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COMPETITION WITHIN AND BETWEEN HATCHING COHORTS OF A TREEHOLE MOSQUITO[1]

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Abstract. Aspects of population growth rates are examined for cohorts of the treehole mosquito *Aedes triseriatus*, reared in field containers under different conditions of larval density, concentration of natural treehole fluid, and in the presence or absence of a late-hatching cohort of larvae. Density and fluid concentration produced similar and independent significant competitive effects on each measure of cohort success, including female mass, development time, and survival to the adult stage, as well as on a composite index of performance. The sensitivity of first-cohort development time to high density and dilute fluid was reduced by the presence of the second cohort, and first-cohort survivorship responses to fluid concentration and density suggest an enhanced performance in dilute medium and intermediate density due to the addition of the second cohort. A composite index did not detect these complex interactions of experimental factors, suggesting that overall population growth rates for the first cohort respond to density and food in a very simple manner. The second cohort experienced total mortality in the presence of high first-cohort density, dilute medium, or the incidental oviposition of another treehole mosquito, *Anopheles barberi*, and never matched the success of the first cohort, even under conditions of low density and concentrated fluid.

Key words: Aedes triseriatus; Anopheles barberi; *competition; density dependence; hatching cohort; installment hatching; mosquito; population regulation; treehole.*

INTRODUCTION

Two types of approaches are commonly used for the detection of density dependence in animal populations. The analysis of sequential census and life table data using key-factor analysis and associated techniques (Varley and Gradwell 1960, 1968) has been applied widely to natural insect populations for which extensive density estimates are available. These techniques permit an assessment of the relative importance of various factors in the regulation of a population (Harcourt 1969). The analysis of life table data is useful for populations that possess discrete generations, or for populations in which generations overlap so completely that a stable age distribution can be assumed. Other methods must be sought when neither of these assumptions applies. Southwood et al. (1972) used key-factor analysis to examine *Aedes aegypti* survivorship data by isolating each larval instar in separate containers. Their approach permits the analysis of a population with partial overlap between generations, by forcing the larval cohorts into discrete groups. However, possible interactions among instars are prevented. Chubachi (1979) proposed a modified method for analyzing survivorship data that holds substantial promise for investigations of species with incompletely overlapping generations. All of these a posteriori approaches depend on correlations using nonindependent observations, and the data requirements for successful analyses are frequently prohibitive.

A second class of approaches is the direct manipulation of density and environmental factors that are suspected regulatory agents. Some of the disadvantages of a posteriori techniques, such as possible spurious correlations, are overcome by direct experimentation. Long-term census programs are not required, and there may be more power to detect density dependence. Other problems must be overcome, such as the prevention of immigration and emigration in experimental populations and the use of experimental densities that occur commonly in natural habitats. Manipulations of density have been applied successfully to enclosed populations (e.g., Eisenberg 1966, Neill 1974, Stiven and Kuenzler 1979) and to relatively sessile organisms (e.g., Connell 1961, Wise 1974) to yield understanding of interactions within and between populations that would not be available without direct experimentation. Organisms that inhabit small enclosures seem particularly suited to such investigations.

A posteriori analyses of insect life tables in natural settings are now fairly abundant, but there are remarkably few documentations of the importance of intraspecific competition by such methods (Stubbs 1977). In some cases, intraspecific competition is inferred when density dependence is detected and no natural enemies are apparent (Southwood et al. 1972, Chubachi 1979). Direct experiments with field insect populations are not common, although Istock et al. (1976) obtained very clear evidence of intraspecific competition in the pitcher plant mosquito, *Wyeomyia smithi*. Further examples are necessary before generalizations can be made about the regulatory importance of intraspecific competition in natural insect populations. I present the following investigations for that purpose.

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I have chosen an experimental approach to inspect intraspecific interactions in the treehole mosquito *Aedes triseriatus,* for several reasons. Primarily, the larva of this species is adapted to life in containers (water-filled tree cavities) which provide a convenient situation for manipulation. Standardized facsimiles of treeholes can be established that have an essential similarity to the natural habitat. The eggs of this species, like most *Aedes* mosquitoes, hatch at discrete intervals following inundation and a subsequent drop in oxygen tension due to a burst of microbial activity (Gjullin et al. 1941, Borg and Horsfall 1953, Judson 1960). Consequently, the establishment of larval populations in cohorts of an even developmental stage is a convenient and realistic procedure. Natural larval densities are not difficult to estimate, and those densities can be used as guidelines for establishing reasonable experimental densities. *Aedes triseriatus* larvae inhabit bodies of water that vary widely in their contents of protozoans and detritus, suggesting that manipulations of fluid concentration will result in food treatment levels that are experienced by portions of the natural population. Overlap among generations in this species arises from repeated oviposition by adult females and from the phenomenon known as "installment hatching," in which only a fraction of eggs of the same age will hatch in response to a given stimulus. Some eggs will remain dormant through numerous subsequent immersions (Buxton and Breland 1952, Means et al. 1977). These sources of generation overlap produce a situation in which there may be more than one discrete hatching cohort present within a treehole. Interactions between hatching cohorts have not been examined in the laboratory or in the field. Finally, *Aedes triseriatus* is the principal vector of LaCross encephalitis (Thompson et al. 1972), and there are clear practical reasons to investigate determinants of its distribution and abundance.

This paper describes the effects of manipulations of larval density, fluid concentration, and interactions between hatching cohorts on the performance of small groups of *Aedes triseriatus* larvae.

Methods

This project was designed as an analysis of variance with three experimental factors: larval density (three levels), fluid concentration (three levels), and cohort structure (two levels). Food availability should be strongly affected by manipulations of fluid concentration, although there are chemical factors that are also affected by this treatment. The cohort structure treatment consists of the addition of a second hatching cohort of larvae to half of the experimental habitats. I established three replicate containers for each combination of experimental treatments to permit inspections of larval success in response to interactions among the following treatments.

Larval density

It should always be possible to impose intraspecific competition in populations simply by using very high density treatment levels, although this approach provides little insight into the role of intraspecific competition in natural populations. Competition may be more difficult to detect, but more applicable to reality if density levels are dictated by larval densities that are experienced frequently by natural populations. I chose the mean larval density encountered in my study area and the upper and lower 95% confidence intervals of that mean as guides for the establishment of three density treatment levels.

In the 2nd wk of July 1979, I removed the entire fluid contents of 29 treeholes in a beech–maple forest in Princeton, New Jersey, USA, that has been relatively undisturbed, possibly for >200 yr (H. Horn, *personal communication*). The fluid was removed with a wide-mouthed Banta pipette, while the contents of each treehole were vigorously stirred. This procedure assured that the rich benthic ooze would be present for the experimental medium, and that larval counts would not be biased by escape movements of the larvae. Total counts of all larvae and pupae divided by the volume of fluid provided a density observation for each treehole. A logarithmic transformation of these densities produced a normal distribution about a mean density of 152 larvae/L. Upper and lower 95% confidence limits for this mean were 241 and 96 larvae/L, respectively. These are certainly underestimates of initial density conditions for first-instar larvae because mortality is not accounted for, and all larval instars were included in the counts. Experimental cohorts were established at initial densities of 20, 40, or 80 larvae/300-mL container. I obtained first-instar larvae for the experiment by scraping the walls of treeholes with a sculptor's gouge and immersing the scrapings in a yeast suspension for 24 h. Newly hatched larvae were pipetted into 300-mL, wide-mouth Nalgene® bottles, each containing one of three natural fluid concentrations.

Fluid concentration

After removing all larvae from the treehole samples, the fluid of all samples was pooled. Eighteen larval containers were filled with this pooled fluid, which was dispensed after thorough mixing to provide a control level of fluid concentration. Another 18 containers received 150 mL of this fluid, and 150 mL distilled water were added to fill each container, providing a dilute medium. The remaining 18 containers received 300 mL of fluid that had been concentrated to half its original 600-mL volume by filtration through a sieve of bolting cloth. This manipulation of medium concentration was intended to affect food levels primarily, although even the dilute concentration contained enough suspended detritus to produce a layer of ≈1 cm of benthic ma-

terial after settling. Therefore, food levels did not appear to be unrealistically low for this filter-feeding species, although an assessment of natural food conditions was not feasible in this study.

Cohort structure

The larvae were allowed to develop within the bottles for 7 d, after which 27 of the bottles received in addition a cohort of 20 first-instar larvae, obtained in the same manner as the first cohort. Three bottles for each density and fluid concentration combination received this second cohort. In each case, the first cohort had sufficient time and resources to achieve a substantial size advantage over the second cohort. Larval counts every 2 d for the duration of the experiment made it possible to distinguish the larvae of each hatching cohort. Consequently, the performance of each cohort can be examined separately, and effects of each cohort on the other can be obtained.

Physical conditions and sundry techniques

The bottles were placed in nearly natural physical conditions immediately after the initiation of the first hatching cohort on 21 July 1979. Each container was half buried in the forest floor immediately adjacent to the north sides of large trees, where treeholes are generally found. Positions in the forest were randomized for all experimental treatments.

The entire contents of each bottle were inspected every 2 d in a white enamel pan. Pupae were removed to the laboratory, where they were placed in water-filled scintillation vials covered with clear plastic cups. These pupal containers were checked daily for emergent adults, which were killed by desiccation in vials and dried in an oven for at least 1 mo. Adults were kept separate according to their original culture and their day of emergence, and individual dry masses were determined to the nearest 0.01 mg.

To isolate each population from ovipositing females, I placed a tongue depressor in each bottle, which provided a suitable substrate for oviposition. When eggs were found, the tongue depressors were replaced. This technique averted recruitment into the experimental habitats and provided a vertical substrate for microbial colonization.

This experiment terminated on 21 October 1979, when the last adult emerged after 92 d.

Analysis

The important observations in a study of factors affecting population growth should be features that relate closely to the rate of increase of a population. In principle, the per capita rate of change should be computed for each experimental population by standard life table methods to produce the best possible quantification of population performance. In practice, this parameter is unattainable because *Aedes triseriatus* requires conditions for mating and oviposition that could not be provided for 54 separate populations. However, three important components of the per capita rate of change can be estimated by examining the effects of experimental treatments on: female dry mass, a reliable predictor of eventual fecundity; female time to emergence, which sets the lower limit on reproductive age and should correlate well with generation time; and the fraction of larvae surviving to the adult stage. Mass and development time of individual females are not likely to be independent of the masses and development times of other individuals within a population if intraspecific competition occurs. Consequently, these separate correlates of the per capita growth rate (r) are analyzed below by using the mean female mass and the mean female development time for each replicate population as dependent variables, rather than individual masses and times. The use of mean values entails a large sacrifice of potential degrees of freedom for the sake of preserving the independence of observations. The use of mean values should also normalize the data within a given experimental cell.

TABLE 1. Analysis of variance for mean female mass of the first cohort in response to first-cohort density, fluid concentration, and the presence or absence of a second hatching cohort (cohort structure).

Source	SS	df	MS	F	P
Density (D)	6.45	2	3.23	12.31	<.001
Concentration (C)	3.96	2	1.98	7.56	<.005
Cohort structure (CS)	0.11	1	0.11	0.41	NS
$D \times C$	0.68	4	0.17	0.26	NS
$D \times CS$	0.89	2	0.44	1.69	>.25
$C \times CS$	0.20	2	0.10	0.39	NS
$D \times C \times CS$	2.06	4	0.52	1.97	>.10
Error	9.43	36	0.26		
Total	23.77	53			

Information about development time, adult mass, and larval survivorship can also be synthesized into a composite index of performance that combines the three features of cohort performance in a manner similar to the contributions of generation time, fecundity, and survivorship to the per capita rate of growth. The index is computed as follows:

$$r' = \frac{\ell n\left[\frac{1}{N_0}\sum_x \bar{w}_x A_x f(\bar{w}_x)\right]}{D + \left[\sum_x x\bar{w}_x A_x f(\bar{w}_x)\right] / \sum_x w_x A_x f(\bar{w}_x)}, \quad (1)$$

and is a direct analogue of the familiar approximation of per capita growth rate:

$$r \simeq \frac{\ell n \sum_x \ell_x m_x}{\tau}. \quad (2)$$

In Eq. 1, x is a given day of adult emergence, $\bar{w}_x$ is

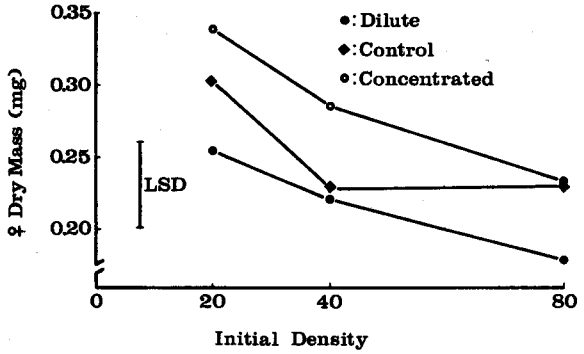


FIG. 1. Responses of the mean female dry mass per bottle for the first hatching cohort to the initial density of the first cohort (number per bottle) at three different concentrations of fluid. Points represent means summed across both treatments of cohort structure. Tukey's least significant difference (LSD) is shown for comparison of cell means.

the average mass of females emerging on that day, A_x is the number of females emerging on day x, N_0 is the initial number of female larvae in the cohort (assumed to be half the initial total), and $f(\bar{w}_x)$ is a function converting female biomass into the expected per capita number of female offspring. D is a constant determined by the delay between adult emergence and oviposition. A least-squares line has been found to describe the relationship between dry mass and the number of female eggs produced:

$$f(\bar{w}_x) = 7.13 + 45.85\bar{w}_x. \quad (3)$$

A crude estimate of $D = 7$ is used in all computations of r', to allow time for mating, bloodmeals, and egg maturation. One of the advantages of the composite index is that possible ambiguities in responses, such as retarded development coupled with increased survivorship, are avoided. I present composite indices as the best available descriptive quantities of cohort performance. Analyses of separate correlates of r are also presented for completeness.

RESULTS

Mean female mass of the first cohort

The analysis of variance for the effects of the three treatments and their interactions on mean female mass appears in Table 1. The initial density of larvae and the concentration of fluid are significant determinants of female mass. The addition of a second cohort has no detectable effect on the size of females from the first cohort. Consequently, the two cohort treatment levels are pooled in Fig. 1 to illustrate the response of mean female mass per bottle to density and fluid concentration. The nonsignificant interaction between larval density and fluid concentration indicates that the abundance of food, while a significant factor, acts independently of larval density (Fig. 1).

TABLE 2. Analysis of variance for mean female development time of the first cohort in response to first-cohort density, fluid concentration, and cohort structure.

Source	SS	df	MS	F	P
Density (D)	1085.88	2	542.94	20.86	<.001
Concentration (C)	730.50	2	365.25	14.03	<.001
Cohort structure (CS)	8.07	1	8.07	0.31	NS
$D \times C$	179.43	4	44.86	1.72	>.10
$D \times CS$	224.39	2	89.72	3.45	<.05
$C \times CS$	238.72	2	119.36	4.59	<.025
$D \times C \times CS$	236.32	4	59.08	2.27	>.10
Error	936.91	36	26.03		
Total	3640.22	53			

Mean female development time of the first cohort

A more complex result is obtained by examining the mean female development time for each culture (Table 2). Again, the initial density of larvae and the concentration of fluid produce strong effects, and there is no significant main effect due to the addition of a second cohort. The significant interaction between the fluid concentration and cohort structure treatments can be interpreted by inspecting the mean female development time, summed over all density levels, in response to fluid concentration (Fig. 2). The interaction results from a more rapid development of females in dilute fluid when the second cohort is added. Development time at the other two concentration levels does not differ significantly between the two treatments. The significant density × cohort structure interaction is shown in Fig. 3. The only significant difference between the two cohort structure groups occurs at the highest density (80 larvae/300-mL container), at which the addition of a second cohort enhances the rate of development of the first cohort.

The significant main effects due to fluid concentration and density are apparent in Figs. 2 and 3, respectively, and there is no evidence that food and density have interdependent effects on female development time.

The accelerated development of the first cohort in response to low levels of food per individual may be due to cannibalism of the small larvae by the larger ones, or it may result from starvation of the small larvae followed by saprophagy. At both the high density and low fluid concentration levels, no larvae of the second cohort survived to adulthood in any of the 15 cultures.

Survivorship of the first cohort

The analysis of the fraction of the initial cohort that survived to the adult stage is given in Table 3. Again, there are strong main effects from density and fluid concentration, with no overall effect due to the addition of a second cohort. Again, the effects of fluid

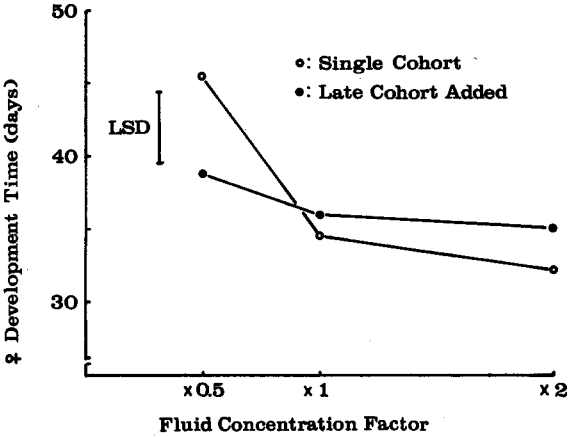


FIG. 2. The effect of the interaction between the concentration of fluid and the cohort structure treatment on first-cohort mean female development time. Points represent means summed across all density levels. Tukey's LSD is shown for comparison of means.

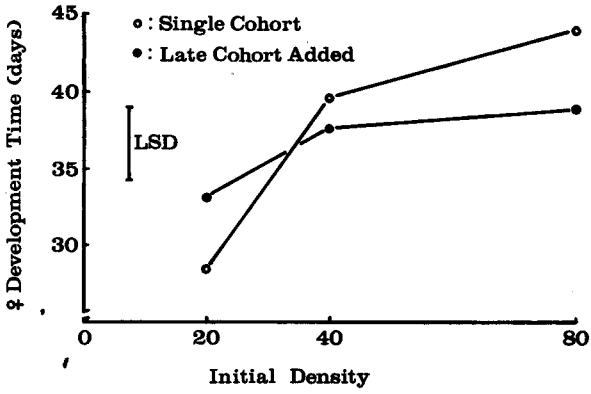


FIG. 3. The effect of the interaction between density of the first cohort and the cohort structure treatment on first-cohort mean female development time. Points represent means summed across all levels of fluid concentration. Tukey's LSD is provided for visual comparisons.

concentration are not affected by density, although the significant three-way interaction among all treatments must now be explored.

Mean survivorship is plotted against fluid concentration and initial density for each of the cohort treatment groups in Fig. 4. The nonsignificant density × concentration interaction indicates that the two surfaces are generally symmetric. The significant main effects indicate that each surface tilts downward in response to high density and dilute fluid. The nonsignificant main effect for cohort structure implies that the two surfaces have similar elevations. The source of the three-way interaction is obtained by subtracting the mean survivorship of the single-cohort treatment level from the value for the double-cohort group at each density and fluid concentration combination. These differences between cells depict the effect of the second cohort on the first at each combination of treatments (Fig. 5). Most of the significant three-way interaction appears to arise from the single significant difference between the two response surfaces of Fig. 4, which is found for the dilute fluid at the intermediate density. It is apparent that the combined effects of high density and dilute fluid are so severe that first-cohort survivorship is insensitive to the addition of 20 new larvae even if they can be consumed by the first cohort, while at low density and dilute food there are sufficient resources available for the first cohort, and the second cohort does not produce an effect. Only at the intermediate density is there a significant response to the second cohort, and it is a positive response. Such a result might be expected from a strategy of facultative cannibalism, in which small larvae are eaten by large ones only as a last resort during hard times. Alternative explanations are that the interaction represents a type II statistical error (i.e., the difference is really due to chance), or that the mixing of fluid did not result in sufficient randomization of the concentration treatment. It is clear that further investigations of responses to food shortage and high densities are needed to resolve the possible causes of the higher-order interactions affecting survivorship and female development time.

Composite index of performance for the first cohort

A more holistic view of performance is provided by the surrogate of the per capita rate of growth obtained from Eqs. 1 and 3. Analysis of variance for this quantity (Table 4) indicates that the separate features of performance inspected above may cancel each other to obscure any interactions among independent variables. Only the main effects of density and fluid concentration are significant. There is no significant effect from the addition of the second cohort, and there is no evidence that the limitations imposed by fluid con-

TABLE 3. Analysis of variance for first-cohort survivorship to the adult stage in response to first-cohort density, fluid concentration, and cohort structure. The angular transformation for proportionate data was applied.

Source	SS	df	MS	*F*	*P*
Density (*D*)	9377.75	2	4688.87	44.30	<.001
Concentration (*C*)	4190.02	2	2095.01	19.79	<.001
Cohort structure (*CS*)	10.01	1	10.01	0.09	NS
$D \times C$	159.82	4	39.96	0.38	NS
$D \times CS$	37.42	2	18.71	0.18	NS
$C \times CS$	54.73	2	27.37	0.26	NS
$D \times C \times CS$	1146.63	4	286.66	2.71	<.05
Error	3810.32	36	105.84		
Total	18 786.70	53			

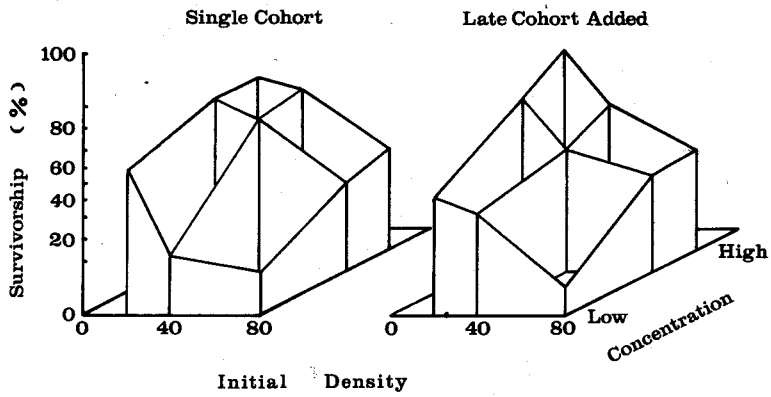


FIG. 4. Survivorship (%) of the first cohort in response to fluid concentration and first-cohort density for the two cohort structure treatments. The vertical scale converts survivorship into the more appropriate arcsin $\sqrt{y}$ transformation for proportionate data to remove the dependence of the variance on the mean.

centration become more or less intense as density is changed. The latter finding implies that food and density have similar and independent effects, i.e., a doubling of population density has approximately the same effect on performance as a halving of food level.

If fluid concentration and density have similar effects, the effective density per unit of resource may be expressed as the ratio between density and concentration. The composite index r' is plotted against the number of larvae per original volume of treehole fluid before dilution or concentration (a measure of density per unit food) in Fig. 6, which shows that performance at all densities and fluid concentrations is well described by a single regression line. Analysis of covariance to test for differences among the separate least-squares lines of the three density groups supports this finding ($F_{2,50} = 2.22$, $P > .10$).

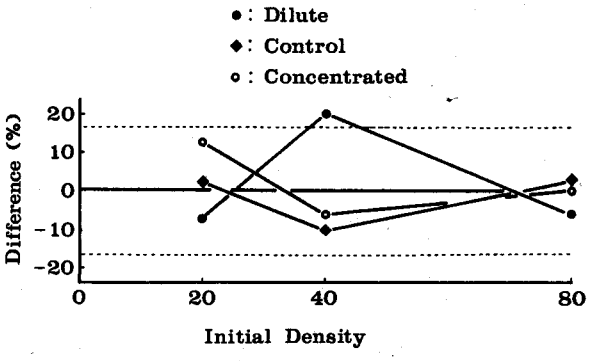


FIG. 5. Interpretation of the effect of the density × concentration × cohort structure interaction on first-cohort survivorship (%). Each point was obtained by subtracting the mean survivorship of the single-cohort group from the mean survivorship of the double-cohort group at each combination of density and fluid concentration. Points falling between the dashed lines, which depict Tukey's LSD, indicate nonsignificant differences.

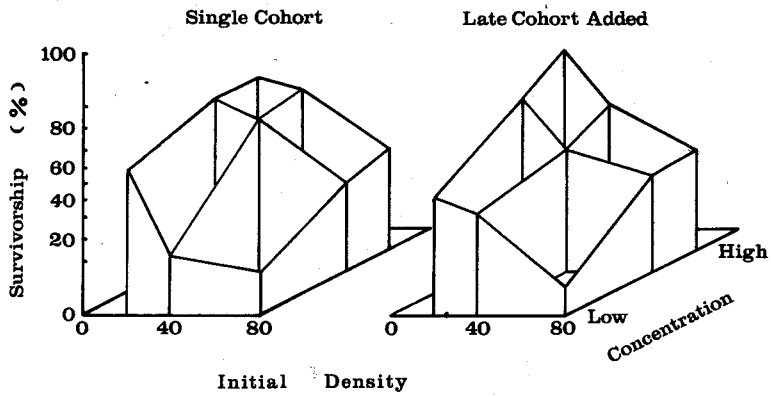
Performance of the second cohort

Of the 27 bottles containing second cohorts, only 9 bottles produced any second-cohort survivors at all. Consequently, statistical analyses of aspects of performance for this cohort are awkward, although the results are quite clear. The distribution-free method proposed by Wilson (1956) is analogous to a two-way analysis of variance and permits a test of the interaction between factors, which are in this case fluid concentration and density of the first cohort. This method is used because of clear heterogeneity of variance within cells.

The percentage of second-cohort members that survived to the adult stage is shown in Table 5. The extreme effects of high first-cohort density and dilute fluid are obvious from inspection. Both main effects are significant ($\chi^2_{[2]} = 7.0$; $P < .05$ for each treatment). The interaction between fluid concentration and first-cohort density is not significant by Wilson's test ($\chi^2_{[4]} = 5.0$; $P > .25$). Exactly the same statistics are obtained in an analysis using the composite index r' (Table 6), because this nonparametric technique is

TABLE 4. Analysis of variance for a composite index of growth performance, r', of the first cohort in response to density, fluid concentration, and cohort structure.

Source	SS	df	MS	F	P
Density (D)	2.02×10^{-2}	2	1.01×10^{-2}	33.24	<.001
Concentration (C)	1.22×10^{-2}	2	6.12×10^{-3}	20.12	<.001
Cohort structure (CS)	1.39×10^{-4}	1	1.39×10^{-4}	0.46	NS
$D \times C$	1.97×10^{-3}	4	4.92×10^{-4}	1.62	>.10
$D \times CS$	6.66×10^{-4}	2	3.33×10^{-4}	1.09	>.25
$C \times CS$	4.29×10^{-5}	2	2.15×10^{-5}	0.07	NS
$D \times C \times CS$	1.77×10^{-3}	4	4.42×10^{-4}	1.45	>.10
Error	1.09×10^{-2}	36	3.04×10^{-4}		
Total	4.78×10^{-2}	53			



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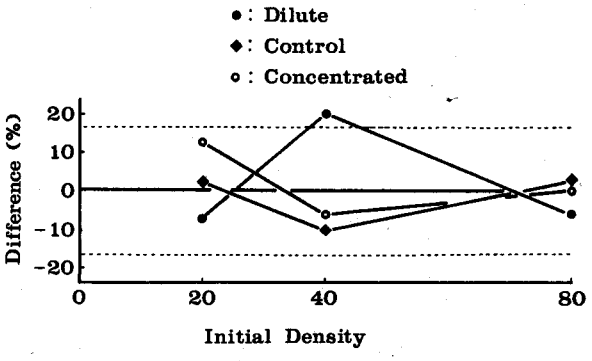

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Total	4.78×10^{-2}	53			

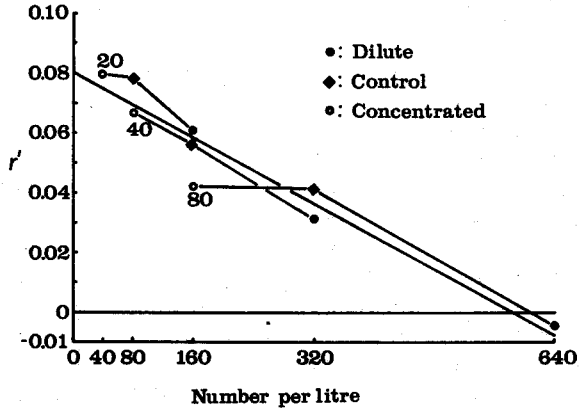

FIG. 6. The response of a composite measure of growth performance, r', of the first cohort to the ratio between first-cohort density and fluid concentration, expressed as the number of larvae per litre of original treehole fluid (prior to dilution or concentration). See Methods section for definition of r'. Points represent means summed across both cohort structure treatment levels. Groups of equivalent numbers of first-cohort larvae per bottle are connected and labelled. The least-squares regression line for the entire collection of points is shown.

based on frequencies within each cell that are above and below the overall median.

Eighteen of the cultures have missing values for mean female mass and mean female development time because no adults emerged (Tables 7 and 8), so analyses of these features are confined to the combinations of concentrated or control fluid with low or intermediate first-cohort densities. No significant effects occur for development time or female mass within this limited domain of first-cohort densities and fluid concentrations, possibly because of the restricted number of observations.

We might expect that the cell with the most concentrated fluid and the sparsest population of the first cohort would produce the most successful second cohorts, yet this did not occur. In fact, two of the three replicates within that cell had no surviving members of the second cohort, which probably resulted from oviposition by another treehole mosquito, *Anopheles barberi*. Bottles in which *A. barberi* oviposited are noted in Table 5. These episodes of oviposition occurred during the week before the second cohort was added. *A. barberi* larvae had sufficient time to gain a size advantage over the late cohort, and I suspect that the complete mortality of the late cohort in all cultures that contained *A. barberi* resulted largely from predation. *A. barberi* oviposits on the surface of the water, so my technique for preventing *Aedes triseriatus* oviposition was ineffective for this species. I have observed predation by fourth-instar *A. barberi* larvae on first-instar *Aedes triseriatus* in the laboratory. However, I did not expect *A. barberi* to be a factor in these experiments because the species is consistently difficult to find in natural habitats. *A. barberi* does seem to have a preference for the most turbid treehole fluids, which is apparent from its choices of oviposition sites among the available experimental habitats. *A. barberi* was never found in dilute or control cultures, and oviposition occurred in 7 of the 18 concentrated cultures.

TABLE 5. Survivorship to adulthood of the second cohort (percent) in response to first-cohort density and fluid concentration. Cultures that were colonized by *Anopheles barberi* are noted with asterisks.

First-cohort density	Fluid concentration		
	Concentrated	Control	Dilute
	Survivorship (%)		
20	20	25	0
	0*	30	0
	0*	50	0
40	5	15	0
	10	5	0
	10	0	0
80	0*	0	0
	0	0	0
	0	0	0

TABLE 6. Composite indices of performance, r', for the second cohort in response to first-cohort density and fluid concentration.

First-cohort density	Fluid concentration		
	Concentrated	Control	Dilute
		r'	
20	0.054	0.032	$-\infty$
	$-\infty$	0.023	$-\infty$
	$-\infty$	0.042	$-\infty$
40	0.028	0.029	$-\infty$
	0.025	0.016	$-\infty$
	0.022	$-\infty$	$-\infty$
80	$-\infty$	$-\infty$	$-\infty$
	$-\infty$	$-\infty$	$-\infty$
	$-\infty$	$-\infty$	$-\infty$

TABLE 7. Mean female dry mass (milligrams) per culture for the second cohort under conditions of first-cohort density and fluid concentration that permitted adult emergence. Dots indicate cultures from which no second-cohort adults emerged.

First-cohort density	Fluid concentration	
	Concentrated	Control
	Mass (mg)	
20	0.41	0.59
	...	0.80
	...	0.41
40	0.45	0.37
	0.34	0.44
	0.34	...

TABLE 8. Mean female development time (days) per culture for the second cohort under conditions of density and fluid concentration that permitted adult emergence.

First-cohort density	Fluid concentration	
	Concentrated	Control
	Development time (d)	
20	47	45
	...	74
	...	45
40	52	65
	63	67
	64	...

DISCUSSION

These results permit three firm conclusions, raise at least four approachable questions, generate a methodological caveat, and provide two theoretical lessons.

Competition among larvae is demonstrated clearly by the strong responses of each measure of success to larval density. I expect that this phenomenon occurs routinely in natural habitats because the effect appears among density levels that were set according to natural densities in a very conservative manner.

The larvae most probably compete for food, which is the factor most likely to be altered by the manipulation of fluid concentration. Each aspect of larval performance responds positively to an enriched medium. Food and density manipulations appear to have interchangeable effects.

Cohorts of early hatching larvae can gain a sufficient size advantage to impose a strong adverse effect on cohorts hatching only a week later. This adverse effect does not appear to be reciprocated by the later cohort. In fact, there is some indication that the early cohort benefits from the presence of small larvae during hard times (Figs. 2, 3, and 5). It is not clear how these benefits arise, or how significant the benefits are in biological terms. The adverse effect of the early larvae on the second cohort is consistent with the "size-efficiency hypothesis" posed by Brooks and Dodson (1965), who contend that large filter feeders should compete successfully with smaller filter feeders because they are more efficient and can exploit a wider range of food particles. In this case, the late cohort was completely eliminated in all but the highest levels of food per individual, and it never approached the success of the first cohort (compare Fig. 6 with Table 6). In light of these findings, it should not be surprising to find a strong inhibitory response of egg hatching to high larval densities in natural habitats, because the time spent in waiting for less-crowded conditions would be compensated by improved chances of survival. Gillett et al. (1977) suggest that hatching delays are induced by larvae that graze the bacteria from the egg surface, thus removing the source of oxygen reduction that stimulates hatching. This well-documented response of hatching to declines in oxygen content among aedine mosquitoes appears highly adaptive, when the bleak prospects for success of a late-hatching cohort in dilute fluid are considered.

Of course, this study does not eliminate alternatives to intraspecific competition as agents in the regulation of *Aedes triseriatus* populations, although the results may be useful as a guide to further work. Now that intraspecific competition has been detected, it is likely that inspections of competitive interactions with other species will be of interest. The correlation of late-cohort mortality with the presence of *Anopheles barberi* merits a much closer look, although that species is so rare that it will be difficult to use in large experiments without successful laboratory colonization. Several other potential competitors also share the treehole habitat, including *Orthopodomyia signifera* (another uncommon treehole mosquito), syrphids, psychodids, and ceratopogonids among the Diptera, and helodid beetle larvae. This experiment should be duplicated in the Southeast (USA), where an interesting difference might be proposed because of the presence of the predatory mosquito *Toxorhynchites rutilus*. Repetition of these fluid and density manipulations at different times during the growing season could detect temporal changes in the importance of competition. A long-term key-factor study could reveal the importance of other modes of regulation, such as larval parasites.

The questions concern the evolution of installment hatching and the possible role of density-induced hatching delays in population regulation. Has installment hatching evolved as a response to a competitive environment? If so, we should expect that hatching delays have the net effect of reducing intraspecific competition. This effect does not seem likely without the accumulation of a massive pool of dormant eggs during periods of high density. While this response should retard population growth below the carrying capacity, there is no mechanism for population declines to adjust to seasonal reductions in the carrying capacity. Consequently, we should not expect regulation to be achieved solely by hatching delays, and the reduction of larval competition by delayed hatching alone does not seem likely. However, a source of mortality in the dormant bank of eggs could create a plausible mechanism for reducing larval competition, in addition to providing a precise means of resource tracking. The fate of dormant eggs in natural habitats deserves a thorough inspection, and schedules for hatching during the growing season should be constructed.

My methodological caveat concerns the use of separate correlates of the per capita rate of change, including survivorship and measures of individual success, to test hypotheses about factors affecting population growth rates. The detection of effects on separate components of the per capita rate does not

necessarily imply that the rate is affected, because there may be subtly ambiguous effects on the separate components. Consequently, the most biologically significant measure of population success may not respond to statistically significant effects on its separate correlates. My results provide three examples of this possibility. When survivorship is considered separately, a complex interaction among the three factors is detected (Table 3). A separate analysis of female development time reveals two significant interactions between pairs of independent variables (Table 2). Separate judgments about factors affecting population growth by these criteria would lead to a conclusion that the complexities of intraspecific interactions are severe. However, when we attempt to combine separate components of the per capita rate into a composite quantity for each population (r'), the higher order interactions among experimental factors disappear (Table 4), leaving us with a relatively simple conclusion about the factors affecting growth rates of the first cohort (Fig. 6).

The experimental dilemma regarding the choice of a dependent variable is only solved when we have confidence in its predictive capacity for population dynamics. The composite index (r') appears to offer more information about per capita success than do any of its components, but a firm judgment about its predictive powers must await experimental validation. This paper is not intended to discourage the inspection of separate features of performance, because such analyses can provide important insights into mechanisms by which growth rate is changed. The lesson of this paper is that simple population growth models may be robust to deep internal complexities that govern the mechanics of population regulation.

Inferences about the validity of theoretical treatments of population dynamics, including the assumptions and predictions of models, have been made on the basis of judgments arising from analyses of separate components of r. Wilbur (1977) analyzed the rates of individual biomass accumulation in frog cohorts to demonstrate nonlinear responses to food and density, and a significant food × density interaction. However, we should not reject the hypothesis of linear responses and independence between food and density effects without incorporating survivorship into the dependent variable. A similar analytical approach was used by Smith-Gill and Gill (1978), in which coefficients of competition between species were found to vary with density. Again, one must wonder if a violation of the theoretical assumption of linear competition effects would be observed if survivorship entered into the measures of population success. Wilbur (1972) inspected separate correlates of r (individual mass at metamorphosis, individual time to metamorphosis, and survival to metamorphosis) to obtain statistically significant higher-order interactions among coexisting cohorts of amphibian species. This finding was interpreted as an example of the inadequacies of the Lotka-Volterra competition equations, although an analysis of an aggregate measure of population success, similar to r', in which the three variables are combined, might lead to a radically different conclusion.

The simple response of the first cohort to density and fluid concentration (Fig. 6) suggests that a simple linear model of density-dependent population growth may apply to situations in which there are no larvae present at the time of a hatching stimulus, such as early spring or following droughts. However, predictions of success for hatching cohorts that enter an occupied habitat will require substantially more information pertaining to food conditions, the density of the established larval populations, and the size distribution of the larvae. The latter constraint will depend on the quantification of interactions among larval groups at various developmental stages.

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