

Egg hatching inhibition: field evidence for population regulation in a treehole mosquito

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ABSTRACT. 1. We investigated the influence of larval density on egg hatching in the treehole mosquito, *Aedes triseriatus* Say (Diptera: Culicidae) by submerging laboratory-produced egg batches in natural habitats stocked with fixed densities of larvae.

2. We found a significant decrease in hatching rate as larval density increased. The results suggest that inhibited eggs refrain from hatching into environments in which competitive effects of large larvae are potentially severe, and in which cannibalism may occur.

3. The amount of time the egg batches were exposed to experimental conditions (between 2 and 16 days) had no significant influence on hatch rate.

Key words. Culicidae, *Aedes triseriatus*, mosquito, hatching stimulation, hatching inhibition, population regulation.

Introduction

Asynchronous egg hatching in *Aedes* mosquitoes permits overlap among individuals of different developmental stages within the same habitat, presenting possibilities for interactions among stages. We show, through manipulation of larval density in natural habitats, that eggs are inhibited from hatching into crowded larval habitats. This interaction has a potentially stabilizing influence on population dynamics.

Many studies of insect population regulation have focused on competition for resources among individuals within the same developmental stage (e.g. Istock *et al.*, 1976; Stiling *et al.*, 1984) and interactions with other species, particularly parasitism or predation (e.g. Varley, 1947; Varley & Gradwell, 1960, 1968; Morris, 1963; Lounibos, 1983). Other forms of intra-

specific interactions may also impose limits to population growth, including various actions to reduce the success of other individuals. Documented interference phenomena include chemical mechanisms of interference (e.g. Park, 1934; Park & Wolcott, 1937; Kidder, 1941; Rose, 1960; Groner, 1974), and varied behavioural interactions, such as territoriality (e.g. Moore, 1964; Wilson, 1971), cannibalism (e.g. Fox, 1975; Polis, 1981; Koenekoop & Livdahl, 1986) and murder (e.g. Stiling & Strong, 1983; Corbet, 1985). A recent review of density-dependence in mosquitoes has been presented by Service (1985).

A tendency for eggs to refrain from hatching during times of larval abundance or resource shortage can reduce population growth by extending the age of first reproduction. This plastic response of the population growth rate to density does not fit into the categories of population regulation described above, but it may have importance in any species that possesses a dor-

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mant stage with variable termination and overlapping developmental stages. Density-dependent change in generation time will not reduce a population that has exceeded its equilibrium level, but it can retard growth below the equilibrium level which may minimize overshooting. This process can also combine with mortality in the dormant stage to reduce population size to an equilibrium level. Competition among larvae, density-dependent dormancy periods, and mortality during the dormant period may act in concert to produce precise concordance of larval abundance with resource conditions. These conditions may hold for many species of *Aedes* mosquitoes. Earlier laboratory work (Livdahl *et al.*, 1984) suggests that larval inhibition of hatching is possible in *A. triseriatus* Say.

Hatching of *Aedes* eggs is stimulated by a decline in the concentration of dissolved oxygen caused by microbial growth in the vicinity of the eggs (Gjullin *et al.*, 1941; Judson, 1960; Fallis & Snow, 1983). Larvae graze on microbial surface growth (Fish & Carpenter, 1982; Carpenter, 1983) and while doing so may weaken the hatching stimulus (Gillett *et al.*, 1977). In the laboratory, the magnitude of hatching suppression is influenced by interactions among resource availability, density and instar of larvae (Livdahl *et al.*, 1984). Because the adverse influence of large larvae on small may be severe through competition for resources (Livdahl, 1982) and cannibalism of newly larvae (Koenekoop & Livdahl, 1986), the inhibition may be adaptive for the eggs. We planned the following field experiment to test for a reduction in the rate of *A. triseriatus* egg hatching in the presence of high densities of larvae.

Materials and Methods

Density estimation and treatment levels

During July 1986 we determined the volume of treehole fluid and number of *A. triseriatus* larvae of each of four larval instars for forty treeholes located in beech and maple trees at two sites in Worcester, Massachusetts, U.S.A. Field data were used to construct experimental larval densities based on a mean and standard deviation after logarithmic transformation of larvae/litre that produced a normal frequency distribution. We chose the following experimental

densities: 0 larvae/litre, to serve as a control; 72 larvae/litre, which was the geometric mean density; 13 and 394 larvae/litre, which were 1 standard deviation (SD) below and above the geometric mean; and 800 larvae/litre, which was approximately twice the mean+1SD and half the highest density observed. The ratio of first and second to third and fourth instars in the experimental groups (18:82) reflected the ratio of these instars found in our field collection of 4277 larvae. Experimental larval densities were assigned randomly to the numbered treeholes. Because of a shortage of larvae, only thirty-nine treeholes could be stocked. All density levels received eight replicates, except the 800 larvae/litre level, which was replicated in seven treeholes.

Establishment of experimental populations

Resident mosquito eggs and larvae were removed 1–2 days prior to the beginning of the experiment by rinsing each treehole with boiling distilled water. On 15 August 1986 we added to each treehole: treehole fluid, obtained from the combined contents of forty treeholes; *A. triseriatus* larvae, at previously determined densities; and four batches of eggs oviposited in the laboratory on basswood sticks. These eggs had been housed under damp conditions at 20°C in constant illumination, and were between 14 and 28 days of age. Control treeholes received only egg batches and fluid. The wooden sticks bearing between ten and eighty-nine eggs were lowered into the treeholes with plastic coated wires, which were anchored to nails at the treehole opening.

Determination of hatch rate

Egg masses were collected, one from each treehole, after 2, 4, 8 and 16 days of immersion, returned to the laboratory, kept under a natural photoperiod, and examined for hatching within 2 days of collection. Egg batches collected on days 2 and 4 of the experiment were placed in 6×50 mm glass tubes, which were filled with 1 g/l nutrient broth solution and immersed in additional broth solution within 14×150 mm test tubes. This procedure promotes hatching of nearly all non-diapausing eggs (Novak & Shroyer, 1978). Thus, we were able to determine the number of eggs that failed to hatch in

treeholes due to diapause. To assess viability, we examined all eggs that failed to hatch following stimulation, along with all unhatched eggs collected on days 8 and 16, by bleaching them with a 9% sodium chlorite solution, a process that renders the embryos visible through the egg shell.

To determine if larval density influenced hatch rate, we employed standard techniques of regression and analysis of variance. The angular transformation, $y' = a \sin \sqrt{y}$ was used to avoid an assumed non-normality and dependence of the variance on the mean in proportionate data.

Results and Discussion

The amount of time the egg batches were exposed to experimental conditions did not significantly influence egg hatch (Fig. 1; regression: $t_{141}=0.04$, $P>0.97$). Therefore, we pooled results across all exposure times. The absence of an effect due to time of exposure may have resulted from a rapid entry of eggs into diapause after exposure of the egg batches to the mid-August photoperiod. Based on the number of unhatched eggs that we were able to hatch under the Novak & Shroyer hatching stimulus, we determined that 74.8% of 457 unhatched eggs had entered diapause after 2 days, and 94.3% of 421 unhatched eggs had entered diapause after 4 days. This result concurs with the findings of Shroyer & Craig (1980), who observed entry of

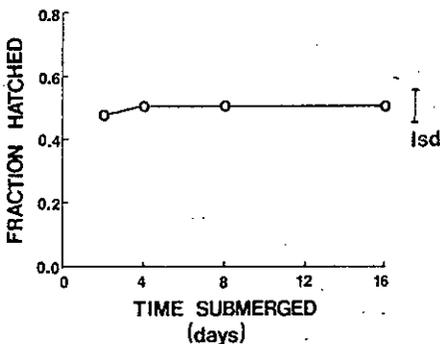


FIG. 1. Fraction of *Aedes triseriatus* eggs hatching as a function of time submerged in natural treeholes. Mean hatch rates are shown, pooled across all levels of larval density. Hatch rate is shown in units of radians, which resulted from the use of the transformation $y' = a \sin \sqrt{y}$, where y is the fraction of eggs hatched per batch. Tukey's least significant difference (lsd) is shown for visual comparison of means.

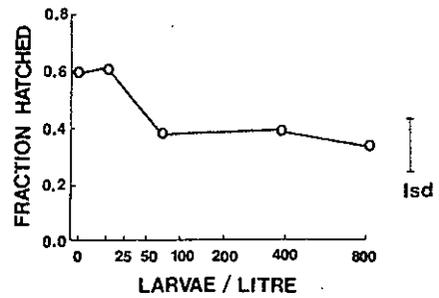


FIG. 2. Fraction of *Aedes triseriatus* eggs hatching in response to density of larvae in natural treeholes. Density is plotted on a square root scale. Mean hatching fractions are shown, pooled across all time intervals. Hatch rate is shown in units of radians, which resulted from the transformation $y' = a \sin \sqrt{y}$, where y is the fraction of eggs hatched per batch. Tukey's least significant difference (lsd) is shown for visual comparison of means.

most eggs into diapause within 4 days after exposure to a short photophase. We found a significant decrease in hatching rate as larval density increased ($F_{4,137}=3.33$, $P=0.01$) (Fig. 2).

Our results support the hypothesis that an increase in larval density affects hatching. The effect appeared at 72 larvae/litre (Fig. 2), the mean density for forty natural treeholes, suggesting that the density effect acts in many treeholes in our study population. This density effect occurred despite the declining photophase, between 13 and 13.5 h, which corresponded approximately with the critical photophase for populations of *Aedes triseriatus* at 41°N latitude (Sims, 1985). This entry into diapause produced an inadvertent source of bias against a significant effect of larval density on hatching rate. The absence of a significant increase in hatch rate due to time of immersion, coupled with our observation that a substantial number of eggs were viable but resisted even a strong laboratory stimulus, indicates that the eggs entered diapause quite rapidly. Most eggs that did not hatch within the first 2 days had entered diapause by the fourth day of immersion. Despite the brief pre-diapause interval during which a response to density could be observed, larvae imposed a significant negative effect on hatching rate. A more intense response might have resulted had the experiment been initiated earlier in the year.

This study demonstrates some of the complexity that may lurk among the mechanisms for

population regulation when individuals of different developmental stages interact. The inhibition of egg hatching by larvae bears potential consequences to the eventual success of the inhibited eggs. Specifically, hatching inhibition can radically alter the seasonal and competitive circumstances that the inhibited eggs will encounter. Inhibited eggs do not hatch into environments in which competitive effects of large larvae have been shown to be severe (Livdahl, 1982), and in which cannibalism may occur (Koenekoop & Livdahl, 1986); the price of this avoidance is a potentially lengthy period before these individuals can reproduce, particularly if diapause intervenes. Furthermore, this delay will expose the dormant or diapausing eggs to additional hazards of mortality.

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