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# Feeding Ability of *Micronecta grisea* Nymphal Instars and Adults on Third Instar *Aedes aegypti* Larvae

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**ABSTRACT.– Pygmy waterboatmen, *Micronecta grisea*, were collected and used to establish laboratory cultures in order to study the predation rates and feeding behavior of nymphal instars (N) and adults upon third instar larvae (L3) of *Aedes aegypti* to assess their potential for biological control. The body length, head capsule size and head length of 330 nymphs and 71 adults of *M. grisea*, collected from Nonthaburi Province, Thailand, were measured using a stereo microscope. In contrast to head capsule size and head length, which yielded overlapping size distributions, five discrete nymphal instars (N1 – N5) plus adults could be classified by body length; the 1st (N1; 0.54 – 0.65 mm), 2nd (N2; 0.69 – 0.84 mm), 3rd (N3; 0.9 – 1.11 mm), 4th (N4; 1.29 – 1.56 mm) and 5th (N5; 1.74 – 1.98 mm) nymphal instars plus adults (2.07 – 2.43 mm). Using body length to define developmental stadia, nymphs were classified as three discrete size categories, small (N1 & N2), medium (N3 & N4) and large (N5), and together with adults these four clases were examined for predation rates and prey handling times when fed L3 *Ae. aegypti* at different predator to prey ratios. Prey searching and handling times decreased with increasing *M. grisea* size (developmental stadia), and were consistent with a Type II functional predator-prey response.**

**KEY WORDS:** *Micronecta grisea, Aedes aegypti,* nymphal instars, Thailand

## INTRODUCTION

*Aedes aegypti* L. (Diptera: Culicidae), the yellow fever or dengue mosquito, is the principal vector for transmission of the dengue flavivirus in humans across the tropics and subtropical regions, including within Thailand, as well as for yellow virus in endemic regions. Infection with one of the dengue virus strains can cause Dengue or the more serious Dengue Hemorrhagic Fever (DHF), and has been estimated to cause an average morbidity in some 40 million and 2-300,000 people annually worldwide, respectively, and some 30,000 cases a year in

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Thailand, although regional epidemics can be large. Urban *Ae. aegypti* populations are well adapted to human (their principal blood host) environments and breed in both indoor and outdoor water-storage containers, as well as a diverse array of other suitable temporal sources of relatively stagnant water, including empty coconut shells, flag pole holders, gutters, plant pot trays, refuse bins, etc. This makes total larval elimination or control unrealistic and restricts these measures to the control of the main breeding sites (human water storage containers) and attempted reduction of the minor sites so as to reduce adult mosquito numbers in the proximity of human habitations. To this end, larval control programs in Thailand are traditionally performed using a mixture of three imperfect approaches, that is physical (water container covers, upturning to drain dustbins, prevention of blockage of gutters and similar drainage channels to reduce pooled rainwater), chemical (e.g. use of the insect growth regulator pyriproxyfen) and biological (e.g. use of bacterial toxins such as *Bacillus thuringensis israelensis* or predators such as *Mesocyclops* copepods) control methods (Vu et al., 1998). However, the choice(s) available are somewhat restricted by whether the water is intended for human consumption or not and the ability to locate, and so treat, all such temporally dynamic potential breeding sites for *Ae. aegypti*. Thus, for example household water jars may be easily covered (physical) but external natural and man made water sources cannot all be located let alone be covered, whilst chemical control suffers from the same problem of the inability to locate and treat all breeding sites, increasing insecticide and larvicide resistance, and the fact that the larval rearing sites are in close proximity to humans, such as in the house or even drinking water reservoirs, and so is restricted by the risks of long term human exposure in addition to environmental and economic concerns. There is thus an increasing demand for alternative treatments, including environmentally acceptable biocontrol based methods, for the treatment of non-household water container based breeding grounds. Many biological control methods are, however, not mobile and so again are limited by the problem of locating and treating all the temporally dynamic breeding grounds, with loss of the control agent as a given site dries up or the mosquito moves away as a pharate adult. Thus mobile, prey seeking (or imago phoretic, such as, perhaps, microsporidia) biological agents would potentially offer an alternative weapon, being able to also control neighboring unlocated breeding sites and also new larval rearing sites as they become available.

*Micronecta grisea* Fieber (Heteroptera: Micronectidae), the pygmy waterboatmen, are true aquatic hemipteran insects with paddle-like legs that have all their developmental stages in water. They are excellent and active swimmers both on the surface and under water, feeding on insect larvae, including mosquito larvae, by capturing the prey in the water and then sucking out the haemolymph from the prey body. Combined with their widespread distribution from India and Sri Lanka to Taiwan and Indonesia and Java (Nieser & Chen, 1999), their relatively small size and water size requirements, mobility between water sites, their natural habitat being stagnant water or parts of streams with little current flow, broadly the same as *Aedes aegypti* larvae, and that they are potential predators of *Ae. aegypti* larvae (Lekprayoon, 2006), makes them potential alternative biocontrol agents in mosquito control programs.

A survey on the distribution of *Micronecta* spp. in all regions of Thailand, including

inspections of water-storage jars which were breeding sites of *Ae. aegypti*, reported that the presence of *Micronecta* spp. was found to be 100, 89, 62 and 25 % of the outdoor jars in the central and eastern, north-eastern, northern and southern parts of Thailand, respectively (Suphapathom et al., 2002).

As *Micronecta* spp., including *M. grisea*, are common species found throughout the country, this study was carried out to examine the body length, head capsule size and head length of *M. grisea* nymphal instars and adults, together with their predation efficiency and feeding ability upon L3 *Ae. aegypti* larvae as a potential biocontrol agent.

## MATERIALS AND METHODS

### *Micronecta grisea*

*M. grisea* were collected from water-storage clay jars at the Research Station for Mosquito Biology and Control, Department of Medical Sciences, Ministry of Public Health, Bang Bua Thong District, Nonthaburi Province, Thailand. All samples were brought to, and used to establish laboratory cultures at, the Department of Biology, Faculty of Science, Chulalongkorn University. Laboratory cultures and experiments were performed at 25 ± 1 °C and an L: D period of 12:12. Five adult female and male *M. grisea* were kept in a 1.2 l clay jar for stock cultures and fed on *Ae. aegypti* larvae *ad libitum*. All clay jars used were covered with nylon meshes (1 mm mesh size).

### *Aedes aegypti* larvae

Freshly laid *Ae. aegypti* eggs, attached on filter paper and dry, were obtained from the laboratory cultures of the Department of Medical Sciences, Ministry of Public Health, and were reared in 2 l plastic rectangular containers in the laboratory at Chulalongkorn University to obtain late L3 *Ae. aegypti* for use in the experiments. Excess larvae were not reared further but killed and in-house *Ae. aegypti* cultures were not established.

Tap water, for rearing all stadia of *M. grisea* and *Ae. aegypti* larvae, was dechlorinated by leaving to air in containers for 24 h. For *Ae. aegypti*, the dried eggs on filter paper were added to this water whereupon they hatched within 30 minutes and the larvae were fed daily with crushed "Pedigree" dog food at 0.1 g of food per 2 l of water containing 200 larvae, and maintained at 25 ± 1 °C and an L: D period of 12:12. After 3 – 4 days under these conditions, larvae molted to the L3 stadia, and when observed under a stereo microscope were within the size range of 6 – 8 mm long, which were then selected and used in the experiments.

### *M. grisea nymphal* instars and adult

Measurements of the body length, head capsule size and head length were made on individual *M. grisea* nymphs and adults using a stereo microscope (32 x). *M. grisea* were put individually on the petri dish by using a dropper. After that, tissue paper was used to dry up the water surrounding the insect body. The three characters were measured once for each individual by a stage micrometer attached under the microscope. To determine the size range of each of the *M. grisea* nymphal instars, as well as adults, the data for the three measured characters for the nymphal instars and adults were analyzed by scatter graph plots. The individuals were then ascribed to a given nymphal instar or adult category based upon the size distribution scatter plot analysis (see results) and then each category was reanalyzed for size range and variation within and between each defined nymphal instar and adults. The nymphal instars were grouped into three

sizes; small (N1 & N2), medium (N3 & N4) and large (N5) for evaluation of their predation and feeding efficiencies and handling time of the different developmental stages of *M. grisea* at different predator (*M. grisea*) and prey (L3 *Ae. aegypti*) densities.

***Mosquito larvae* consumption**

Feeding tests were conducted to determine the ability of the three nymphal size categories, plus adults, of *M. grisea* to feed on L3 *Ae. aegypti* as determined by predation efficiency and handling times.

To broadly standardize the hunger level of *M. grisea* (predator), and thus the potential hunting desire and, when prey is not limiting, the total consumption rate, the three size categories of *M. grisea* nymphs (small, medium and large) and adults were selected randomly from stock cultures and kept separately without food for the same period of time, that is for 24 h prior to experimentation. *M. grisea* were housed at three different densities, *viz.* 5, 10 and 20 nymphs or adults in 1.2 l clay jars filled with 1 l of dechlorinated tap water. After that, third instar *Ae. aegypti* larvae at one of three different densities, *viz.* 10, 20 and 40 larvae, were put into each clay jar containing the *M. grisea* at different densities to start the tests. After 24 h the number of living larvae and the cadaver remains in the jars was recorded. In all experiments three replicates were performed for each combination.

The mortality numbers were adjusted by Abbott's formula (Abbott, 1925), and then used to calculate the percentage mortality of mosquito larvae in all experiments, and as a measure of predation levels. Plots of prey density against prey attacked were plotted to determine if the predator-prey relationship best fitted a type I or type II functional response.

**Statistical analyses**

Data for the mean sizes of the three measured parameters for nymphal instars and adults were subject to ANOVA and Duncan's multiple means tests with significant differences accepted at the $P \leq 0.05$ level. For evaluation of the predation levels, the data obtained from all experiments were calculated as the mean percentage mortality from all replicates and then the percentage means of *Ae. aegypti* L3 mortality induced by each *M. grisea* category (nymphal instar and adults), as a measure of predation efficiency, was analyzed by Mann –Whitney U-test to compare predator efficiency within 24 h.

**Feeding behavior of *M. grisea***

During the feeding tests with L3 *Ae. aegypti* mosquito larvae as the prey, the searching and handling times of individual *M. grisea* waterboatmen nymphs and adults were recorded separately using a timer for each of the four size categories. Searching time started from the time when the prey (L3 *Ae. aegypti*) were put into containers until the time that the first prey was captured by a predator. The handling time started from the time the prey were captured until the time that predators released their prey or prey remnants, and included capturing, killing, eating and digesting (Holling, 1959).

The feeding time of *M. grisea* nymphs and adults were calculated by summation of both the searching and handling times.

**TABLE 1.** The range and mean (± S.E.) body length, head capsule size and head length of each developmental stage of *M. grisea*.

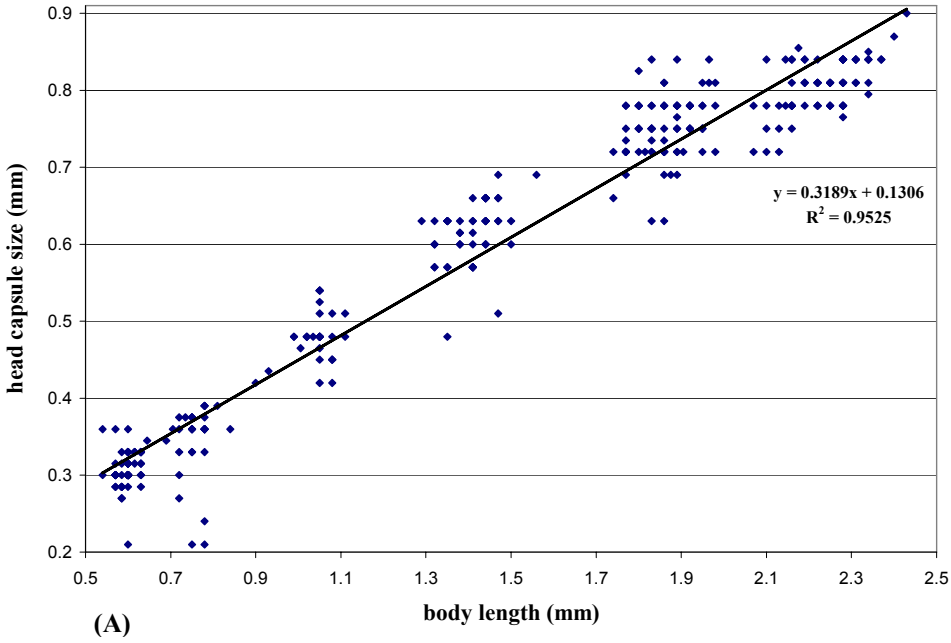
| | N | Body Length (mm) | | | Head Capsule (mm) | | | Head Length (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MIN | MAX | MEAN±SE | MIN | MAX | MEAN±SE | MIN | MAX | MEAN±SE |
| 1st nymphal instar | 73 | 0.54 | 0.65 | 0.60±0.002a | 0.21 | 0.36 | 0.31±0.003a | 0.06 | 0.15 | 0.11±0.002a |
| 2nd nymphal instar | 34 | 0.69 | 0.84 | 0.76±0.005b | 0.21 | 0.40 | 0.35±0.008b | 0.09 | 0.18 | 0.13±0.003b |
| 3rd nymphal instar | 63 | 0.90 | 1.11 | 1.03±0.005c | 0.42 | 0.54 | 0.48±0.003c | 0.12 | 0.23 | 0.17±0.003c |
| 4th nymphal instar | 70 | 1.29 | 1.56 | 1.40±0.006d | 0.48 | 0.69 | 0.61±0.005d | 0.12 | 0.27 | 0.22±0.004d |
| 5th nymphal instar | 90 | 1.74 | 1.98 | 1.86±0.007e | 0.63 | 0.84 | 0.75±0.004e | 0.18 | 0.38 | 0.28±0.003e |
| Adult | 71 | 2.07 | 2.43 | 2.23±0.010f | 0.72 | 0.90 | 0.81±0.004f | 0.21 | 0.39 | 0.28±0.005e |

* Means in the same column with different letters are significantly different ($P \leq 0.05$; One-way ANOVA)

**TABLE 2.** Mean (± 1 S.E.) mortality (%) of third instar *Ae. aegypti* mosquito larvae observed within 24 hours using different instars and densities of *M. grisea* (M*; predator) with different densities of *Ae. aegypti* larvae (A*; prey).

| M* | S | | | M | | | L | | | ADULT | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A* | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 | 5 | 10 | 20 |
| 10 | 0a[1*] | 0a[1] | 0a[1] | 33.3 ± 8.8a[1] | 40 ± 5.8a[1] | 46.7 ± 3.3a[1] | 50 ± 5.8a[1] | 80 ± 5.78a[2] | 80 ± 5.8a[2] | 96.7 ± 3.3a[1] | 100 ± 0.0a[1] | 100 ± 0.0a[1] |
| 20 | 0a[1] | 0a[1] | 0a[1] | 31.7 ± 7.3a[1] | 30 ± 8.ab[1] | 43.3 ± 4.4a[1] | 45 ± 2.9a[1] | 71.7 ± 4.4a[2] | 80 ± 5.8a[2] | 95 ± 5.0a[1] | 100 ± 0.0a[1] | 100 ± 0.0a[1] |
| 40 | 0a[1] | 0a[1] | 0a[1] | 19.2 ± 4.2a[1] | 25.8 ± 1.7b[1] | 35.8 ± 5.1a[1] | 28.3 ± 0.8b[1] | 50.8 ± 0.8b[2] | 69.2 ± 6.0a[3] | 95.8 ± 3.0a[1] | 95.8 ± 2.2a[1] | 100 ± 0.0a[1] |

* Means in the same column with different letters, or across rows (within the same size of predator) with different superscript numbers are significantly different($P \leq 0.05$; *Mann-Whitney U*-test).



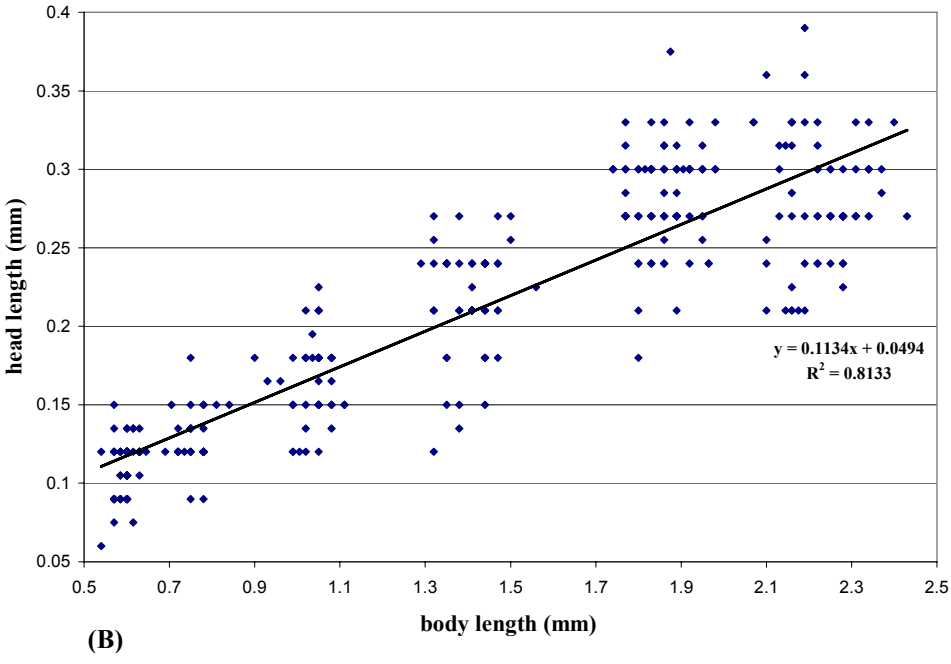




**FIGURE 1**. The (A) body length and head capsule size (mm) and (B) body and head lengths (mm) of *M. grisea*. Data are shown for 401 individuals of mixed developmental stadia.

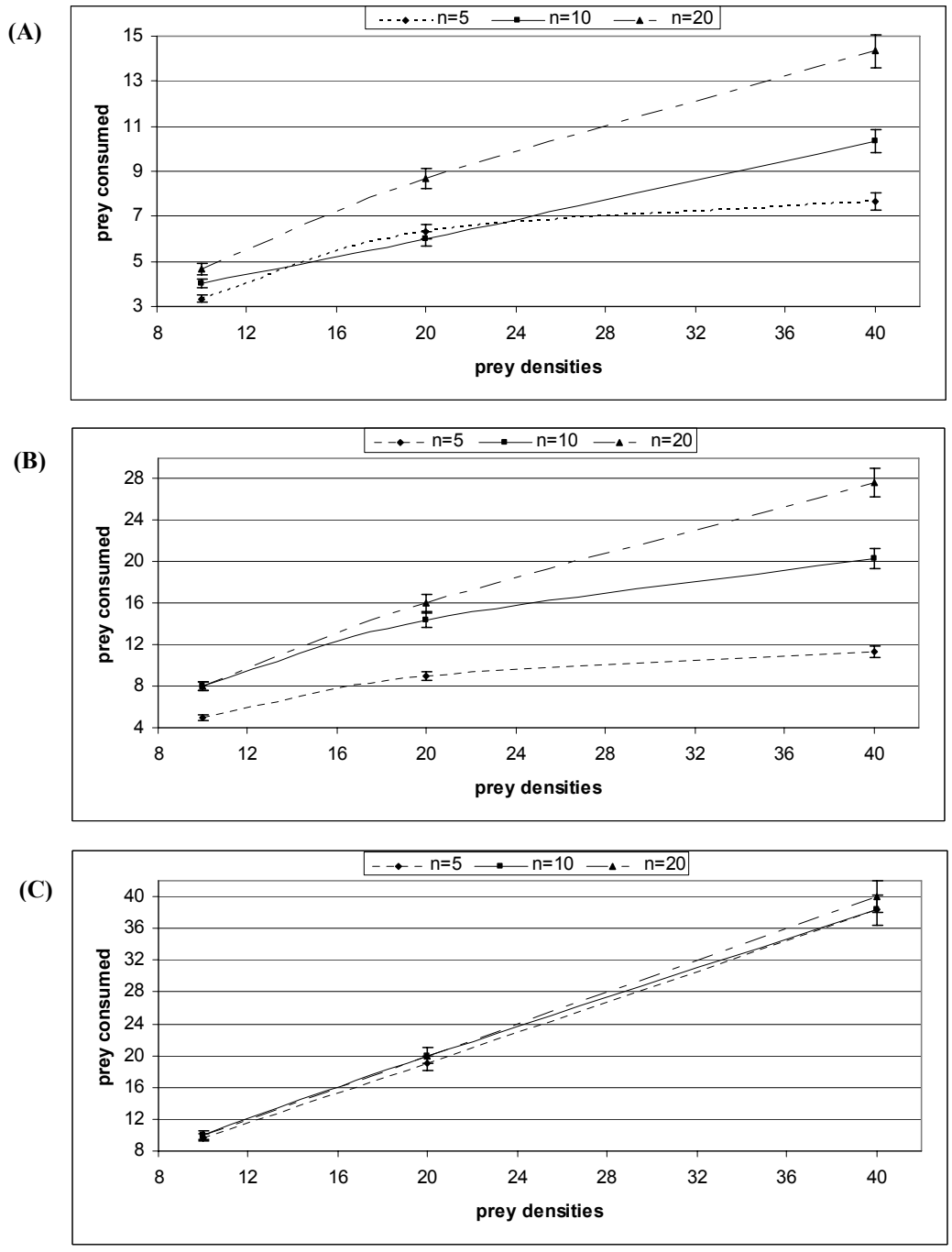



**FIGURE 2**. Type II functional response of the predator (*M. grisea*) – prey (L3 *Ae. aegypti*) relationship at different predator densities (n). Data, shown as the mean ± S.E. and derived from 3 repeat trials, are shown for (A) medium nymphs, (B) large nymphs and (C) adults of *M. grisea*.

## RESULTS

Measurements of the body length, head capsule size and head length of 401 *M. grisea* specimens of mixed developmental stadia revealed six discrete developmental stages of *M. grisea*, comprised of five nymphal instars (N1–N5) and the adults, were recognized from the three measured characters when the data was analyzed by scatter graphs, as shown in Figs. 1A and 1B with $r^2 = 0.95$ and 0.81, respectively. No overlap in the body length distribution was found between each nymphal instar of the examined pygmy waterboatmen, whereas some overlap in the head capsule size and head length size distributions was noted between nymphal instars and also adults.

The normality test and homogeneity test showed that the data were normally distributed and the variances were significantly homogenous at $P \leq 0.05$. A one-way analysis of variance (ANOVA) with Duncan's multiple comparison tests on the data for the three different measurements for each instar of the pygmy waterboatmen showed a significant difference in the mean body length between each instar (df = 5, 395, F = 9383.19, $P = 0.000$), but no significant difference was found within each instar of pygmy waterboatmen. With discrete non-overlapping distributions between the five apparent nymphal instars plus the adults, the body lengths could be and were subsequently used for classifying these six discrete developmental stages of *M. grisea*, and as such split the 401 specimens into 73, 34, 63, 70, 90 and 71 individual N1, N2, N3, N4, N5 and adults of *M. grisea*, respectively. Analysis of the body length, head capsule size and head length within these six categories (developmental stadia) revealed that each nymphal instar and the adults were significantly different ($P \leq 0.05$), except for head lengths between the N5 and adults (Table 1).

Using the discrete body size distributions as markers for the different developmental stages of *M. grisea*, the nymphal instars were grouped into small (N1 & N2), medium (N3 & N4) and large (N5) size groups for the predatory feeding tests with L3 *Ae. aegypti*. The feeding tests were performed with four size categories of *M. grisea* at three different densities (5, 10 and 20 per l container), each of which was supplied with live L3 *Ae. aegypti* as prey at three different densities (10, 20 and 40 larvae per l). The mean *Ae. aegypti* larval mortality within 24 h, used as the marker for predation rate, was evaluated and the data is summarized in Table 2. Analyses of the predator-prey relationship by plots of prey density against prey attacked were consistent with a Type II functional response (Fig. 2), and were tested using the Holling's disc equation. Predator satiation occurred to a slight extent in the M and L nymphs' functional response curves, but not in the adult's curve. The proportion of prey consumed by predators declined with increasing prey densities. Functional response curves revealed that the decreasing rate, as for five medium nymphs, five large nymphs and ten large nymphs, was due to prey saturation of the pygmy waterboatmen, while the constant consuming rate showed unsaturated conditions, higher prey densities were still available for predators to consume. The adult, medium and large nymphal categories predated the L3 *Ae. aegypti* in all experiments, whilst in contrast the small nymphs showed no evidence of predation upon L3 *Ae. aegypti* in all tests.

The normality test showed that the data were not normally distributed and the homogeneity test also showed the variances

to differ significantly at $P \leq 0.05$. Using *Mann-Whitney U*-test, the results revealed that as the prey numbers (and thus the prey: predator ratio) were increased no significant difference in the predation level (larval mortality) was observed for *M. grisea* of the same developmental stage at the same density ($P \leq 0.05$). Comparisons of larval mortality, as a marker for predation levels, amongst the different nymphal stages of *M. grisea* at different predator densities showed that only the large nymphal size category consumption caused a significantly different mortality amongst the different predator densities (Table 2).

For a density of five *M. grisea* with 10, 20 and 40 prey (L3 *Ae. aegypti*), the consumption by medium and large nymphs were numerically, but not statistically significantly, different, whereas adults, caused a significantly higher predation level than both medium and large nymphs at all prey densities (Fig. 3).

When the *M. grisea* density increased to 10 there were significant differences amongst the predation levels (mortality of L3 *Ae. aegypti*) between each size category of nymphal instars and adults of pygmy waterboatmen, with the predation levels increasing with increasing predator size (developmental stage).

**TABLE 3.** Mean (± S.E.) searching, handling and feeding times of medium (M) and large (L) size category nymphs and adults of *M. grisea* upon third instar *Ae. aegypti* larvae (One-way ANOVA) ($P \leq 0.05$)

| ***M. grisea* stage** | **No. of *M. grisea* : No. of mosquito larvae** | **Searching time (s)** | **Handling time (s)** | **Feeding time (s)** |
|---|---|---|---|---|
| **M-size** | 10 : 20 | 92.8 ± 13.1a* | 1680 ± 312a | 1773 ± 314a |
| **L-size** | 10 : 20 | 57 ± 10.1b | 867 ± 182b | 924 ± 185b |
| **Adult** | 10 : 20 | 13.8 ± 1.0c | 480 ± 42b | 494 ± 43b |

* Means in the same column with the different letters are significantly different ($P \leq 0.05$; One-way ANOVA).

At predator densities of 20, the prey consumption of medium sized nymphs was significantly lower than that of large nymphs, with that of adults being significantly higher still.

The laboratory no-choice feeding tests with M. grisea (predator) feeding on L3 Ae. aegypti (prey) revealed that at a predator: prey ratio of 10:20 the searching and handling times of predators were significantly different between the medium and large nymphal stages and the adults (Table 3), with adult and medium sized nymphal M. grisea having the shortest and longest, respectively, searching and feeding times. Therefore, with feeding time being simply the summation of searching and handling times, then the same trend was noted with adults having the shortest feeding time and medium stage nymphs the longest. Note, however, that no data on searching, handling and feeding times is presented for the small M. grisea nymphs since no prey were ever caught or killed.

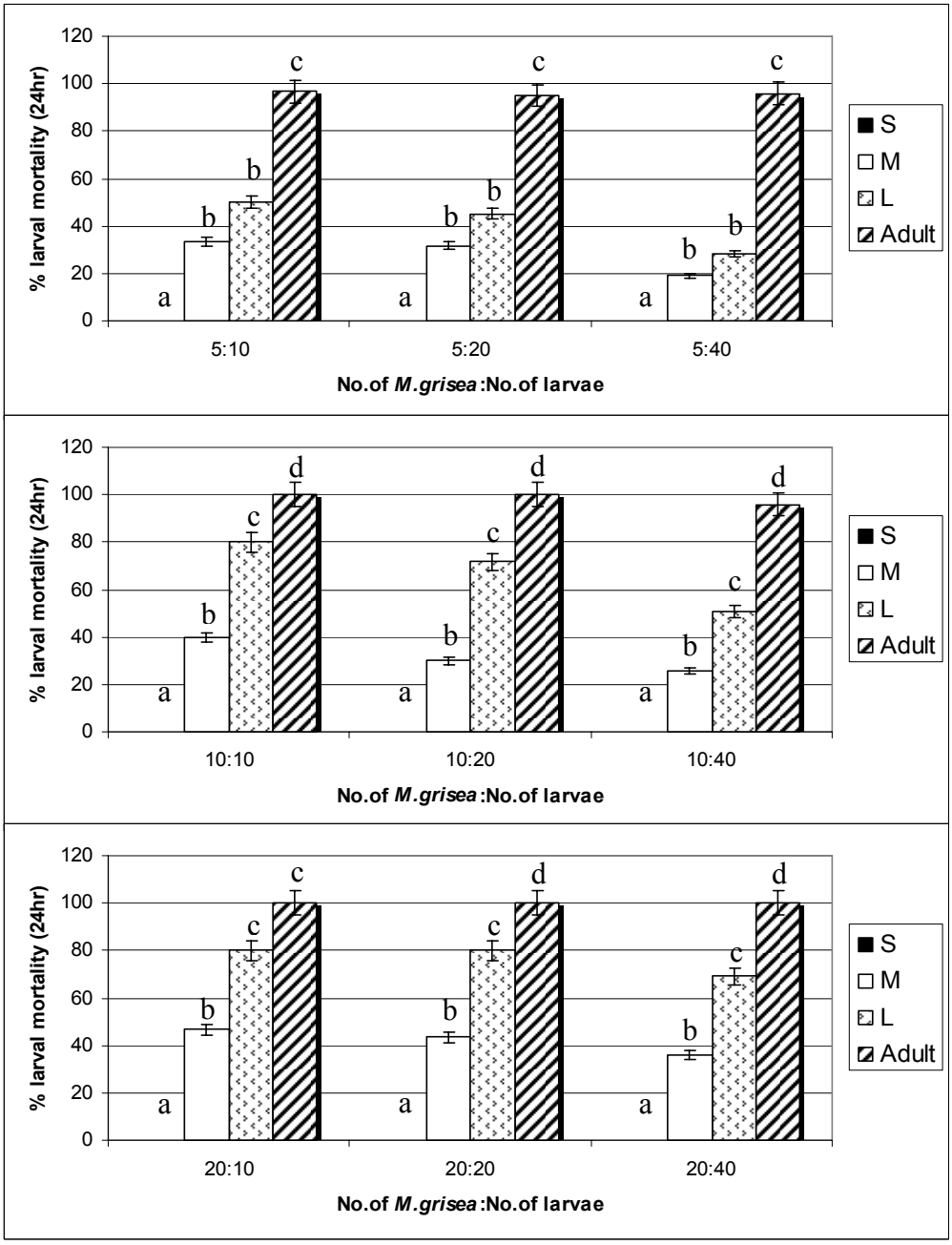



**FIGURE 3**. Mean mortality (predation) of third instar *Ae. aegypti* larvae by different instars of *M. grisea* at different densities (5, 10 and 20) with different densities of mosquito larvae (10, 20 and 40). Data are shown as the mean $\pm$ S.E. and derived from 3 repeats. Means with a different letter are significantly different ($P<0.05$; Mann-Whitney U tests)

## DISCUSSION

Measurement of the body and head lengths and the head capsule size of different nymphal instars and adults of the pygmy waterboatman, *M. grisea*, revealed that five discrete nymphal instars, plus the adult stage, could be distinguished. Although no allometry was found between the three characters measured (analysis not shown), the body length was found to have a discrete and well separated distribution pattern between these six developmental stages, whereas both the head capsule size and head length showed some overlap in their size distribution profiles between related developmental stadia. This notion of five nymphal instars is consistent with studies in the biology of the related waterboatmen (Gerridae) and the backswimmers, *Enithares* sp., which have also been reported to have five preadult nymphal instars in their development (Chittihunsa, 1980).

The body lengths of each instar, showing no overlap, were used for classifying each *M. grisea* instar. The non-overlapping discrete body length ranges were 0.54 – 0.65, 0.69 – 0.84, 0.9 – 1.11, 1.29 – 1.56 and 1.74 – 1.98 mm for the $1^{st}$, $2^{nd}$, $3^{rd}$, $4^{th}$ and $5^{th}$ (N1 – N5) nymphal instars, respectively, and 2.07 – 2.43 mm for adults. Although the adult size range is lower than that previously reported from the study of *M. grisea* in Singapore and Peninsular Malaysia, such as 2.6 – 3.2 mm (Nieser, 2002), it is in agreement with the results of a recent survey on the distribution of *Micronecta* spp. across all the regions of Thailand which found that adult *Micronecta* spp. were approximately 2 – 3.5 mm long (Suphapathom et al, 2002). This may be due to environmental conditions and diet sufficiency at different distribution regions.

In this study differences in the body length, head capsule size and head length (with no evidence of allometry between these three parameters) of each nymphal and adult stage were noted, except for the head lengths between the N5 and adult stages which were approximately the same. This may be due to the fact that the anterior part of the head segment in adults was hidden underneath during measurement leading to variable underestimates in their size.

Within the 24 h feeding window, the predation and feeding ability of the pygmy waterboatmen (*M. grisea)* on L3 *Ae. aegypti* differed amongst each size category. Adult waterboatmen showed the highest predation rates (as *Ae. aegypti* L3 mortality) followed by large, medium and small nymphs, in that order, with small nymphs revealing no detectable predation of L3 *Ae. aegypti* in all tests. Consistent with this observation is that under laboratory conditions larger sized backswimmers (*Enithares* sp.) caused a higher larval mortality (predation rate) than those of a smaller size (Chittihunsa, 1980). For the small sized nymphs of *M. grisea* in this study it seems likely that they were too small, compared with the prey size available (L3 *Ae. aegypti*) in these no-choice experiments, to capture their prey, whilst the newly hatched instar nymphs would also still have nutrients from the yolk available to them.

*M. grisea* of the same size categories (small, medium and large nymphal and adults) and at the same densities caused no significant difference in the larval mortality percentages (predation levels). Rather, within each size category *M. grisea* (predators) when at the same predator number (density), they consumed about the same amount of L3 *Ae. aegypti* prey items even when at higher prey densities, and so higher prey: predator ratios, suggesting that prey saturation may influence predator consumption. Of course, as the prey densities are increased for a given number of predators, and the total numbers

of prey consumed remain the same (satiation), the evaluated prey mortality percentages would appear to decrease.

Although in general, predators may spend time on three types of activities, searching, handling their prey and then satiation related activities, we observed the former two in these assays. At the same prey density, the searching times of adults, large and medium sized nymphs of *M. grisea* were significantly different. Adults showed the shortest searching time followed by large and finally medium sized nymphs, respectively. The handling time showed a similar relationship, except that the handling time for large nymphs and adults were not significantly different. Consequentially, feeding time paralleled the predator handling time.

Overall, adult *M. grisea* provided the highest L3 *Ae. aegypti* mortality in all tests with the shortest feeding times. This may be due to adults being more active and larger than nymphs, but it may also reflect the relatively large prey size used. Thus, whilst it remains important to evaluate the predation efficiency and feeding times of all developmental stages of *M. grisea* upon all larval developmental stages of *Ae. aegypti*, it also remains of interest to evaluate if different developmental stages of *M. grisea* preferentially feed upon different larval developmental stages of *Ae. aegypti,* as well as other prey items, since *Ae. aegypti* development in urban water resources is frequently derived from multiple females and is asynchronous.

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