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**Rodney L ITAKI**[*1] MBBS, B. Med Sci. and **Setsuo SUGURI**[2] PhD

[1]Immanuel Lutheran Rural Hospital, P.O Box 420, Wabag, Enga Province;
[2]International Medical Zoology, Faculty of Medicine, Kagawa University, Miki, Kita, Kagawa, 761-0793 Japan

*Corresponding author: email. itaki7@gmail.com.

# DIFFERENCES IN SUBMERGENCE TIME AND ALAM REACTION RATE IN *Anopheles stephensi*, *Aedes egypti* and *Culex pipiens* FOURTH INSTAR LARVAE IN RESPONSE TO VIBRATION STIMULUS

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[2]International Medical Zoology, Faculty of Medicine, Kagawa University, Miki, Kita, Kagawa, 761-0793 Japan
*Corresponding author: email. itaki7@gmail.com.

## ABSTRACT:

Studying mosquito larvae behavior can provide insights into developing new ways in controlling vector borne diseases. Feeding is an aspect of larval behavior that has been studied extensively. Surface and underwater feeding behaviors are linked by diving. The larvae use dive behavior to feed underwater or to escape predators. We elicited dive responses in fourth instar larvae of three different mosquito species using vibration stimulus and compared their response rate and submergence time. Marked differences in the response rate and submergence time were recorded for the three species of mosquitoes. These differences can be exploited to develop new larvae control methods to reduce mosquito borne diseases.

**Key words**: dive behavior, *Anopheles stephensi, Culex pipiens, Aedes aegypti*, vibration stimulus, alarm reaction
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## INTRODUCTION:

In an effort to develop new ways to control mosquito borne diseases, larval behavior has been studied extensively [1-5]. Walker and Merritt [2] made an exhaustive list of larval behaviors. Generally, larval behaviors consist of a group of surface behaviors and a group of under water behaviors [2, 6]. These two groups of behaviors are linked by dive and rise behaviors. A mosquito larva after diving may remain submerged for some time. This period is known as the submergence time [2, 6]. Some factors that may determine the submergence time of a mosquito larva include, gas exchange requirements, presence of a predator on the surface of the water and the need to feed while underwater; also included are the difference in anatomical structures and the physiological needs of different mosquito species [6].

A number of studies have examined the diving behavior or alarm reaction [1, 3, 7, 9]. To elicit a dive behavior various stimulus have been used to provoke a mosquito larva to dive [6]. It has also been found that if a stimulus is given continuously, the mosquito larva becomes desensitized to that stimulus [6]. After diving, a mosquito larva tends to stay submerged for some time before re-surfacing (personal observation). Studies examining dive behavior have established that different mosquito species may have different submergence time [1, 3, 6, 7, 9], but published data comparing the submergence time of different mosquito species under the same experimental conditions is scanty.

In the present study vibration stimulus was used to elicit dive response (alarm reaction) in fourth instar larvae of three different mosquito species (*Anopheles stephensi*, *Aedes albopictus* and *Culex pipiens*). The submergence time under controlled environment and the response rates for each of the three species of mosquitoes were calculated.

**MATERIALS and METHODS:**

Larvae and adults were maintained using standard protocols in an insectary with temperature maintained at 26$^{o}$C and relative humidity 65 % with a 15 hours 8 hours day night cycle [2, 6]. Light was provided by four 40-watt fluorescent light bulbs. Eggs were hatched in 250ml of de-chlorinated tap water in plastic cups (surface area = 95 cm$^2$). At the late second to early third instar stage, larvae were transferred to 33- x 24- x 7-cm pans. Larvae were fed on ®Tetra Min baby fish food. Water was changed every other day. Only fourth instar larvae were used in this study.

All observations were done in a cylindrical tube, length = 200cm and internal diameter = 14.9cm, water surface area = 174.28cm$^2$ and containing 374.28cm$^3$ of water. The tube was filled with de-chlorinated tap water and incubated for 1 week to permit growth of microorganisms on the walls including the floor, in the water column and at the air-water interface [2]. Larvae at the fourth instar stage were individually pipetted into the glass tube and allowed to acclimatize for one hour. A total of 65 larvae (*Anopheles stephensi* = 23, *Aedes egypti* = 21, *Culex pipiens* = 21) were used.

The vibration stimulus used was a single tap made on the side of the tube. When the larvae did not dive, the taping was done in quick successions. A non response was recorded if the larva did not dive after two minutes of tapping.

Preliminary observations showed that *Anopheles* larvae required longer period for the stimulus to be applied before eliciting a dive response. Thus, unlike the other five minutes was allowed before recording non response for the *Anopheles* larvae. Hand held stop watch was used to record the submergence time.

**RESULTS and DISCUSSION:**

Table 1 shows fourth instar *Aedes aegypti* (100%) larvae were more sensitive to the vibration stimulus compared to *Anopheles stephensi* (0.17%) and *Culex pipiens* (4.91%) larvae.

Preliminary experiments showed that *Aedes aegypti* larvae used the entire microcosm to feed while *Culex pipiens* larvae fed by filtering while attached to the water's surface with occasional dives to feed on the walls of the cylinder. Diving in response to a vibration stimulus was a rare behavior in fourth instar *Anopheles stephensi* larvae. This species spent most of its time at the water's surface. Although *Anopheles* larvae had the lowest response rate (0.17%), they typically remained submerged for almost five minutes compared to *Culex* and *Aedes* larvae which remained for 1.75 and 2.38 minutes respectively.

*Anopheles* larvae remained under water twice as long as *Aedes* and nearly three times as long *Culex* larvae. It was also noted that giving the stimulus intermittently did not evoke further diving response in larvae that responded to the initial stimulus. This is in line with previous studies showing mosquito larvae becoming accustomed to a stimulus if given repeatedly [6].

Larva of *Anopheles* species have a higher density compared to *Culex* and *Aedes* species [6]. The higher density could be one reason for their longer submerged time. *Culex* and *Aedes* species are less dense thus they tend to float up to the water's surface.

The *Anopheles* larvae sank to the bottom of the observation cylinder when the larva was not actively swim to the surface. *Aedes* and *Culex* larvae floated back to the water's surface even when not actively swimming. The larvae of these two species swam actively to stay submerged.

Table 1: Alarm reaction response rate and mean submergence time in fourth instar *Anopheles stephensi, Culex pipiens* and *Aedes aegypti* larvae

| **Variables** | ***Anopheles stephensi* (n = 21)** | ***Culex pipiens* (n = 21)** | ***Aedes aeygypti* (n = 21)** |
|---|---|---|---|
| **Mean submergence time (minutes)** | 4.25 | 1.75 | 2.38 |
| **Response rate (%)** | 0.17 | 40.91 | 100.0 |
| **Non-response rate (%)** | 83.0 | 50.09 | 0.00 |

The difference in the alignment of the larvae to the water surface may also be a reason why there was a marked difference in the response rate. *Aedes* and *Culex* larvae hang obliquely to the water surface compared to *Anopheles* species which lie parallel to the surface. This difference in orientation may result in differences in the surface tension force acting on the siphon of the larvae. As a result, *Anopheles* larvae may require a slightly greater force to break free from the water surface compared to *Aedes* and *Culex* larvae which may need less strength. A simple wriggle of their body may be sufficient to break free from the water's surface. Differences in feeding behavior between the three species are well documented [1, 2]. The difference noted in the response rate and submergence time may be a reflection of the difference in feeding behavior. Differences in gas exchange requirements influence the duration that the larvae stay submerged. Gray and Bradly [10] have shown that adult mosquitoes breathe discontinuously. Hetz and Bradly [11] further hypothesized that the discontinuous breathing may be a mechanism to prevent injury from oxygen radicals. It is not known if mosquito larvae also breathe discontinuously. However, it is possible that a similar mechanism observed in insects that live underground [12] may exist in mosquito larvae and this mechanism may be different in the larvae of different mosquito species. Thus the differences observed in the larvae of the three mosquito species in the present study may reflect differences in gas exchange requirements.

The differences in surface and submerged time could be determined by frequency in diving episodes (preliminary observations). This study did not examine the time larvae spent at the water surface. However, we can speculate that larval species that tend to spend most of their time feeding at the water surface may be affected by larvicides that float while larval species that dive frequently and or spend most of their time submerged may be affected by larvicides that are dissolved in water. Thus according to some researchers [13-15], this explains some of the differences observed in natural oil toxicity against mosquito larvae.

One can suggest that laboratory experiments studying larvicides need to consider differences in larval behavior in different mosquito species and incorporate these differences in their experiment designs. In addition, users and manufacturers of commercially available larvicides need to consider differences in larval behavior, then reformulate the constituents and vary the application protocols to suit targeted mosquito species. The changes in the use of commercially available larvicides may allow larvae of specific mosquito species to be targeted.

**CONCLUSION:**

Our results showed difference in the alarm reaction rate of *Anopheles*, *Culex* and *Aedes* fourth instar larvae in response to vibration stimulus. The three species also showed differences in their mean submergence time. These findings confirmed pervious studies on mosquito larva behavior [1-9]. The difference in submergence time and alarm response rate may be due to differences in the anatomy and physiological requirements in the species studied. The differences observed can be exploited to develop new larval control methods or to improve existing ones. More detailed studies should be done to re-examine mosquito larvae behavior.

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