# THE INFLUENCE OF DISSOLVED OXYGEN CONTENT ON THE SURVIVAL OF SUBMERGED MOSQUITO LARVAE

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ABSTRACT. The survival times of 3 species of mosquitoes were observed when they were kept submerged in water of fixed dissolved oxygen content. The influence of temperature and larval size on survival times was also noted. Results suggest that larvicides which kill by anoxia are likely to be effective only when the water is less than 30% saturated with oxygen, the exact value depending on the species. In most circumstances these conditions are likely to occur only at night.

## INTRODUCTION

It has long been recognized that the effectiveness of larvicidal oiling is influenced by the amount of oxygen dissolved in the water of the larval habitat (Sen 1914, Scott Macfie 1917, Ramsey and Carpenter 1932, Wang 1938, Richards 1941, Sautet et al. 1946, Micks et al. 1974). Recent work on insoluble monolayers (Reiter and McMullen 1978, Reiter 1978) has shown that these substances act efficiently as larvicides only in water of low dissolved oxygen content. This paper reports observations on the survival times of mosquito larvae when kept submerged in water of a fixed dissolved oxygen concentration.

## METHODS

A number of methods for adjusting the dissolved oxygen concentration were tried. Most satisfactory was found to be chemical reduction by sodium sulphite—

$$2SO_3^{2-} + 0_2 \rightarrow 2SO_4^{2-}$$

The WHO standard larval susceptibility test (WHO Technical Report Series No. 443, 1970) was used to test whether the reducing agent or its product were toxic to larvae. No significant mortality was observed even at 10 times the concentrations used in the experiments.

Larvae were observed in tall glass bottles (internal diameter ca 7cm., height ca. 30 cm.) completely filled with water (volume ca, 1150 ml.) and sealed with a ground glass stopper. At the start of each set of observations the bottles were filled with distilled water saturated with air at the required temperature. Rapid stirring of this water just prior to filling served to eliminate super-saturation produced during aeration. A fine wire brush was used to dislodge bubbles if they formed on the sides of the bottles. Sodium sulphite was added in a quantity calculated to reduce the dissolved oxygen by the desired amount, and then the bottles were stoppered, care being taken to insure that no air was trapped beneath the stopper. The solution was stirred for one minute with a magnetic stirrer, after which the bottles were transferred to a glass-fronted water-bath at constant temperature (± 0.05°C). Reduction was fairly rapid (> 95% complete in 2 to 3 hr), but the bottles were always left undisturbed for 18 hr to insure complete reaction.

A mechanical separator (Fay and Morlan 1959, modified by Grose et al. 1966) was used to provide larvae of a standard size for each replicate. After separation, larvae were washed and counted into 10ml. beakers, 25 per beaker. A dropping pipette was used to transfer the contents of the beakers, in a minimum of water, to the test bottles. All 25 larvae had to be projected into the bottle at once, and with some force, so that they were clear of the stopper when it was inserted. This was especially true when the dissolved oxygen content of the water was low, as in this

case the larvae were markedly reluctant to swim away from the surface.

Six bottles were used in each test, 5 with reduced oxygen content and the 6th with air saturated water. Bottles were examined at regular intervals, and larvae classed as dead if they showed no sign of movement. At the end of the test, the dissolved oxygen content of the water was measured with a Dissolved Oxygen Meter (YSI 51B, Yellow-Springs Instruments Company, Yellow Springs, Ohio 45387, U.S.A.). Measurements with this instrument showed that larval respiration had a negligible effect on the dissolved oxygen content of the water in the bottles.

Three species of mosquito were used: *Anopheles (Cellia) gambiae* Giles, species A, from a culture originating in Pala Upper Volta, obtained from the Ross Institute of Tropical Hygiene. *An. stephensi* Liston from a lab culture, CDE Porton, Wilts, U.K. and *Culex quinquefasciatus* Say (= *fatigans* Wiedemann), from a culture originating in the Gambia. All were reared in LD 12:12 (Alternating 12h light: 12h dark) at 27°C.

## RESULTS AND DISCUSSION

The survival of larvae when submerged depends on their ability to absorb oxygen through the cuticle (Wigglesworth 1933). In addition, the tracheal system acts as an air store, for it was observed that larvae which did not survive the submergence gradually lost buoyancy before they died, indicating a reduction of the volume of the gas within them. The rate of loss of buoyancy was very rapid when the dissolved oxygen concentration ($dO_2$) was low suggesting that the outward diffusion of gas into the water is significant in these circumstances. This was confirmed when the larvae were placed in nitrogen-free, oxygen-saturated water; the insects lost buoyancy within seconds and were unable to rise from the bottom of the bottle.

Fig. 1 shows the relation between $dO_2$ and the time when 75% of the larvae ($IV^{th}$ instar) had ceased to move. (It must be mentioned that if larvae were removed to shallow, aerated water shortly after they ceased to move they did revive, i.e. the true time of death is about an hour later than that indicated by the observations in the bottles.) Clearly the "straight" portion of each curve is at a $dO_2$ which is close

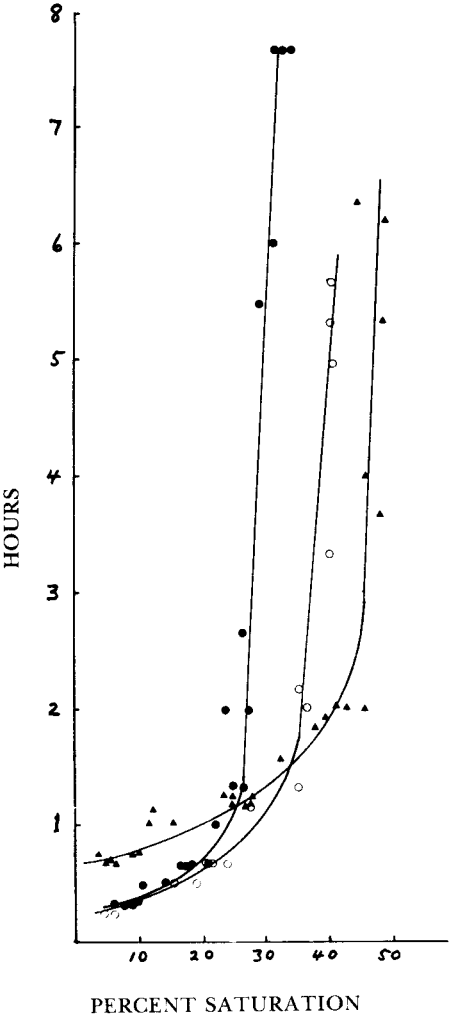


Fig. 1. Time to 75% mortality for 3 species of mosquito larvae (4th instar) when submerged in water of fixed dissolved oxygen content. (Δ) *Culex quinquefasciatus* (○) *Anopheles gambiae* (●) *An. stephensi.*

to a critical concentration above which cutaneous respiration adequately compensates for the absence of siphonal respiration. This critical concentration was higher for *Cx. quinquefasciatus* than for the other species, which may indicate that its cuticle is less permeable to oxygen. This would be supported by the fact that at lower $dO_2$, *Cx. quinquefasciatus* was able to survive for considerably *longer* than the other species; a less permeable cuticle would reduce the rate at which oxygen diffuses out from the insect into the water. This would be a useful adaptation in an insect which often breeds in highly polluted (low $dO_2$) water.

Fig. 2 shows the survival times of submerged *An. stephensi* larvae at 3 different temperatures. At low $dO_2$ larvae survive longer at lower temperatures, presumably due to the reduced respiration rate. Similarly at 32°C the critical $dO_2$ is about 50% higher than at 27°C. The fact that the critical $dO_2$ at 22°C is higher than that at 27°C may be due to the unacclimated insects ceasing to move earlier as a result of the low temperature. The close similarity between curves for 2nd and 4th instar larvae (Fig. 2) suggests that size differences (and surface to volume ratio) are relatively unimportant in determining the survival time of submerged larvae.

## CONCLUSIONS

The results reported here indicate that larvicidal methods which depend on anoxia are likely to be effective only when the $dO_2$ of the breeding water is considerably below saturation level. In highly polluted standing water such conditions may occur throughout the day, but in sunlit water containing photo-synthetic organisms they are likely to occur only during the night, when animal and plant respiration utilizes the dissolved oxygen.

Very little information is available on the $dO_2$ of the larval habitat. However, in a survey of over 300 anopheline breeding sites in India, Iynegar (1930) found that more than 80% had a $dO_2$ of 1–4 mg litre$^{-1}$ (<50% saturated) between 08.00h and 10.00h each day and the level dropped to below 25% saturation during the night. Muirhead-Thomson (1942) found that in the shaded pools where *An. minimus* Theobald breed, $dO_2$ ranged be-

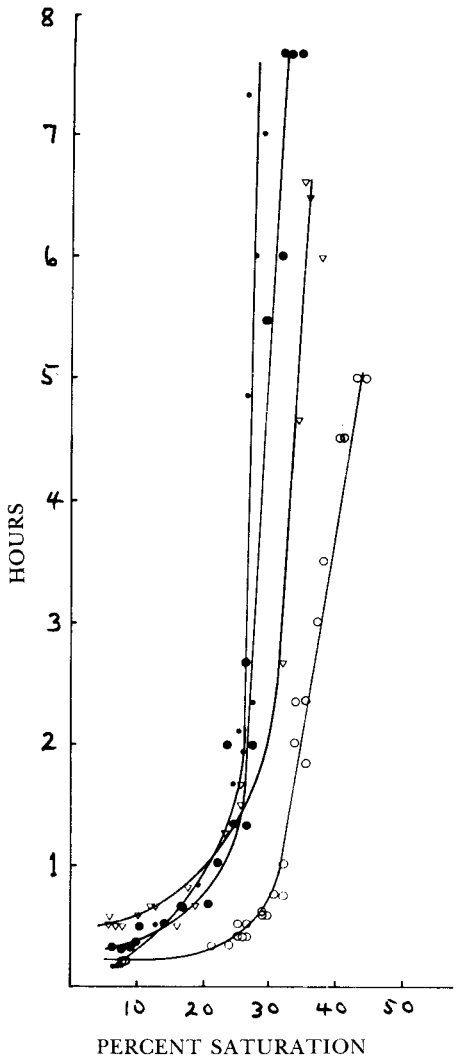


Fig. 2. Time to 75% mortality for *Anopheles stephensi* at 3 different temperatures: (Δ) 22°C; (•) 27°C; (○) 32°C and percent $dO_2$ shown. All larvae 4th instar except (.) which were 2nd instar larvae at 27°C.

tween zero and 25% saturation. Lastly the $dO_2$ in rice field water in Kisumu, Kenya was found to drop below the 20% saturation level for about 6 hr of the night, and under these conditions insoluble monolayers showed good larvicidal activity against *An. gambiae s.l.* (Reiter, unpublished work). Further work on the role of dissolved oxygen on the efficacy of monolayers as larvicides in similar breeding places is planned.

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