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Response of Mosquitoes to Mechanical Stimuli

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by

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Response of Mosquitoes to Mechanical Stimuli

1. Response of the larvae and pupae of *Aedes aegypti*, *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae) to surface disturbance*

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ABSTRACT

The response of the larvae and pupae of *Aedes aegypti* (L.) *Anopheles stephensi* Liston and *Culex pipiens* L. to surface disturbance, for which a surface ripple was produced by water drops of peristaltic pump was studied. Oualitative and quantitative estimates and ontogenic aspects of the response were considered. All the species studied here, in general, showed a diving down response to this stimulus, but a small number of *Anopheles stephensi* showed a sideways run. In the three species, the response changed with the age; it gradually decreased in *A. aegypti* and *C. pipiens* but increased in *A. stephensi*.

INTRODUCTION

In broad sense, mechanoreception includes the perception of any mechanical distortion of the body detected by sense organs. This may result from touching an object or from the impact of vibration borne throungh the air, water or substrate. The ability of animals to perceive mechanical stimuli in their surroudings allows them to avoid danger, locate food and communicate with members of their own species.

Male mosquitoes detect the wing beat frequencies of females and use this to locate the females (16). Larvae and pupae of mos-

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quitoes also respond to the mechanical stimuli in their surroundings, such as disturbance (10, 1, 12).

Surface vibration is a mechanical stimulus which evokes a high percentage of response in mosquito larvae and pupae. The reactions of larvae to surface vibration differ in different species; the response is always a diving down movement to the floor of the dish in Culicines larvae (10), while it is usually a performance of the sideways run in Anophelines larvae (1, 12).

Hocking (10) working on *Aedes communis* larvae in the field found that the larvae dived down to the floor of the pool in reponse to visible surface ripples on the surface of the pool. Such ripples or vibrations were also experimentally produced by several means, e.g. touching the surface with a stick, dropping a very small stone or dropping water artificially with pipette (1) or naturally such as rain drops (12).

Allen, Hansell and Hood (1) working on *Anopheles d'thali* larvae found that the main response of this species to water drops released from a bulb pipette 6 cm above the water surface was a rapid sideways run of short duration and without loss of contact with water surface. In each test, less than 1 % of the larvae dived down. However, Hansell (pers. comm.) in C. *pipiens* and *Anopheles gambiae* observed that the most frequent response of the larvae to the surface ripple stimulus was to dive downwards, rather than to run sideways.

Kulhorn (12) working on *Anopheles messeae* and *Anopheles claviger* found that the response of the larvae to rainfall or to the imitation of rainfall in the laboratory by falling water drops of various sizes was either a short sideways run or diving down to the floor. He observed that these two response were given by the larvae according to the strength of the falling drops; the heavy or repeated drops elicited diving down response whereas the light drops elicited only a short sideways run. The rain drops or artificial water drops often did not affect the larvae when they fall onto the rear end or to the middle of the body. When they fell on or near the head the given responses were very high, this suggests that drops not hitting the body are unlikely to evoke

any response at all. Külhorn (12) pointed out the importance of the strenth of the stimulus on the response, in which case the size and the releasing height of the drops are the factors which could easily change the level of the response. So that to establish the level of the response it is important to give same size of drops from the same height. In the work of previous authors, the stimulus (water drops) which was released from hand-controlled pipettes was not well standardised and also no pupae of any species were studied. Therefore, the following experiment was undertaken to test the response of the larvae and pupae of the three species of mosquitoes to the regular release of a standard drop controlled by a peristaltic pump and to compare the responses of the different species to the same stimulus.

As the larvae and pupae grow, their responsiveness would be expected to show an increase due to the increase in central nervous and sensory capacity . Conversely it was found in late larval and pupal stages that the responsiveness to visual stimuli significantly decraesed (11). If visually and mechanically mediated responses share same common central mechanizm and taht mechanism is, late in larval and pupal life, being compromised for adult needs, then we would expect to see a decline in response to mechanical stimuli parallel to that for visual stimuli. It is therefore of interest to investigate the responsiveness of different age groups within a species in order to establish the changes in responsiveness with age to surface ripple stimulus.

MATERIAL AND METHODS

The mosquito larvae and pupae used in these experiments reared in an insectary, with a controlled temperature (at 24–25° C), humidity (at 75–80 % RH) and photoperiod (at 12 hr dark and 12 hr light).

In this experiment a peristaltic pump with 0.8 mm diameter tube was used to produce a standard water drop . A five lt water reservoir was connected to one end of the tube to give a continuous flow of water for the whole duration of the test (fig. 1).

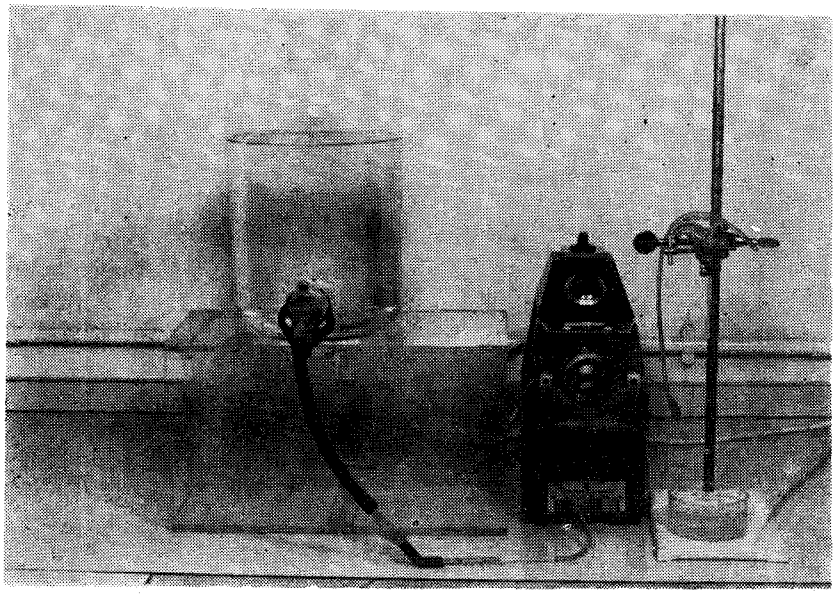

Fig. 1. The apparatus used to conduct the surface ripple experiment. On the left is the water reservoir, leading to a peristaltic pump which drives the water to the end of the tube held over the dish by a clamp (right). The peristaltic pump regulates water flows and produces standart water drops at regular intervals, adjustable according to the speed of the pump, (see also Material and Methods).

The weight of a standard drop of water was measured by a sentitive balánce. It was calculated to be 0.058 mg.. In order to weigh a drop of water five drops of water vas released on to a piece of filter paper of known weight and weighed in a sentsitive balance, then this process was repeated 10 times to give a total weight of 50 drops and the average of this was taken as the weight of a standard drop.

In all tests the standard drops were released from a height of 20 cm on to the centre of the experimental dish of 6 cm diameter.

Six groups, each containing 6 of the 3 rd and the 4 th instar larvae and pupae of each species were tested every morning between 9.00–12.00 hrs and every afternoon 14.00–17.00 hrs throughout the instar to determine the ontogeny of the response. Each stimulus was applied after at least 5 minute since the previous

stimulus and for *A. aegypti*, when half the number of the larvae and full number of pupae and, for *A. stephensi* and *C. pipiens*, when the full number of larvae and pupae were at the surface. The reason for taking half the number of the larvae of *A. aegypti* was because the larvae of *A. aegypti* were very active and very rarely all six larvae were at the surface at one time. Five minute intervals were given between two stimuli to conduct the experiments in unhabituated conditions; preliminary experiments showed that even in the repeated stimulus situation most of the animals responded immediately after application of this stimulus, only the number of the animals which moved in the first five seconds after the application of the stimulus was counted. The counts were repeated six times for each groups in each test. From these counts the percentage response, overall mean and standard deviation was estimated. The correlation coefficient test was also used to determine the significance of decrease or increase in the response with age.

Besides the level of the response, the manner of the response was also recorded.

RESULTS

1. *Aedes aegypti*

Behaviour observed

The larvae and pupae of this species are normally very active; they spend most of their time in swimming and only coming to the surface to breath. If a drop of water was released while they were breathing at the surface they swam downwards very rapidly. On reaching the floor the larvae slowed down, continued to swim near the floor for about a minute before returning to the surface by active swimming as they had done when descending.

The pupae of this species also dived down very rapidly in response to the stimulus produced by the water drops. But owing to their great buoyancy they could only maintain themselves below the water surface by a succession of dives produced by the strokes of the abdomen with its terminal paddles. The return to

the surface was passive. As soon as pupae stopped swimming they floated back to the surface of water.

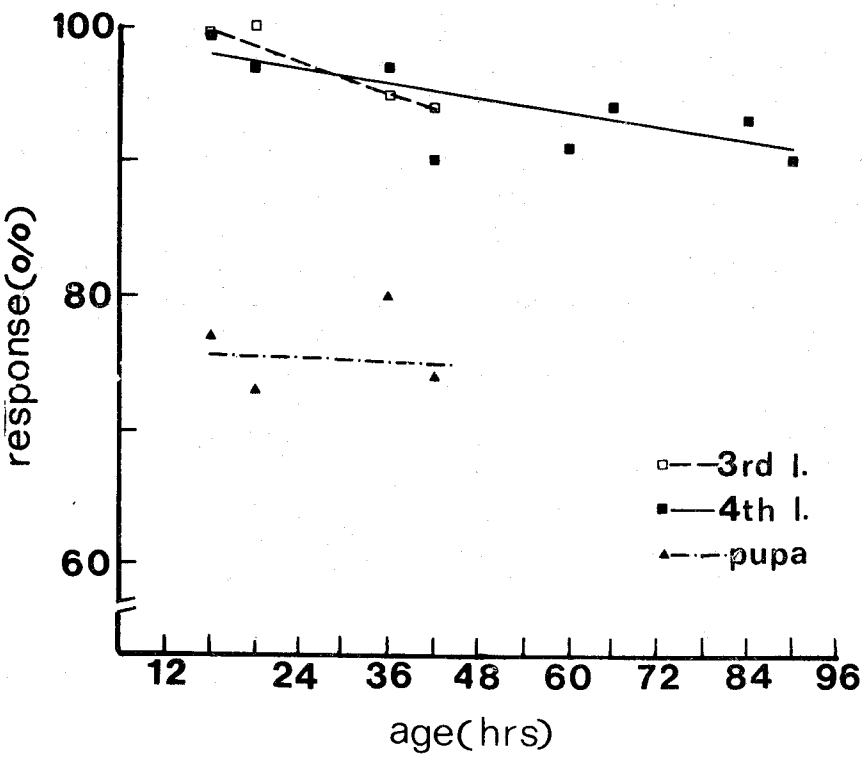

Fig. 2. *Aedes aegypti*: The ontogeny of the response of the larvae and pupae to surface ripple stimulus. The age of the animals is plotted against the percentage response. Each point in the figure is the mean of 6 groups. The regression line was fitted according to the correlation coefficient test (14).

Quantitative findings (*Fig.* 2)

The response of the larvae gradually but significantly decrased with age ($p < 0.002$ for the 3rd instar and $p < 0.001$ for the 4 th instar larvae). There was no significant decrease in response of the pupae with age ($p > 0.5$). As clearly seen from the figure the overall responsiveness of pupal stage was lower than the response of larval stage ($p < 0.01$).

2. *Anopheles stephensi*

Behaviour observed

The larvae of this species lie parallel to the water surface and only leave the surface in response to a sudden stimulus. When

a drop of water was released in the centre of the experimental dish most of the larvae rapidly sank down from the surface, after breaking contact with it by an active swimming. Only a small number of them ran across the surface for a short distance keeping contact with the water surface (sideways run).

The *A. stephensi pupae responded to the surface ripple stimulus by rapidly diving* dawnwards *in the same* way *as A. aegypti.*

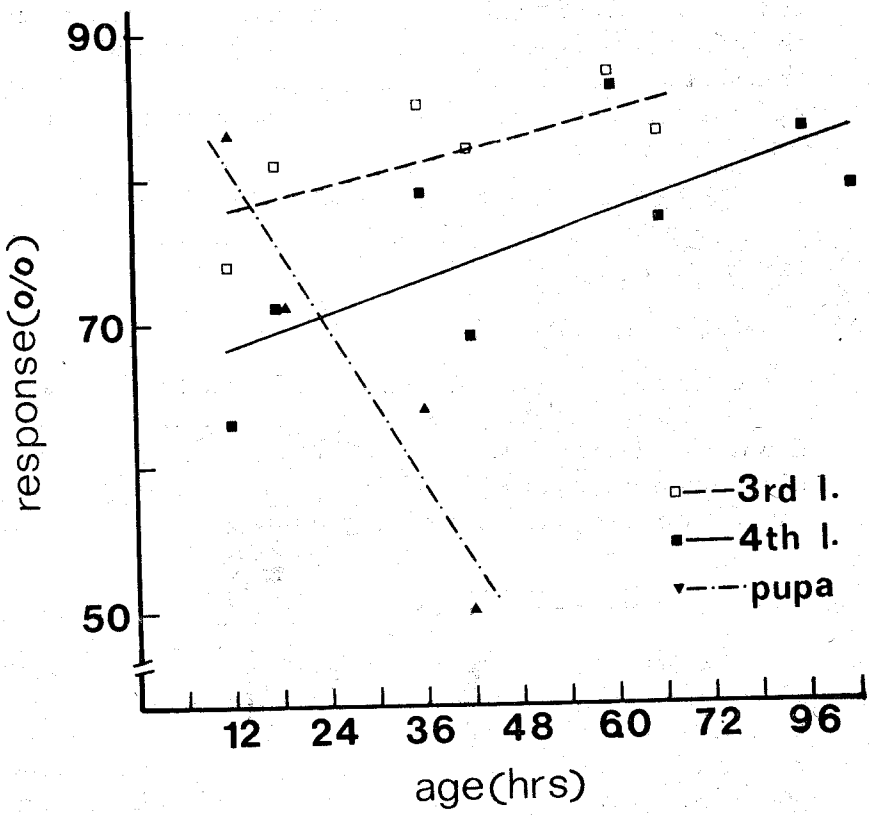

Fig. 3. *Anopheles stephensi*; Explaination as in Fig. 2.

Quantitative findings (*Fig.* 3)

The response of the 3rd and 4 th instar larvae significantly increased with age ($p < 0.01$). But response of the pupae significantly decreased ($p < 0.001$).

3. *Culex pipiens*

Behaviour observed

The larvae of this species, like *A.aegypti* hang down from the water surface, however they spend most of their time at the surface and only swim down for feeding or in response to a sudden stimulus. Larvae responded to water drops in either of two ways: (i) they swam actively to the floor and came back to the surface at the other side of the dish without stopping, (ii) they swam actively for a short distance to break contact with the water surface then sank passively down to the floor ultimately returning to the surface by active svimming after staying motionless on the floor for 1–2 minutes.

The pupae of this species responded to the surface ripple stimulus by rapidly swimming downwards, in a manner similar to taht of *Aedes aegypti.*

Quantitative findings (*Fig.* 4)

A significant decrease was found in the response of 3 rd and 4 th instar larvae with age ($p > 0.001$). There was no singnificant decrease in pupae ($p > 0.5$). As it is seen from the figure the level of overall responsiveness of pupae was significantly lower than the level of overall responsiveness of larvae ($p < 0.05$).

CONCLUSIONS AND DISCUSSION

In the three species studied the most easily elicited response of the larvae and pupae to surface ripple stimulus was to escape from the water surface by diving downwards. *A. stephensi* however showed two kinds of response, dive and sideways run; a small proportion of the larvae of this species responsed with sideways run while others responded by diving down. According to findings of Külhorn (12) if the stimulus is weak the larvae showed sideways run, but if it is strong then the larvae showed diving down response. In this experiment as the drop was standardised all larvae should have the same stimulus intensity from the same drop. Therefore this difference in response may be due to a difference in rea-

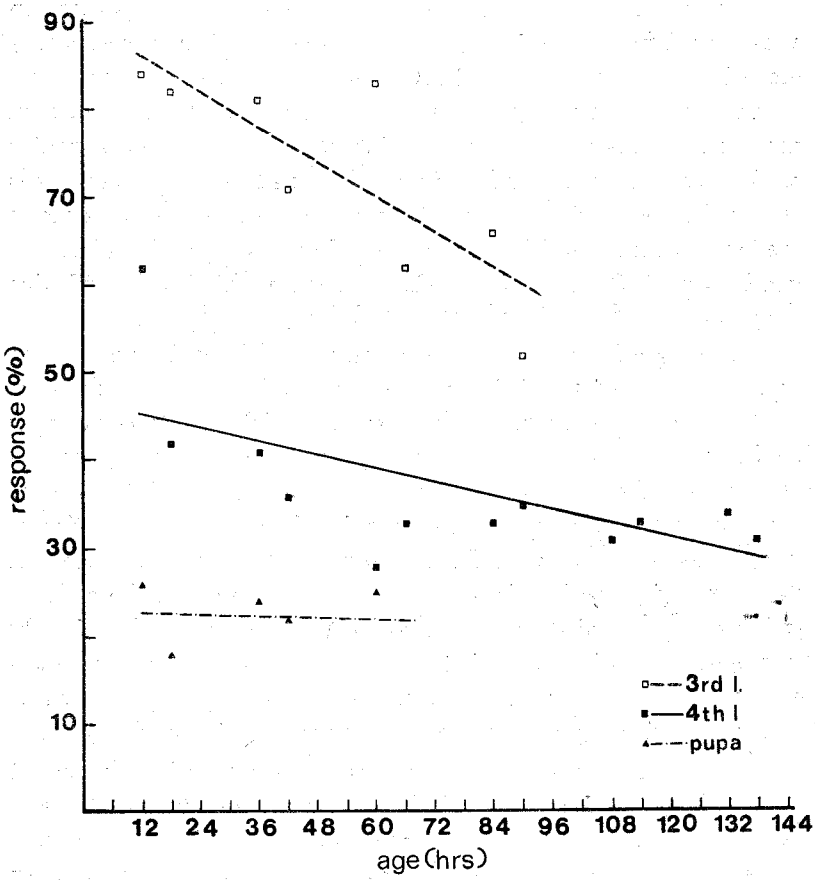

Fig. .4 *Culex pipiens*: Explaination as in Fig 2.

diness of individuals to respond, but in this experiment as the animals were not tested individually, this point can not be strongly argued.

It seems reasonable to assume that the surface ripple stimulus produces appriciable wave action at the water surface and slight pressure waves within water. This being the case, *Aedes* and *Culex* will recieve weaker stimulation from surface ripple than *Anopheles*, because the former, hanging from the water surface, will have less contact with the water surface than *Anopheles* lying parallel to the water surface.

The nature of the stimulus may also be very important in evoking a different kind of response. It was found that the animals moved to the floor of the dish in response to the surface ripple stimulus where there may be less disturbance for them. If so, then the stimulus may represent a predator. Considering the stimulus produces strong water surface disturbance and weak pressure waves within the water it is very likely to present an extra-aquatic predator, which could be surface film dwellers such as spiders (3); valid bugs (9, 8); or aerial predators such as flies of Ephydridae (19), Antomyiidae (9) and Dolicopodidae (19, 13).

Although the same response pattern was followed by the larvae and pupae of *Aedes* and *Culex*, the larvae and pupae of *Aedes* were more responsive than those of *Culex*. This difference may simply be due to difference in sensory capability between the species since there are differences between them in number and location of chordotonal organs, which are considered to contribute to detection of mechanical stimuli. In *A. aegypti* these organs are found on the first and second abdominal segments and also on the siphons and are not shielded (5) Whereas in *Culex* they are found only on the siphon and are shielded (6). Although the response of *Anopheles* is lower than that of *Aedes*, the results can not be comparatively argued as such organs were not yet recorded in this species.

The difference in responsiveness of *Aedes* and *Culex* could also be influenced by their different larval and pupal habitats. The larvae and pupae of *C. pipiens* were collected from semi-permenant pools containing emergent aquatic vegetation, mainly reeds and floating dead leaves. Shannon (17), Berner (2) and Chan, Ho and Chan (4) recorded that the larvae of *A. aegypti* live in very small water collections in domestic places such as tin cans, buckets and bowls not containing vegetation. In the habibat of *Culex* as the emergent vegetation would produce mechanical disturbance even in sliht windy conditions the intensity of the artificially produced stimuli, e.g. the stimuli used in this experiment, might not be strong enough to set a high level of response, whereas in the habibat of *Aedes* with no vegetation the effect of the wing should be slight in producing mechanical disturbance and therefore even in a slight mechanical stimulus might be expected to evoke a high level of response.

The response of the larvae of *A.aegypti* and *C.pipiens* and the pupae of the three species decreased wiht age in the same way as shown to the visual stimuli (11). Only the response of the larvae of *A. stephensi* increased. Although significance of these results is hard to explain, a few suggestions could be made (i) As the responses are likely to be anti-predatory, in some circumstances being inactive may be more protective than being active, because movement may produce visual or mechanical or both kinds of stimuli which may be detected by a predator and release a predatory behaviour, (ii) As the growth is an important process in larval phase then larvae will concentrate their growing towards the end of each instar resulting in a reduced response to stimulus, (iii) Thomas (18) found a correlation between oxygen requirement and the age of larvae of a *Culex* sp., if such is the case then response would be expected to decrease towards the end of each instar.

The response to studied stimuli suggests that irrespestive of stimulus whether, mechanical or visual, it acts upon the same part of central nervous system (CNS). Such a common centre receiving inputs of various sensory channels could mediate similar responses to various stimuli. Electrophysiological evidence showed that there are a variety of visual and auditory single units and non-specific multimodal units in the optic lobes of many insects (15). These multimodal units of *Locusta* respond to vibration or touch stimuli or all kinds, to a wide range of visual stimuli via compound eyes. Those of *Periplaneta* respond to light-off, to gentle touches of abdomen and pterothorax or brief displacement of cerci and tarsal segments (7). It, therefore, is quite possible that these multimodal units are also present in the optic lobes or elsewhere in the CNS of mosquito larvae and pupae and could recieve sensory channels from both eyes and mechanoreceptors. In which case these visual and mechanical stimuli could act upon the same part of the central nervous system to general escape responses.

Mosquito larvae and pupae generally live in small water collections where may be a limited number and limited variety

of predators. If so, in such habitats there would probably be limited variety of potential external stimuli representing predation threat to which larvae and pupae could respond. Therefore the variety of exogenous factors acting upon the sensory mechanism of animals may be somewhat limited and this may in turn cause a limited range of response.

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ÖZET

Ae. aegypti, *An. stephensi* ve *Cu. pipiens* larva ve puplarının suyun yüzeyinde oluşturulan dalgalanmaya karşı gösterdikleri davranışları incelendi. Yüzeydeki dalgalanma su yüzeyine su damlatılarak oluşturuldu. Bu etkiye karşı gösterilen davranışlar ve bunların yaşla olan ilişkileri göz önünde tutularak grafikler elde edildi. Genel olarak tüm larva ve puplar bu etkiye karşı yüzeyden tabana doğru dalarak davranış gösterdikleri halde az sayıdaki *An. stephensi* larvaları su yüzeyinde yana doğru kaymak suretiyle davranış gösterdi. *Ae. aegypti* ve *Cu. pipiens*'te gösterilen davranış yaşla azaldığı halde *An. stephensi*'de arttı.

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