

Oviposition Habitat Selection of Dengue Vectors, *Aedes aegypti* and *Aedes albopictus* in Response to Fish Predator

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Abstract: To understand the effects of fish predator's kairomones on *Aedes* mosquitoes' oviposition, we established an experiment using gravid *Aedes* females. Kairomones concentrations were established using *Hampala macrolepidota*. One individual fish was placed inside containers with varying water levels (1 L, 5 L, and 10 L of water). The fish were kept in the containers for 24 hours and were removed immediately at the start of each trial in order to have the kairomones remnants. Twenty gravid adult females of *Aedes aegypti* and *Aedes albopictus* were allowed to lay eggs on oviposition site with various treatments: (1) control without any kairomones; (2) kairomone remnant in 1 L of water; (3) kairomone remnant in 5 L of water; and (4) kairomone remnant in 10 L of water. There are significant differences between the numbers of eggs laid by both *Aedes* species for each different treatment ($F = 9.131$, $df = 16$, $p < 0.001$). However, fewer eggs were laid by *Ae. albopictus* compared to *Ae. aegypti* in the presence of kairomone remnants. This suggested that *Ae. albopictus* are significantly affected by the kairomones itself and have ability to detect the residual kairomone presence from *H. macrolepidota*.

Keywords: *Aedes albopictus*, *Hampala macrolepidota*, Kairomone, Mosquito, Predator

INTRODUCTION

Regardless of the control efforts done by various parties, mosquitoes (Diptera: Culicidae) still plays a major role in transmitting vector borne diseases in many parts of the world, with over 1 million cases of death in children annually involving malaria (World Health Organization 1999; Breman 2001) and the outbreak of dengue fever and dengue haemorrhagic fever worldwide due to spreading of vector *Aedes aegypti* and *Aedes albopictus* with recently estimate of 390 million

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dengue infections per year (Bhatt *et al.* 2013). Reckless and unmanaged practice of chemical insecticide in pest management sector has given rise to resistance problems in insect (Rafikov *et al.* 2009; Wijesinghea *et al.* 2009; Nyamah *et al.* 2011). Studies showed that in Malaysia, *Ae. aegypti* has developed temephos resistance (Lee 1991), while *Ae. albopictus* was found to be resistant towards DDT and permethrin (Wesson 1990). Since chemical insecticidal approach causes much worry over increasing resistant traits in mosquitoes, not to mention the impact they posed on the environment, biological control has received much attention and interest as an alternative management action on mosquito borne diseases' vector (Collins & Blackwell 2000; Focks 2007; Wijesinghea *et al.* 2009; Nyamah *et al.* 2011). Fish predator especially *Gambusia* species has been one of the interest in biological of mosquitoes and identified as significant predator with wide range of habitat (Griffin 2014). Here, we tested the effectiveness of kairomones remnant from Malaysia endemic predatory fish, *Hampala macrolepidota* on oviposition site choose by two species of dengue and dengue hemorrhagic fever vector; *Ae. albopictus* and *Ae. aegypti*.

MATERIAL AND METHODS

A series of experiments to investigate the effects of *H. macrolepidota* predatory fish kairomone on *Aedes*' oviposition was conducted under field condition at School of Biological Sciences, Universiti Sains Malaysia with temperature of $30\pm 2^{\circ}\text{C}$, $65\pm 10\%$ RH and 12L:12D. A total of 20 full gravid females of *Ae. albopictus* and *Ae. aegypti*, aged 2–3 days old were placed separately in mosquito cages sized 30 x 30 x 30 cm. Two days after emergence, these mosquitoes were provided blood meal from white mice prior to dusk. Oviposition medium which was made of a cone-shaped filter paper fixed in Petri dish was utilised in this experiment as oviposition sites for mosquitoes. Treatments were established as follows with discrepant kairomones concentration: (1) 1 L; (2) 5 L; (3) 10 L; (4) without kairomones (only seasoned water) served as control. Kairomone were established by accommodating one predatory *Hampala* fish into different water levels of 1 L, 5 L, 10 L, and 10 L for 24 h prior to experiment in order to have different kairomones concentrations. Approximately of 10 mL of kairomones remnant from *Hampala* fish was used to moisten the oviposition medium for everyday throughout this study. A total 5 mL of kairomones was added in the morning (0900) and evening (1800). The oviposition medium was observed daily for the presence of eggs deposited and replaced with a new one until no more eggs were laid by *Aedes* mosquitoes. The number of eggs deposited will be counted using light microscope and recorded. These studies were replicated three times for each replicate. Data were tested for normality using a one-sample Kolmogorov-Smirnov test. All data were log transformed prior to analysis to satisfy the assumptions of ANOVA. Data were then analysed using Repeated Measure General Linear Model analysis, which within subject factors types of treatment (1 L, 5 L, 10 L, and control), mosquito species (*Ae. albopictus* and *Ae. aegypti*) and oviposition day (day 1 and day 2).

RESULTS

There were significant differences on the number of eggs oviposited by both adult females *Ae. albopictus* and *Ae. aegypti* ($F = 78.35$, $df = 1$, $p = 0.000$; Table 1). Similarly, significant differences were also detected in all treatment tested for both species ($F = 9.13$, $df = 3$, $p = 0.000$). Thus, female *Ae. albopictus* and *Ae. aegypti* appeared to choose non-treated oviposition site when laid their eggs and avoided oviposition sites with *H. macrolepidota*'s kairomones in it due to sense of predator. Other than that, it seemed that *Ae. aegypti* delayed to oviposit their eggs until day 2 in the presence of kairomone remnant from *Hampala* fish (Fig. 1), whereas *Ae. albopictus* completed their oviposition on day 1. In 10 L of kairomone, *Ae. aegypti* continued to lay significantly more eggs on day 2 under single bloodmeal as compared to other treatments of 1 L and 5 L kairomones (Tukey post-hoc; $p < 0.05$). This maybe due to *Ae. aegypti* sense is less threatening from fish predator after the second day as the kairomones become less strong by the day which allowed mosquitoes to fully oviposit their eggs. Significant differences were also recorded between days of oviposition by female *Aedes* mosquitoes ($F = 416.79$, $df = 1$, $p = 0.000$). These results suggested that both *Ae. albopictus* and *Ae. aegypti* oviposition behaviour was altered in the presence of *H. macrolepidota* fish, either reduced the number of eggs oviposited or delayed their oviposition.

Table 1: Results of repeated measure ANOVA are examining the cumulative number of *Ae. aegypti* and *Ae. albopictus* in response to different types of *H. macrolepidota* kairomones treatments and days of oviposition.

Source	F	df	MS	Significance
Species (S)	78.35	1	8.75	0.000
Treatment (T)	11.88	3	1.181	0.000
Days (D)	416.79	1	41.42	0.000
S × T	9.13	3	1.02	0.001
S × D	59.73	1	6.64	0.000
S × T × D	13.72	3	1.53	0.000
Species × subject within groups		16	0.11	
Species × subject between groups		16	0.09	

Notes: df = degree of freedom, MS = mean squared value. Significant values are in bold. Data were log transformed prior to analysis.

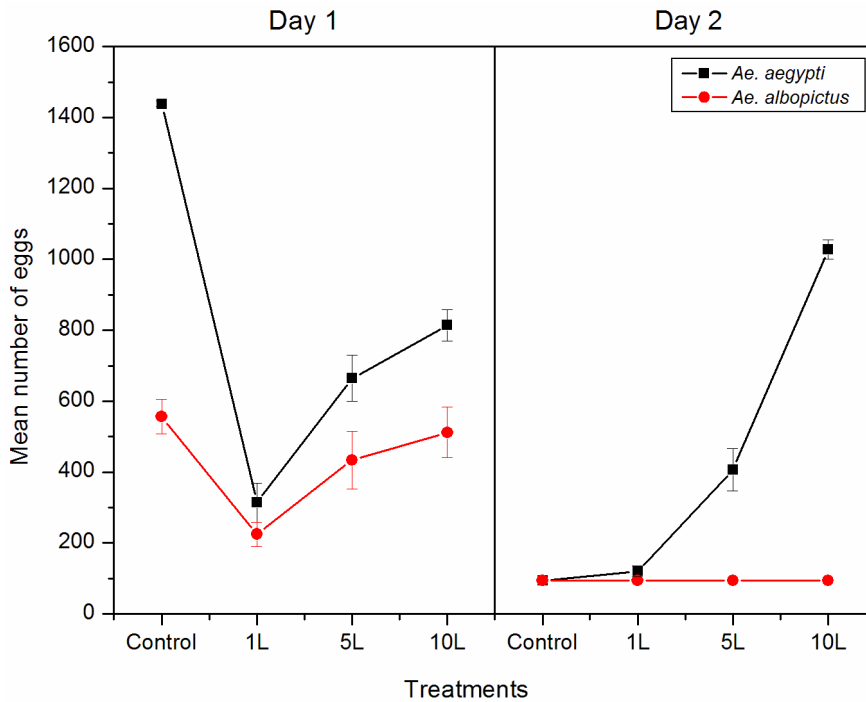


Figure 1: The effects of fish predator (*H. macrolepidota*) on the number of eggs deposited \pm SE by *Ae. aegypti* and *Ae. albopictus*.

DISCUSSION

This study has predicted that both *Ae. albopictus* and *Ae. aegypti* females will alter their oviposition behaviour in response to *H. macrolepidota* predatory fish’s kairomones. This prediction is based on a few studies that found evidence that alteration on mosquito oviposition may happen when facing with the presence of predators or its kairomones (Blaustein *et al.* 1995; Blaustein 1998; Blaustein *et al.* 2004; Kiflawi *et al.* 2003; Eitam & Blaustein 2004; Blaustein *et al.* 2005). A study by Berendonk (1999) also found that not only mosquito, but adult aquatic insects, *Chaoborus* as well avoided to oviposit in waterbodies that contains fish kairomones. However, there is some anomaly to this theory sometimes. For instance, *Culex pervigilans* mosquitoes do not modify their oviposition pattern though the existence of a predator due to kairomones release by *Anisops* predator is not strong enough to mark their presence in water bodies (Zuharah & Lester 2010).

Most of the terrestrial adult insects will turn on the water to oviposit their eggs and it is important to find a suitable and safer place to oviposit their next progeny (Berendonk 1999). In our recent study, we found evidence that *Ae. albopictus* and *Ae. aegypti* oviposition pattern was influenced by the

presence of *H. macrolepidota* fish kairomones. Even 10 mL of kairomones from the lowest kairomones concentration of 10 L can reduce the number of eggs oviposited by both *Aedes* species. Only a small amount of kairomones may alert and give a strong alarm to the female mosquitoes in the presence of predator and avoid to oviposit in this particular oviposition site. This selection should favour *Aedes* mosquito female adults on alteration of oviposition behaviour to maximise the survival of their juvenile.

In regards to the kairomones, mosquitoes such as *Culiseta longireolata* can detect chemical from *Notonecta* for up to 8 days after their removal from the pools (Blaustein *et al.* 2004). Thus, it seems reasonable that *Ae. aegypti* had delayed their oviposition on day 2 for all concentration tested, but it did not happen to *Ae. albopictus*. The way *Ae. albopictus* dealt with this kairomones was different from *Ae. aegypti*, with less number of eggs were deposited by *Ae. albopictus* due to pressure from this fish predator. Therefore, modification of behaviour regarding to oviposition is very essential for mosquitoes to ensure there is a continuous flow of generation without being extinct.

In conclusion, with the presence of *H. macrolepidota* fish predator, the number of mosquitoes in water bodies will be decreased. Other than become a predator, the kairomones remnant from the fish is enough to reduce the number of *Aedes* mosquito larvae in water bodies. Therefore, this fish predator can act as one of the potential biological controls for controlling the rising of dengue fever cases in the future.

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