on Water Quality	
		Class I	Class IIA/IIB
Total Coliform count	1011.2 MPN / 100 ml	100 count / 100 ml	5000 count /100 ml
E. Coli count	9.8 / 2000 MPN /100 ml	10 count /100 ml	100 count / 100 ml

These results are comparable to Class IIA/IIB of Interim National Water Quality Standards (DOE, 2005).

4.2 Results on the Application of *MpN2000* for mosquito control

The scope of this experiment is to identify the presence of *MpN2000* versus mosquito breeding in stagnant water located in natural field environment and to apply *MpN2000* in stagnant water for mosquito breeding control in residential areas.

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4.2.1 Results on Survivability Test

Results for the survivability test on DO, organic pollutant (fish food) and temperature are shown below:

4.2.1.1 Effect of low dissolved oxygen (~2 mg/L)

Dissolved Oxygen (DO) represents the amount of oxygen dissolved in the water. Water with an excellent water quality grade indicates there is enough oxygen available for a wide variety of aquatic organisms. Conversely, water with a poor water quality grade indicates water lacking in dissolved oxygen.

Results for survivability test under reduced dissolved oxygen (DO) for 10 *MpN2000* over a period of 96 hours are shown in Figure 4.10.

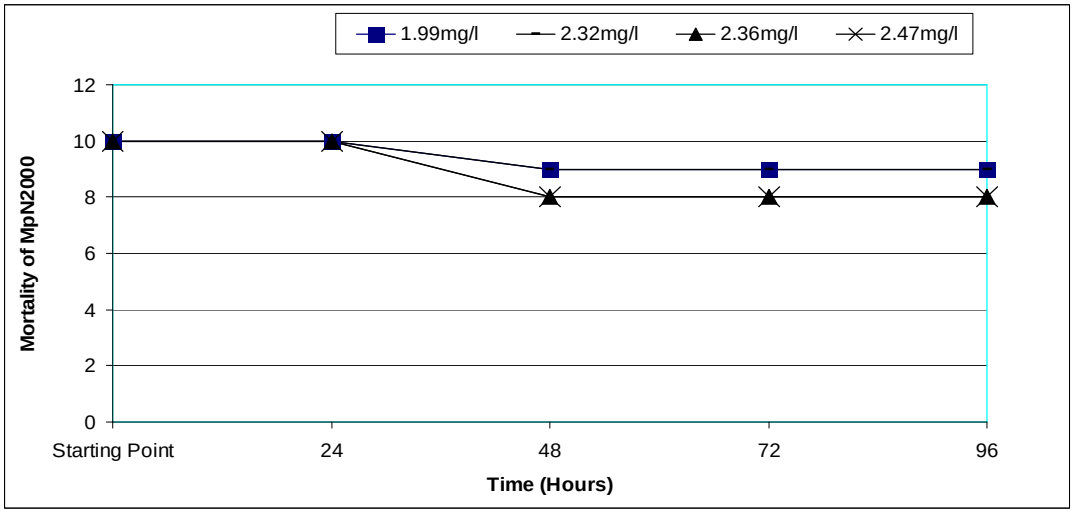

Figure 4.10 Mortality of 10-*MpN2000* in different level of dissolved oxygen

From the stressed test environment, *MpN2000* was found to be able to survive in water with dissolved oxygen as low as 2 mg/L (Figure 4.10). It was shown by the total mortality number of 10 *MpN2000* in four level of Dissolved Oxygen (DO); 1.99 mg/L, 2.32 mg/L, 2.36 mg/L and 2.47 mg/L. After four days of observation, the figure showed

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the total number of *MpN2000* that survived in this condition were 8 *MpN2000*. Eighty percent (80%) of the initial population survived the low DO condition and this could be due to the nature of the *MpN2000* that is capable of taking and carrying air bubble as they swim in the water.

In water environment with the meeting requirement of DO level will provides a huge diversity of organisms living within. This would be considered a diverse environment. If the level of DO decreased, the number of species or the diversity of organisms becomes less because only those creatures that are tolerant of low oxygen levels can survive in such an environment. According to Duffus in 1980, dissolved oxygen that is between the ranges of 2 mg/L and below is not only can be harmful to aquatic life, but it is also increase susceptibility to other environmental stresses to death. At a DO of 2 mg/l the water would be comprised of only carp, leeches, pond snails, protozoans and mosquito larvae. This experiment shows that *MpN2000* able to survive within the range of dissolved oxygen with a condition that the water environment is clean.

4.2.1.2 Effect of organic pollutant (fish food)

The total number of *MpN2000*that survived in 7 L of rain water with 35 mg of fish food is shown in Figure 4.11. The fish food concentration was 5 mg/L.

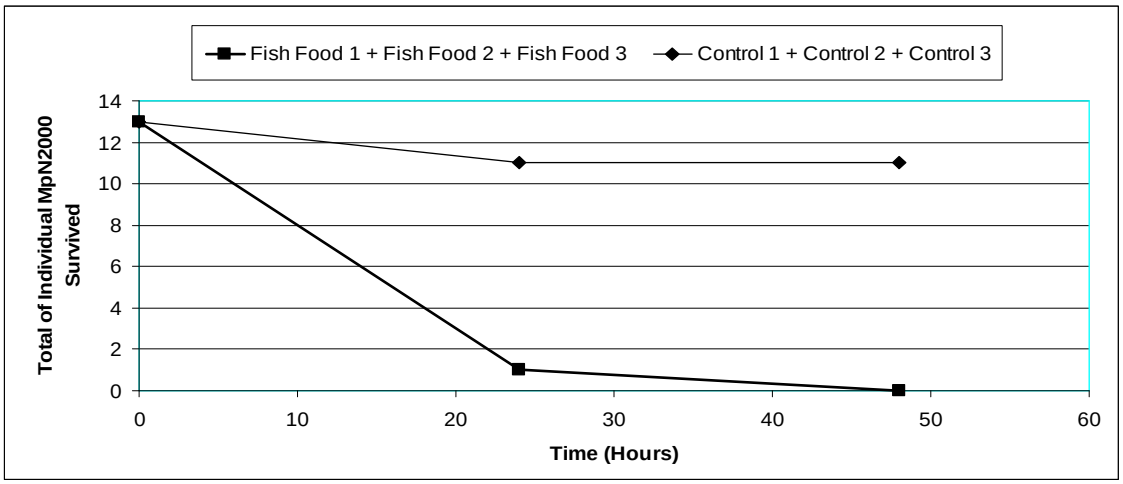

Figure 4.11 Number of *MpN2000* survived in polluted water (Food 1 + Food 2 + Food 3) against control (Control 1 +Control 2 +Control 3)

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The 13 *MpN2000* were not able to survive in the water samples containing of fish food. After 24 hours, the total number of *MpN2000* survived was only one and in another two replicates were zero. As for comparison, *MpN2000* population in control remains seemingly unchanged. The Dissolved Oxygen (DO) readings (mg/L) for rain water contained fish food and Control (rain water) are shown in Figure 4.12.

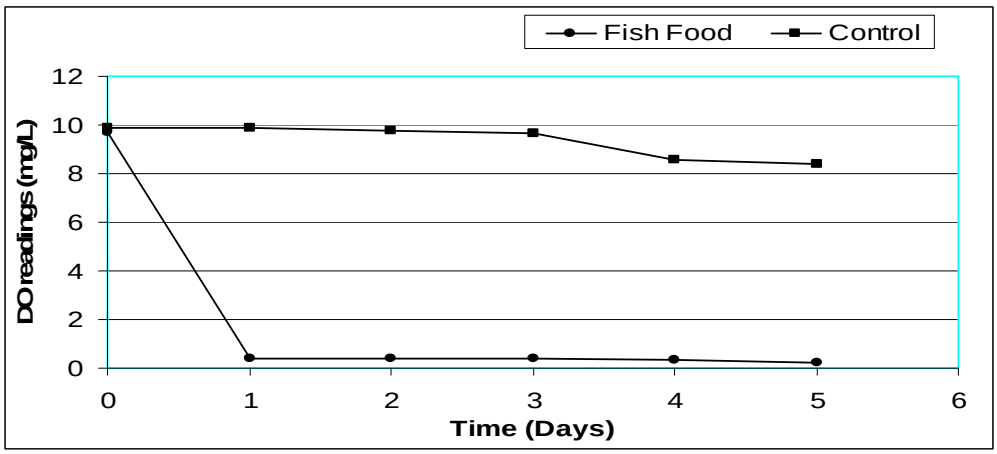

Figure 4.12 Dissolved Oxygen (DO) readings (mg/L) for rain water containing fish food and Control (rain water)

Biochemical Oxygen Demand (BOD) is a standard microbial incubation procedure that measures the oxygen required to oxidize organic material and certain inorganic materials over a given period of time. If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste that leads to high BOD level. Rain water that contained fish food increased the BOD level. It is due to high nutrient content in fish food given. During the first few days, the rate of oxygen depletion was rapid because of the high degradability of the organic matter present as presented in Figure 4.12. The Dissolved Oxygen (DO) readings (mg/L) showed a rapid decline in DO levels for rain water with the concentration of fish food of 2 mg/L. The DO readings for control remain constant between the ranged of 10 to 8 mg/L. Meanwhile, the DO readings for rain water contained fish food were depleted after 24 hours from 9.74 mg/L to less than 2 mg/L. The values of DO within the ranged of 2 to 4 mg/L may results in death to a large number of fish and other aquatic organism. Base on previous research, *MpN2000* population was able to survive a low DO levels as low as

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2 mg/L. The BOD level for the water containing fish food was high. It was 289.5 mg/L. The survivability test shows that *MpN2000* population cannot survive under such BOD level.

Nevertheless, the actual BOD level for this water is higher than 289.5 mg/L because the concentration of fish food in the rain water during the survivability test of *MpN2000* was also high. It was 35 mg of fish food in 7 L of rain water which gives fish food concentration in rain water at 5 mg/L. Therefore, the actual BOD level for the water containing this organic pollutant (fish food) in the survivability test is 723.75 mg/L. The BOD level has exceeded the limit of Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979 (Environmental Quality Act 1974, 1997) which is only 400 mg/L for the parameter limit on BOD.

4.2.1.3 Effect of temperature

Temperature plays a major role in the survival and health of the aquatic organisms in the aquatic ecosystems. It affects the solubility of dissolved oxygen in water. The warmer the water, the less dissolved oxygen is present in the water. Temperature also affects the chemical processes of the aquatic insects like *MpN2000*. Results of the survivability of four *MpN2000* in the four different water temperatures for a total period of four days are shown in Figure 4.13.

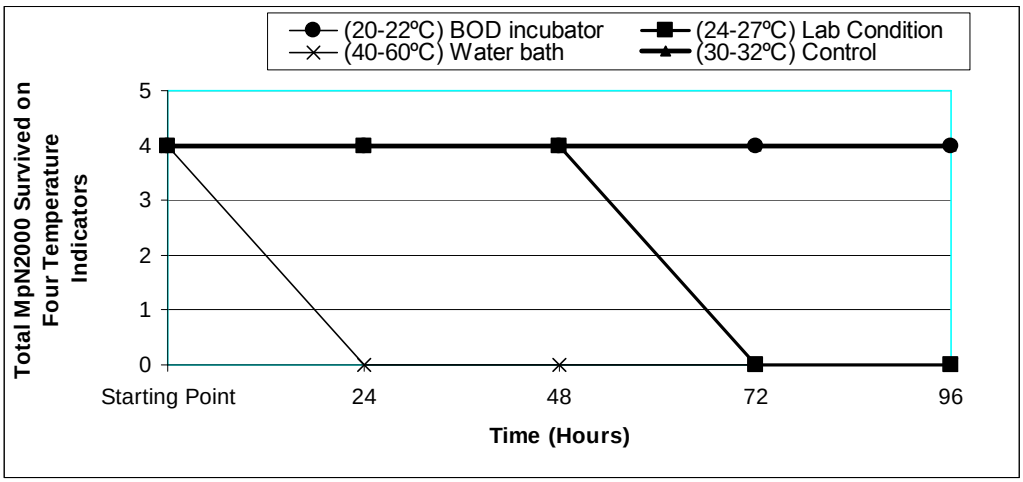

Figure 4.13 The total *MpN2000* survived in four different temperatures

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The temperature ranged from 40 ºC to 60 ºC was not favor the survival of 4 *MpN2000*. High temperature lower the dissolved oxygen level of the water and the continuous thermal pressure eradicated these four *MpN2000* within 24-hours. As for comparison Pandian *et al.*, (1979) reported that dragonfly nymphs which also the natural predator of mosquito larvae was succumbed as the temperature was raised over 37ºC. At 40 ºC, no individual nymphs survived. The dragonfly nymphs are very sensitive towards temperature changes which will affect its predation efficiency. The predation potential of dragonfly nymph depends on the temperature and its body weight. The more body weight it has, the more mosquito larvae consumed (Pandian *et al.*, 1979).

4.2.2 *MpN2000* Habitation and Mosquito Infestation of Stagnant Water

The percentages of established *MpN2000* population and infestation of mosquito in 90 stagnant waters in natural environment that were prepared as model ecosystems in field environment is presented in Figure 4.14.

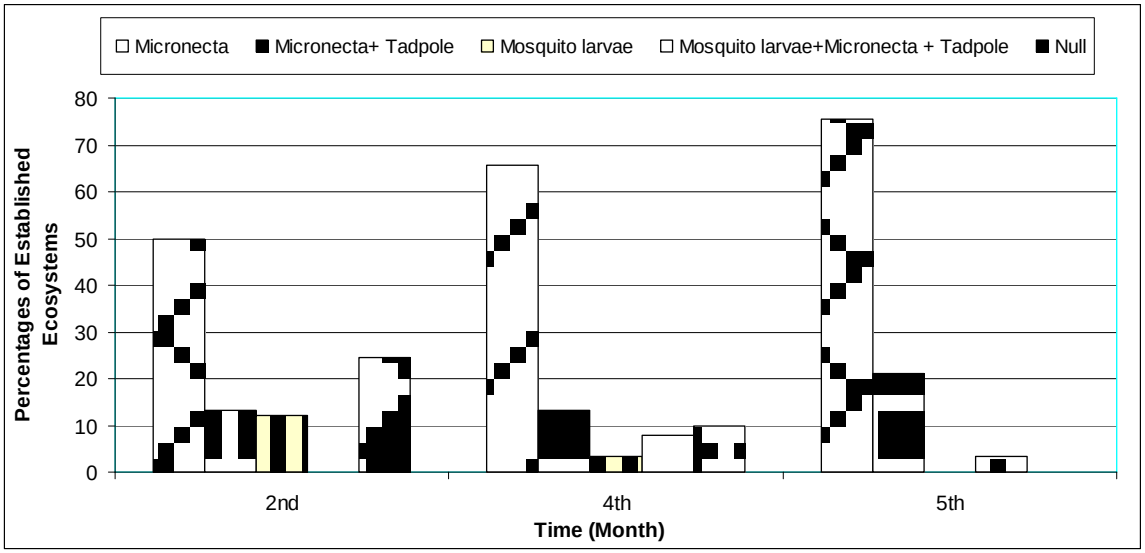

Figure 4.14 Mosquito predators and infestation of mosquito naturally established in 90 stagnant waters

In the occurrence of water stagnation in natural environment, the probability for infestation of mosquito breeding was 12.22% (11). This was happen at the 2nd week of

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observation. At this period, 24.44% of earth jars (22) containing stagnant water was not established a mini model ecosystem and 63.33% stagnant waters (57) were naturally established with *MpN2000* and tadpoles. The observation showed that *MpN2000* has a symbiotic relationship with tadpoles. On the 4th week, the presence of a new population of *MpN2000* and tadpole were observed in 7 stagnant waters containing mosquito larvae (7.78%). At this stage, the percentages of 24.44% (22) non-established stagnant waters were decreased to 10% (9). The percentages of established stagnant water with the natural predators were increased to 86.67% (78). At week 5th, the infestation number of mosquito breeding declined to zero.

The presences of other bio-organisms in infested stagnant waters were 3 (3.33%). The numbers of established stagnant waters were 87 (96.67%). In natural healthy environment, the probability percentage of mosquito breeding can be as low as 3.33% to 12.22%. The presence of its natural predator in stagnant water via natural establishment can be as high as 63.33% to 96.67%. These natural controls keep the ecosystem stagnant water in equilibrium. However, the numbers of mosquito infestation probably will have a high probability to proliferate if the environment is unhealthy for the predator itself.

4.2.3 Introduction of Model Ecosystems (MEs) in Three Housing Areas

The scope of this experiment is to apply *MpN2000* as biological controls of mosquito in urban residential areas. The chosen housing areas were Taman Maju (TM), Bandar Universiti (BU) and Taman Tasek Putra (TTP). The evaluation was made base on the relationship between Micronecta Index and Infested Container Index in these housing areas accordingly.

Survey results for TM were failed because *MpN2000* populations in MEs were found dead due to frequents fogging activity by the vector squad from Health Department of Sri Iskandar. The fogging activity cannot be stop as it is one of Malaysian government policy to combat Aedes mosquito in dengue hot spot area such in TM. Consequently, TM was

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eliminated from the research list. All the containers (MEs and Controls) were turned up side down to prevent mosquito breeding.

Figure 4.15 represents the average readings of Micronecta Index and Container Index in order to evaluate the efficacy of Micronectidae as biological control of mosquito in BU over a period of three months observations (Appendix 2.1).

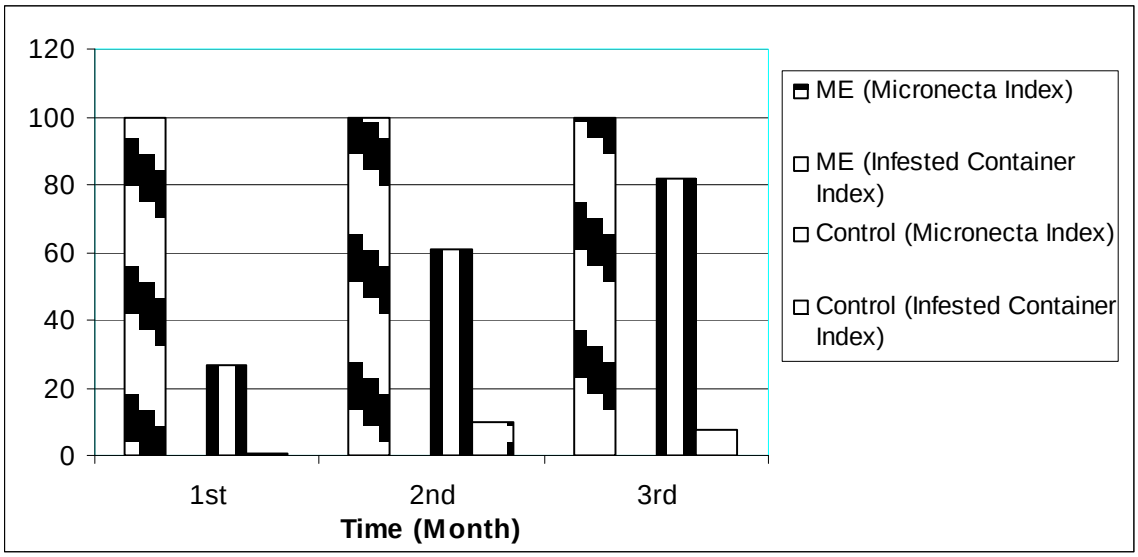

Figure 4.15 The efficacy of Micronectidae as biological control of mosquito using model ecosystem in Bandar Universiti

According to this figure, there was zero infestation of mosquito breeding in MEs. Inherently, the Container Index for MEs also was zero in every month. *MpN2000* population in MEs remains unchanged and gives Micronecta Index of 100. As for controls, the presence of a new population of *MpN2000* is shown via Micronecta index. They were 26.7, 60.8 and 81.8 for the 3 months accordingly. The infested Container Index for the controls were 0.6, 9.9 ad 7.8 respectively.

Figure 4.16 represents the evaluation on the efficacy of *MpN2000* as biological control of mosquito in TTP base on Micronecta Index and Container Index (Appendices 2.1 and 2.2).

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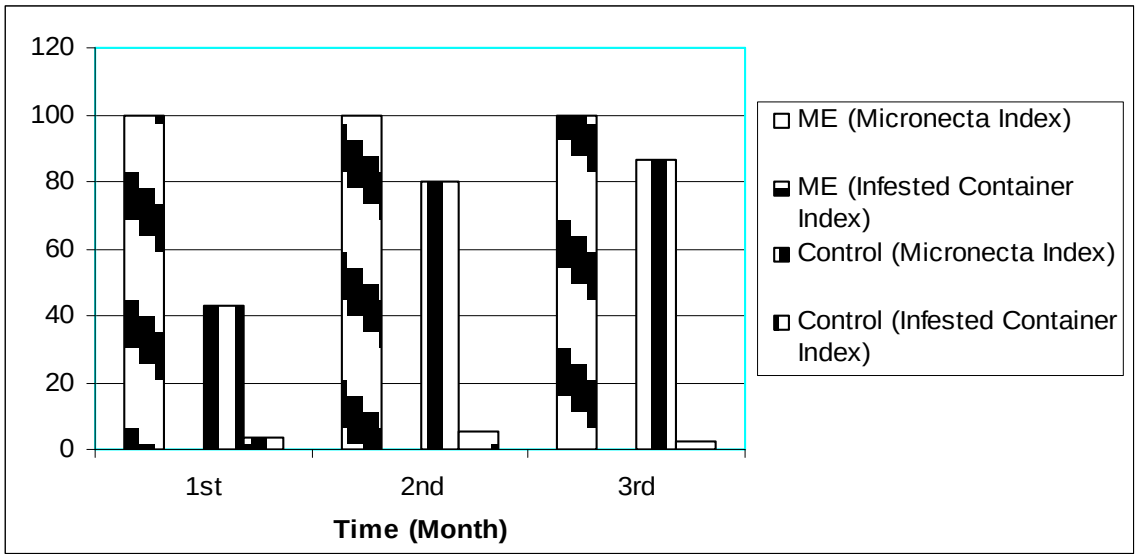

Figure 4.16 The efficacy of Micronectidae as biological control of mosquito using model ecosystem in Taman Tasek Putra (TTP)

Similar pattern to BU was observed in TTP. There was no infestation of mosquito in MEs which is indicated by zero infested Container Index. *MpN2000* also started a new population in controls. Micronecta Index for controls was increased every month with 42.7, 79.8 and 86.6 accordingly. The Container Index for the controls was very low as indicated by these Figures; 3.6, 5.6 and 2.5 respectively.

According to Surendran *et al.,* on 2007, seasonal changes give a lot of impact towards density of mosquito infestation. The infestation record in January 2009 shows the monthly variations. The Infested Container Index for controls increased in both housing areas (BU and TTP) on January 2009 (Figures 4.17 and 4.18). This phenomenon was believed to happen due to high rain fall level (more than 250 mm of rain fall) in January as shown in Table 4.4.

Table 4.4 Rain fall (mm) of the study area from December 2008 to February 2009 (MMD, 2009)

Month	Total Averages of Rain Fall (mm)
December 2008	≥200
January 2009	≥250
February 2009	≥150

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Figures 4.17 and 4.18 show the prey predator relationship between MI and ICI in Bandar Universiti and Taman Tasek Putra for three months.

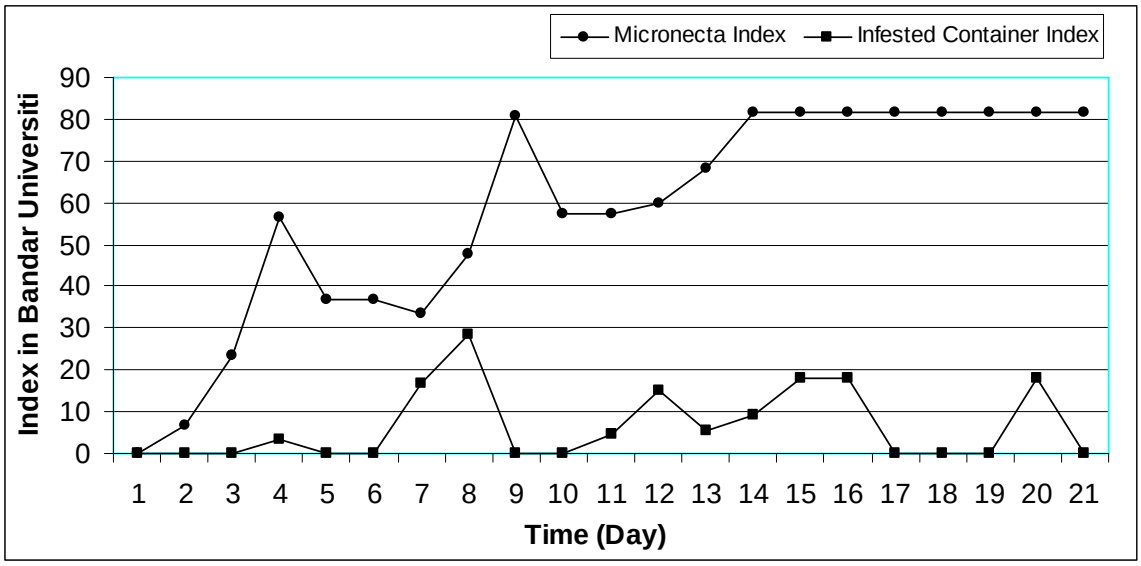

Figure 4.17 Micronecta Index and Infested Container Index in Bandar Universiti

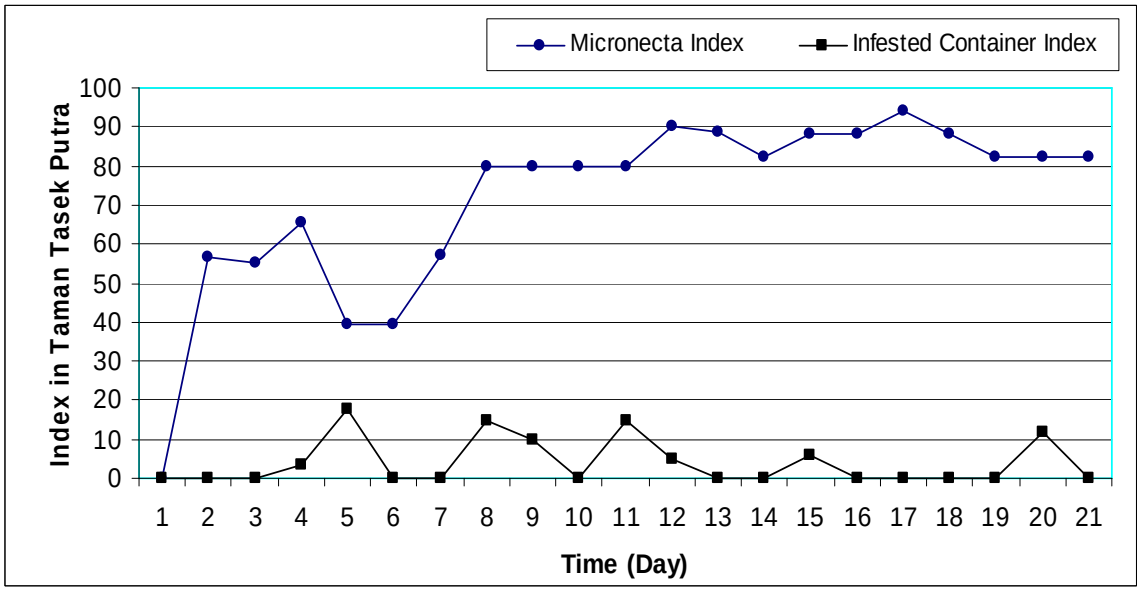

Figure 4.18 Micronecta Index and Infested Container Index in Taman Tasek Putra

Meanwhile, Micronecta Index for control was continuously increased every month with no influence of the rain level. *MpN2000* population was influenced by the presence of its source of food which can be observed through the comparison of Micronecta Index

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and Infested Container Index in BU and TTP. The predator and prey relationship base on Micronecta Index and Infested Container index in BU and TTP are shown in Figure 4.17 and Figure 4.18.

According to Figure 4.17 and Figure 4.18, predator populations (*MpN2000*) typically lag prey populations (Mosquito larvae) both when prey numbers were increased and decreased. Populations of predators responded to changing prey densities in several ways. *MpN2000* started to populate in container (controls) and increased their production of offsprings when the food sources (mosquito larvae) are more abundant. This numerical response explained why predator populations increased after growth in prey population occurred.

Analysis of Variance (Anova) is a procedure for hypothesis testing that compares the variability (Mean Squares: MS) between the samples to the variability within the samples by computing the ratio (Eq.4.1) (Larry, 2003);

$$F = \frac{Variability\ \ between\ \ the\ \ samples}{Variability\ \ \ within\ \ the\ \ samples} \qquad \text{Eq.4.1}$$

The F-statistic is a numerical measure of how much the sample means differ (Eq.8). If it becomes unusually large, H_0 will be rejected directly. The hypotheses for the analysis were H_0 and H_a. (Larry, 2003; Joseph, 2002; Wong & Phua, 2006; Arora *et al.*, 2006): In the analysis, the H_0 represents null hypothesis that showed that there is no statistically difference between MI and ICI. The H_a represents alternative hypothesis that showed that there is statistically difference between MI and ICI with 95% of confidence level.

The measure of variability in the numerator and denominator of the F-statistic are called mean squares (MS). They are extension of the sample variance, s^2, which measures the variability within single sample. In the case of multiple samples in the one-way

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analysis of variance, a reasonable combined measure of variation within the samples is a pooling of all the individual sample variances (Larry, 2003; Joseph, 2002; Wong & Phua, 2006; Arora *et al.*, 2006). For example, if 21 observations (Numbers of MEs or Controls) represents the sample variances (ICI or MI) and 21 are the associated sample sizes, then mean square for error (MSE or within sample variability) is a pooled measure of the variability within the sample (MI or ICI). The numerator of MSE is called the sum of squares (SSE) because it is a combined measure of errors within each sample. The denominator is the degrees of freedom associated with SSE.

To measure the variability between the samples, we simply need to calculate the variation across the sample means. If $mean^1$, $mean^2$….$mean^{21}$, are the sample means of the 21 samples and $mean^G$ is the overall sample mean, then ($mean^1$-$mean^G$)….($mean^k$-$mean^G$) are the 21 deviations of the sample means from their grand mean. Summing the squared deviations ad dividing by their degrees of freedom, 21-1, we obtain the between-sample variation, which is called the mean square treatments (MST or between-sample variability). The numerator of MST is called the sum of squares for treatment (SST) because the various samples arise from the different treatments in an experiment. The denominator is the degrees of freedom (df) associated with SS (Larry, 2003; Joseph, 2002; Wong & Phua, 2006; Arora *et al.*, 2006).

To prove the effectiveness of *MpN2000* in controlling mosquito breeding is by showing the relationship between Micronecta Index and Infested Container Index in both study areas (BU and TTP). The relationship between these two figures was computed and analyzed via statistical analyses program from Microsoft Excel software. The analysis was done using analyses of variance (ANOVA) as shown in Table 4.5 and Table 4.6. With reference to the ANOVA results in Table 4.5 and Table 4.6, the F-value (BU: 7.16×10^1 and TTP: 1.67×10^2) was greater than the F-critical (BU: 4.08 and TTP: 4.08), and the p-value (BU: 1.88×10^{-10} and TTP: 7.08×10^{-16}) was smaller than predetermined α of 0.05. The conclusion was to reject the null hypothesis and accept the alternative hypothesis. The associated risk in doing so is much less than the predetermined risk of 5% (the p-value is much lower than 0.05).

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Table 4.5 and Table 4.6 show the statistical analyses of ICI and MI in Bandar Universiti and Taman Tasek Putra accordingly.

Table 4.5 Statistical analyses of Infested Container Index (ICI) and Micronecta Index (MI) in Bandar Universiti (BU)

Groups	Count	Sum	Average	Variance
MI_BU	21	1.22×10^3	5.80×10^1	7.00×10^2
ICI_BU	21	1.37×10^2	6.53	7.79×10^1

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between MI_BU & ICI_BU	2.79×10^4	1	2.79×10^4	7.16×10^1	1.88×10^{-10}	4.08
Within MI_BU & ICI_BU	1.56×10^4	40	3.9×10^2			
Total	43438.55	41				

Table 4.6 Statistical analyses of Infested Container Index (ICI) and Micronecta Index (MI) in Taman Tasek Putra (TTP)

Groups	Count	Sum	Average	Variance
MI_TTP	21	1.5×10^3	7.10×10^1	5.33×10^2
ICI_TTP	21	8.3×10^1	3.90	3.70×10^1

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between MI_TTP & ICI_TTP	4.78×10^4	1	4.78×10^4	1.67×10^2	7.08×10^{-16}	4.08
Within MI_TTP & ICI_TTP	1.14×10^4	40	2.85×10^2			
Total	59172.72	41				

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There was a statistically difference in the average amount of Infested Container Indices to Micronecta Indices obtained from BU and TTP with 95% confidence level. Thus, the total infestation of mosquito in stagnant water was affected by the densities of *MpN2000* as its predators. Thus, *MpN2000* is a good biological control of mosquito. It can control mosquito breeding problem particularly *Aedes* mosquito in stagnant clear water in urban areas.

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