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Density-dependent interactions within a complex life cycle: the roles of cohort structure and mode of recruitment

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Summary

1. We analysed the effects of cohort structure, density, egg hatch inhibition and cannibalism on estimated per capita growth rate (r') in populations of *Aedes triseriatus* established in artificial habitats in Massachusetts.

2. As density increased from 0·5 K to 0·75 K to the estimated carrying capacity ($K = 60$ larvae per 100 ml), r' decreased, along with other measures of success: survivorship, female size, and development rate.

3. Cohort structure and recruitment schedule significantly influenced r'. Populations initiated as eggs achieved r' values greater than cohorts started as first instar larvae and substantially greater than those populations consisting of single cohorts.

4. We did not observe a significant relationship between hatch rate and larval density. In addition, hatch inhibition did not require direct larval contact with eggs.

5. We found no evidence for cannibalism in our experimental habitats, which were stocked with leaf detritus and treehole water, suggesting that refugia offered protection not gained in a simpler laboratory setting or that large larvae had sufficient levels of alternative food sources (Koenekoop & Livdahl 1986).

6. Egg-initiated cohorts exhibited the greatest developmental asynchrony during the first month of the experiment, whereas cohorts added as 2-day-old larvae displayed highly synchronized development. Increased competitive interactions in this latter treatment may explain the small size attained by adult females emerging from these populations. Differences among multiple cohort groups increased with increasing density.

7. Our results emphasize the need to incorporate overlapping stages into experimental designs for populations that develop asynchronously, and the value of using r' as a descriptor of success rather than the individual components of success (e.g. survivorship, size, and development rate) which can lead to misinterpretations of productivity.

Key-words: density dependence, mosquito, *Aedes*, competition.

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Introduction

Communities of container-breeding mosquitoes have shown substantial promise for testing many kinds of basic ecological and evolutionary questions (e.g. Istock, Wasserman & Zimmer 1975; Bradshaw & Holzapfel 1983; Lounibos 1983; Hard, Bradshaw & Malarkey 1989; references in Service 1985). In this paper, we investigate potential mechanisms of population regulation in the treehole mosquito, *Aedes triseriatus* Say.

In the southern US, where the predatory mosquito *Toxorhynchites rutilus* (Coq.) occurs, the work of Bradshaw & Holzapfel (1983) and Lounibos (1985) suggests that predation, as well as desiccation (Bradshaw & Holzapfel 1988), may alleviate resource competition among mosquito prey. In contrast, beyond the range of the predator (generally north of the Ohio River Valley, Pennsylvania and New Jersey (Jenkins & Carpenter 1946)), manipulation of *A. triseriatus* larval densities in field containers filled with treehole fluid has revealed strong compe-

tition among larvae within experimental cohorts, and a particularly adverse effect of an earlier on a later cohort (Livdahl 1982).

In our experiment, we explore the population consequences of density-dependent interactions among life cycle stages in *A. triseriatus* in Massachusetts. Like most *Aedes*, this mosquito hatches from eggs laid in clusters on the sides of the container, usually just above the water line. Although embryogenesis appears to proceed within a few days with no interruption (Shroyer & Craig 1980), the fully formed embryos will not hatch until inundation and an ensuing drop in oxygen concentration (Gjullin, Hegarty & Bollen 1941; Borg & Horsfall 1953; Judson 1960). Eggs differ in their response to inundation; some hatch following the first rainfall of the spring, while others require multiple dousings (Livdahl & Koenekoop 1985). In addition, hatch rate decreases in response to moderate and high larval densities (Livdahl, Koenekoop & Futterweit 1984; Livdahl & Edgerly 1987). Such asynchronous hatching results in treeholes stocked with multiple cohorts. For example, in a sample of 40 treeholes containing a total of 4277 larvae in Worcester, Massachusetts, we found the ratio of first and second to third and fourth instars to be 18 : 82 (Livdahl & Edgerly 1987).

In spite of the complex life history resulting from asynchronous hatch, previous experimental studies of population regulation in this species have commenced with single, synchronized cohorts. We suspected that staggered hatching may have profound effects on such critical measures of performance as survivorship, development time and fecundity, and on the response of these measures to larval density. Given the potential for strong selective pressure on the timing of hatch, we asked whether hatching will occur in such a way as to maximize overall average fitness as well as the fitness of larvae that delay their entry into the treehole when they are presented with multiple options of hatching times under varying experimental densities.

Our experiment permits an assessment of the relative effect of the following factors on per capita success:

1. egg–larva interactions, where larvae may influence the hatching stimulus (Gillett, Roman & Phillips 1977);

2. larva–larva interactions, including competition and cannibalism.

If larvae do hatch into an environment with other larvae, the potential exists for competitive adversity (Fish & Carpenter 1982; Livdahl 1982) or even death by cannibalism (Koenekoop & Livdahl 1986). If larvae delay hatching, delayed reproduction may reduce their success. We subjected larvae to two different recruitment schedules and three different density levels to examine the influence of hatch delay and cohort structure on competition.

Methods

GENERAL PROCEDURES

Experimental animals. Female mosquitoes, caught while biting in Worcester Co., Mass., were allowed to oviposit on wooden slats in the laboratory. These slats were carved into sticks containing egg batches of the necessary sizes (see below).

Medium. In an effort to construct experimental habitats with essential aspects of natural treeholes, we stocked 125 ml plastic jars with 100 ml of field-collected treehole water on the first day of the experiment. We removed insect larvae from the fluid (approximately 30 l) which we then stirred in a plastic garbage can with a canoe paddle as we dispensed fluid into the jars. To simulate the nutrient levels and add some of the physical complexity of natural treeholes, we added to each jar one beech leaf collected from a treehole and stemflow water, collected from a slit rubber hose caulked about a maple tree in a nearby forest. This stemflow was added to the experimental habitats weekly after the first rains occurred during the week of 22 July. When adding stemflow, we submerged a plastic vial into each jar, withdrew and disposed of 25 ml of the experimental water, and added 25 ml of fresh stemflow. The routine of discarding rearing water was intended to mimic the effect that natural rainfall has on treeholes: removing metabolic wastes and replenishing nutrients by periodic flooding and overflowing (E.D. Walker, D.L. Lawson, R.W. Merritt, W.T. Morgan & M.J. Kluge, unpubl. data).

Seventy-five loosely capped jars (sufficient for five replicates of each treatment combination) were stocked on 22 June 1988 with a first cohort of newly hatched larvae, and were stored on a concrete floor in an open garage in Worcester, so that the mosquitoes were exposed to natural temperature and light conditions. This semi-protected environment prevented disturbance by wild animals, a problem which plagued a previous attempt of this field study (summer 1987; unpubl. data). Additional cohorts (see below) were added to some jars on each of the next three Wednesdays. To obtain hatch rate, development and survivorship data for newly hatched larvae, we censused each jar every Friday in a nearby screened room, by removing each larva with a glass pipette, recording its stage of development, and placing it in a plastic holding-container. Once all larvae were counted, we returned them to their original jars. Treehole water and stemflow exchanges were also performed at those times. Pupae were removed each Wednesday and Friday and placed in glass vials capped with inverted vials in the laboratory at 22°C (16 h : 8 h L : D). Adults were harvested daily and frozen until measurements could be made. Weekly censuses continued until no lar-

vae remained, 120 days following commencement of the study.

EXPERIMENTAL TREATMENTS AND RATIONALE

Density and recruitment pattern

We exposed larvae to different potential sources of competition in a nested design that included three density levels and two recruitment schedules. The experimental habitats were stocked with either a single cohort of larvae or with multiple cohorts added in four batches. Within each of these categories, three density levels were established. Multiple cohorts were added either as eggs or as first instar larvae (see below). We used an estimate of carrying capacity (K) from a previous field study on *A. triseriatus* (Livdahl 1982), in which K was approximately 60 larvae per 100 ml. We established our lowest density at approximately $K/2$, the point on the logistic growth curve where production, or yield, should be maximized (Clark 1981). To create a greater potential for competition, cannibalism and egg hatch inhibition, we established two higher densities at $3/4K$ and K. The total densities of larvae in each jar, distributed as either one or four equal cohorts, were 28, 44, and 60 larvae per 100 ml treehole water.

MULTIPLE COHORT TREATMENTS

Egg hatch inhibition

We designed two different treatments to analyse the mechanism, as well as the consequences to population growth, of asynchronous hatch. The density at which hatch inhibition occurred in a previous field study (Livdahl & Edgerly 1987) was approximately the mean density (7·2 larvae per 100 ml) found in 40 natural treeholes at our field site. Thus, the lowest density in the present study was well above the level needed to trigger egg hatch inhibition. To test the effect of egg–larva interactions we immersed eggs into the artificial treeholes in 1 dram glass vials that were either open to allow larval contact or capped with nylon mesh screening (0·8 mm mesh) secured with a rubber band over the opening of the vial to prevent large larvae contacting the eggs. For identification purposes, we pinned a tiny square of filter paper labelled with India ink onto each stick holding the eggs. All other treatments received as controls wooden sticks, pins, paper labels, empty vials, rubber bands and nylon mesh.

To simulate the staggered recruitment observed in natural treeholes, three cohorts of egg batches were introduced into jars already containing the first cohort. First, each jar was stocked with a cohort of newly hatched first instar larvae of one of the three density levels (7, 11 or 15). To this, three egg batches were introduced so that final densities would equal 28, 44 or 60 larvae per 100 ml as prescribed by the argument above concerning K. To mimic natural fluctuations of drying and inundation within the treehole, we exposed the eggs to periodic immersions by leaving each batch in the water for 1 week, then removing it for 1 week. Egg batches held out of the water were kept separately in uncapped glass vials alongside the artificial treeholes so that lighting conditions were consistent for all eggs and larvae. We repeated this immersion schedule such that each batch was immersed five times during the experiment.

Prior to the experiment, fertility of laboratory eggs was estimated by opening eggs under the dissecting scope and determining percentage embryonated. From this we estimated a fertility rate of 73%, and therefore increased the number of experimental eggs per batch proportionately so that each of the three cohorts of eggs would average 7, 11 or 15 fertile eggs. Following the experiment, we determined that the actual fertility rate was 79·7% by adding the number of fertile unhatched eggs, determined by dissection, to the number hatched. Therefore, our resulting average experimental densities were slightly higher than called for in the initial design. All analyses on the effect of density take this difference into account.

Cannibalism

Cannibalistic behaviour in *A. triseriatus* was discovered previously in a simpler environment where food-deprived fourth instar larvae were placed in 30 ml vials with 1-h-old or 24-h-old first instar larvae; cannibalism was uncommon among the 24-h-old first instars (Koenekoop & Livdahl 1986). To test the importance of cannibalism to density-dependent processes in more complex habitats, we added three cohorts of first instar larvae in the following manner. The jars already contained the first cohort of larvae at the densities described above. To acquire the additional cohorts, eggs were hatched in the laboratory 2 days prior to addition to the jars. The resulting larvae were separated into batches of 7, 11 and 15 and placed in 17-ml plastic containers with deionized water, plus a small amount of nutrient broth that was transferred with each larva. On three consecutive Wednesday mornings, eggs were hatched and the newly hatched larvae were sorted in the same manner as above. All larvae, 2-days old and newly hatched, were then added to the jars. The following Friday, the incidence of cannibalism was assessed by determining the number of larvae lost during the previous 2 days. This number was compared to the mortality rate of first instars in the first cohort after their first 2 days in the artificial habitats. The differences between the two treatments thus reflect the differences between cohorts that are exposed to cannibalism and competition (newly

hatched larvae) and those that are exposed only to competition (2-day-old larvae).

STATISTICAL METHODS

Dependent variables

We employ an estimate of the per capita growth rate of change dN/Ndt, as a convenient overall summary of average individual success for experimental populations (Livdahl & Sugihara 1984). This variable gathers information about separate components of population success (development time, adult size, and survival to adult emergence) in a manner similar to the combination of age-specific survival and fecundity schedules in the calculation of the rate of change for a cohort in which such schedules can be obtained, and is calculated as follows:

$$r' = \frac{\ln\left[\frac{1}{N_0}\sum_x A_x f(W_x)\right]}{D + \frac{\sum_x x A_x f(W_x)}{\sum_x A_x f(W_x)}}$$

in which N_0 represents the initial number of females (assumed to be half the initial number of individuals); x is time elapsed since the population was initiated; A_x is the number of females emerging on day x; w_x is the mean size (measured as wing length in mm) of females maturing on day x; $f(w_x)$ is a function predicting number of female offspring per female based on female body size; and D is the time delay between female emergence and first oviposition. Separate unpublished studies of isolated individual females, offered blood meals daily after emergence, provide an estimate of 11·9 days for D, and a linear function for size-dependent fecundity, $f(w_x) = -51 + 23{\cdot}2w_x$.

Mechanisms underlying differences among treatment groups in their overall r' values, or in response of r' to density, were inspected further by analysing separate components of r': time to adult emergence, the fraction of initial individuals surviving to adult emergence (and the fraction surviving the first 2 days of larval life), adult sex distribution (quantified as the fraction of adult females), and female wing length. For analyses of individual characteristics, such as female wing length or development time, the mean for each replicate was used as the dependent variable to preserve the independence of observations. For proportionate data (e.g. fraction female, survivorship), the angular transformation was applied prior to analysis. In an effort to summarize the degree of developmental synchrony for different treatment groups, we have used the Shannon index, $H' = -\Sigma p_i \ln p_i$ (Brower & Zar 1984), in which p_i represents the proportion of larvae within a given instar.

Analysis

Most of our interpretations rely on procedures of analysis of covariance, which included tests for differences among the vertical positions of the lines of least squares for the separate treatment groups where density was used as a covariate. In addition, we tested for homogeneity of the regression coefficients of the different treatment groups (Armitage 1971, pp. 294–298). Analysis of covariance was made necessary by the higher density levels imposed on the two egg treatments (resulting from an initial underestimate of egg fertility), and permits a correction for that bias.

Overall differences among treatment groups are summarized by calculating the corrected mean values for independent variables at the overall mean density. For visual comparisons of cell means, and as a summary of variability, we provide Tukey's least significant difference based on the error mean square obtained from analyses of variance in which density is assigned only three levels (i.e. the additional density for the egg cohort treatments is ignored).

The assumptions for analyses of covariance include homogeneity of group regression coefficients because non-parallel lines cannot be claimed to differ in their vertical positions. Nonetheless, we consider it a useful procedure to detect vertical position differences among group regressions despite the presence of slope differences, but only as a summary of differences found within the range of the experimental density levels. In such cases, the corrected group mean at the overall mean value of density was calculated using the separate group regression coefficients to depict overall treatment effects.

We have also used a three-factor analysis of variance to test for differences in survivorship among treatments, cohorts, densities (at three categorical levels), and interactions among those factors for the multiple cohort treatments. Emergence patterns for each jar were used to assign individuals to cohorts 1–4. Because males develop more rapidly, each onset of emergent males after a series of females had emerged was used to indicate the emergence of a new cohort. We acknowledge that some cohort assignment errors are unavoidable with this method, and we can only assume that the assignment of individuals to cohorts has not been biased by experimental treatments.

Results

DENSITY, RECRUITMENT AND COMPETITION

For all treatments, estimated per capita growth rate (r') decreased as density increased (Fig. 1a). In addition, populations with multiple cohorts achieved considerably higher r' than those with only a single cohort (Fig. 1b). Irrespective of density, r' was

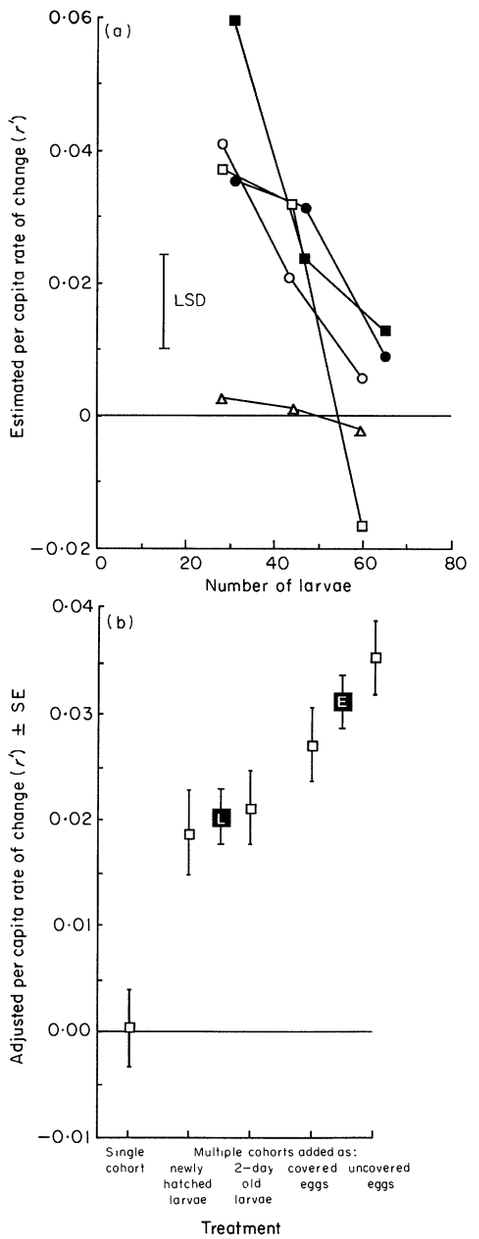

Fig. 1. (a) Response of the estimated per capita growth rate (r') to density for five experimental recruitment modes: single cohort (△), newly hatched (□), 2 days old (○), uncovered eggs (●), and covered eggs (■). Densities of the egg treatments are adjusted to account for the average number of eggs that hatched. An analysis of covariance reveals significant differences among vertical positions of treatment least squares lines ($F_{4,65} = 12{\cdot}72$, $P < 0{\cdot}001$), as well as differences among slopes ($F_{4,61} = 3{\cdot}85$, $P < 0{\cdot}01$). Tukey's least significant difference (LSD), calculated from the error mean square of a two-way analysis of variance using treatment and density as factors, is provided for visual comparison of cell means. (b) r' for the five experimental recruitment modes at the overall mean density. Per capita rates obtained by pooling the two multiple cohort egg recruitment treatments and the two multiple larval treatments are shown as the black squares, labelled E and L, respectively. Predicted values of r' and their standard errors are calculated from individual group regression coefficients.

significantly higher for all populations that had additional cohorts added as eggs rather than as larvae. While the superiority in overall r' values for populations with cohorts added as eggs is of ecological interest, the evolutionary question about the adaptive value of discretionary hatching is obscured because information is combined for all cohorts, and the differences between the single and multiple cohort treatments could have arisen simply from the reduced competition faced by the first cohort.

To address this problem, we subdivided the adult emergence data from the multiple egg cohort populations into two groups: adults from the initial cohort of larvae, and adults from all subsequent cohorts, added as eggs. If benefits to fitness result from discretionary hatching, they should be reflected in higher r' values for the late cohorts relative to the synchronous populations. Figure 2 illustrates these benefits, and shows that discretionary hatching with asynchronous cohorts is advantageous at the lower densities, but that the advantage vanishes at high density.

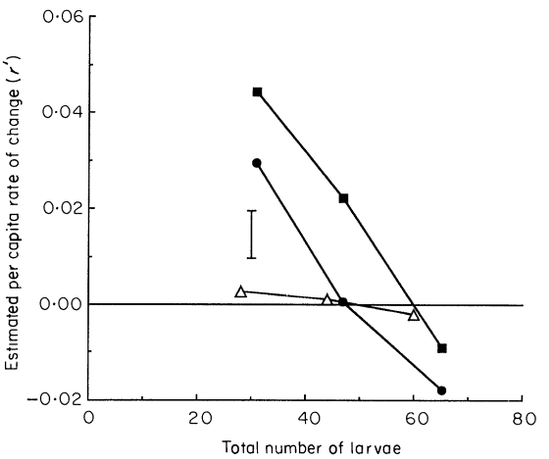

Fig. 2. Per capita growth rate estimates for the single-cohort populations (△) compared with estimates for the late cohorts (2–4 combined) for the egg cohort treatments: uncovered eggs (●) and covered eggs (■). An analysis of variance reveals a significant treatment effect ($F_{2,32} = 8{\cdot}84$, $P < 0{\cdot}001$), a significant density effect ($F_{1,32} = 29{\cdot}56$, $P \ll 0{\cdot}001$), and a significant interaction between treatment and density ($F_{2,32} = 5{\cdot}90$, $P < 0{\cdot}01$). Tukey's least significant difference (LSD) is shown for visual comparison of cell means.

Development

Populations into which 2-day-old larvae were added exhibited the lowest instar diversity during the first 30 days of the experiment (Fig. 3). After this time, individuals introduced as eggs or newly hatched larvae tended to reach the fourth instar and were soon removed as pupae, as reflected in the decline in instar diversity for all treatments after day 30. The decline in diversity was particularly steep for populations with cohorts added as covered eggs.

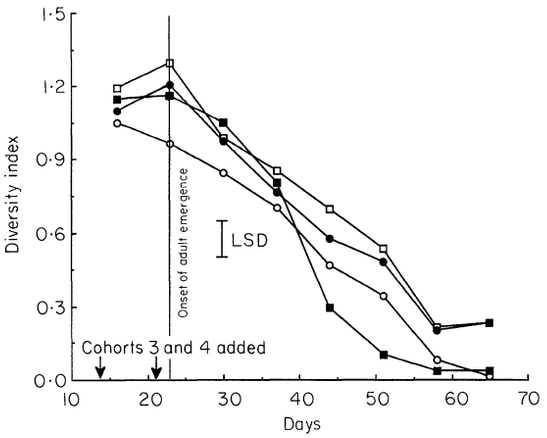

Fig. 3. Temporal changes of instar diversity within each multiple cohort treatment, pooled across densities: newly hatched (□), 2 days old (○), uncovered eggs (●), and covered eggs (■). Tukey's least significant difference is shown for visual comparison of treatment means. The following factors had significant influences on instar diversity: treatment ($F_{3,144} = 14{\cdot}4$, $P < 0{\cdot}001$), density (a positive effect, $F_{2,144} = 50{\cdot}77$, $P < 0{\cdot}001$), time ($F_{3,144} = 87{\cdot}27$, $P < 0{\cdot}001$), treatment × time ($F_{9,144} = 4{\cdot}34$, $P < 0{\cdot}001$).

Time to female emergence was significantly influenced by treatment and density (Fig. 4). Cohort structure is apparent in the pattern of female emergence (Fig. 4). Cohorts added as eggs tended to produce more females from the later cohorts than did the other treatment groups. Females from single cohorts exhibited delayed emergence especially conspicuous at medium and high densities (Fig. 4). Of particular note is that females from single cohort populations did not emerge until after the seventh week, 4 weeks after females emerged from multiple cohort populations.

Survivorship

Pupae from multiple cohort treatments were more likely to survive to adult emergence (1·14 radians (=83%) ± 0·06 radians SE) than those from single cohort populations (0·84 radians (=55%) ± 0·08 radians SE). Differences in survivorship to adult emergence resulted from treatment and density differences (Fig. 5a) as well as from cohort differences, with the greatest differences between treatments at the initial density of 15 (Fig. 5b). For all treatments, the later cohorts suffered more from competitive interactions. The effect of density on survivorship to the adult stage was stronger for treatments where cohorts were added as larvae rather than eggs, with covered eggs achieving the highest survivorship.

Female size

By this measure, the single cohort treatment was less successful than the egg and newly hatched larval cohort treatment. For all cohort treatments, the later cohorts were impaired significantly and consistently by the presence of the initial group of larvae (Fig. 6). Among the multiple cohort treatments, the 2-day-old cohort additions produced the smallest females, which were similar in size to the single cohort treatment.

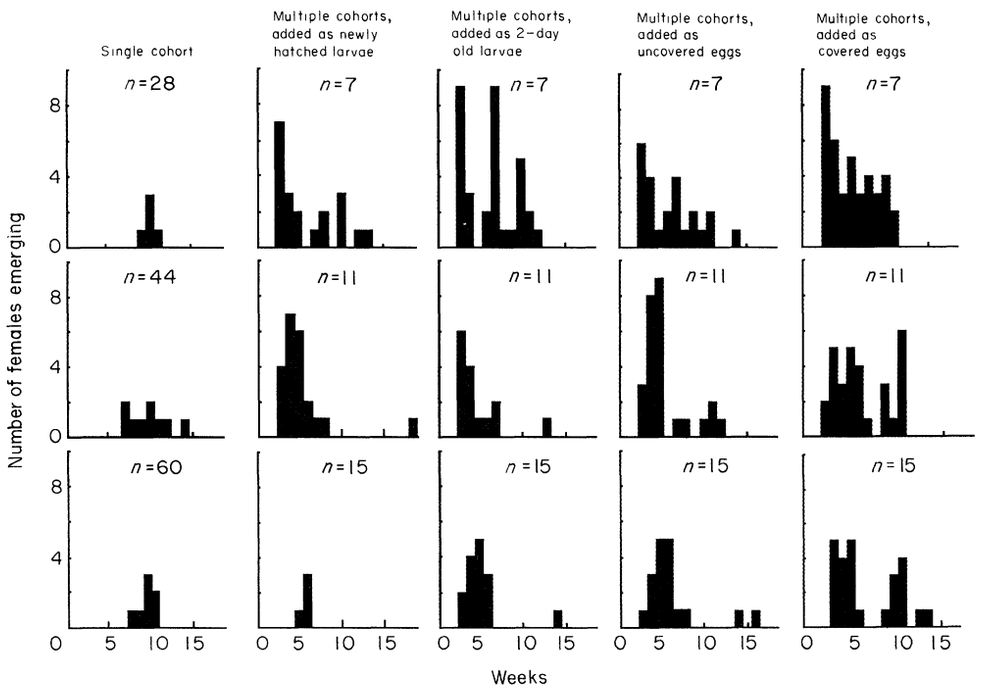

Fig. 4. Emergence of adult females for all treatments, n = initial density.

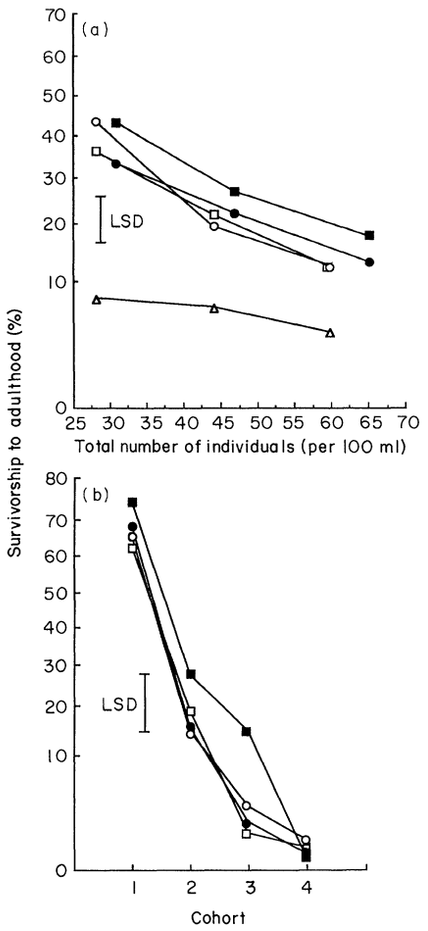

Fig. 5. Survivorship to adult emergence. (a) Response of survivorship to density for each treatment: single cohort (△), newly hatched (□), 2 days old (○), uncovered eggs (●), and covered eggs (■). An analysis of covariance reveals significant differences among vertical positions of treatment least squares lines ($F_{4,69} = 27{\cdot}86$, $P < 0{\cdot}001$), and among slopes ($F_{4,65} = 2{\cdot}67$, $P < 0{\cdot}05$). Tukey's least significant difference (LSD) is provided for visual comparison of cell means. (b) Interactive effects of cohort and treatment on survivorship for the multiple cohort treatments. A three-factor analysis of variance revealed significant effects due to: treatment ($F_{3,192} = 4{\cdot}33$, $P < 0{\cdot}01$), density ($F_{2,192} = 54{\cdot}39$, $P < 0{\cdot}001$), cohort ($F_{3,192} = 211{\cdot}5$, $P < 0{\cdot}001$), density × cohort ($F_{6,192} = 11{\cdot}74$, $P < 0{\cdot}001$). For both (a) and (b), the vertical scale converts survivorship into the more appropriate angular transformation for proportionate data.

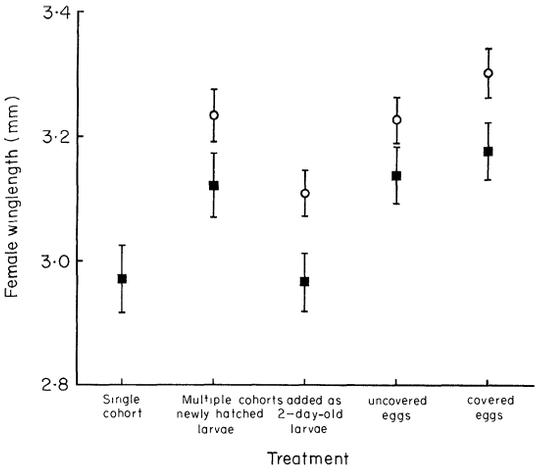

Fig. 6. Female wing length for all treatments, including overall mean wing lengths and those of only the first cohort (○). An analysis of covariance of wing lengths for all cohorts reveals significant differences among vertical positions of treatment least squares lines ($F_{4,62} = 4{\cdot}14$, $P < 0{\cdot}005$), as well as differences among slopes ($F_{4,58} = 3{\cdot}85$, $P < 0{\cdot}025$). An analysis of covariance performed only on first cohort females reveals significant differences among vertical positions ($F_{3,51} = 4{\cdot}42$, $P < 0{\cdot}01$), and no significant slope differences.

EGG HATCH INHIBITION

Eggs hatched asynchronously as evidenced by emergence of first instars on days 30 and 37 for $N_0 = 7$, 11 or 15 and day 44 for $N_0 = 7$. Generally, most eggs hatched during their first immersion into the treehole water on day 9 for egg batch 1, day 16 for egg batch 2 and day 23 for egg batch 3 (Fig. 7). Hatch rate was not related to larval density ($F_{2,83} = 1{\cdot}01$; $P = 0{\cdot}368$) nor to treatment of the eggs ($F_{1,83} = 0{\cdot}8$; $P = 0{\cdot}785$).

CANNIBALISM

First instar larvae, either newly hatched or 2-days old, did not differ from each other or from the first cohort in survivorship during their first 2 days in the jars although there were significant interactions between cohort and treatment (Fig. 8a) and treatment and density (Fig. 8b). Survivorship declined for larvae added as 2-day-old larvae at the highest density level (Fig. 8b) suggesting that competition may have been stronger for larvae that were more synchronized in their development with the established larval population. A surprising difference that resulted from adding newly hatched larvae was that only two of five replicates produced females at the high density level (Fig. 9) giving rise to low r' values.

Discussion

Staggered entry of individuals into the larval environment conferred substantial improvement on average success. Not only did populations of multiple cohorts attain significantly higher r' values than did single cohorts, but within the multiple cohort treatments, those that entered the habitat by hatching at times of their choice outperformed all other combinations of instars (Fig. 2).

Among the multiple cohort populations, those with cohorts added as 2-day-old larvae exhibited the lowest diversity of instars early in the experiment and produced the smallest females of all multiple cohort populations. In addition, the fourth cohort in this treatment group suffered significantly higher

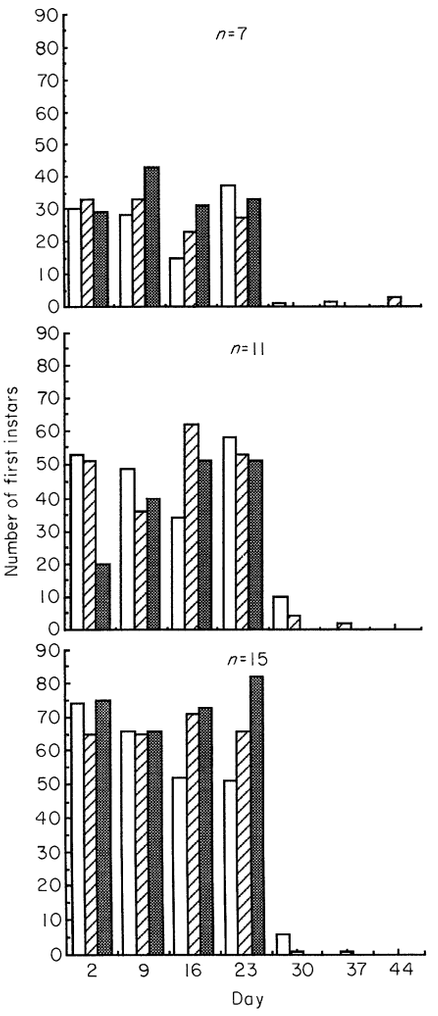

Fig. 7. Occurrence of first instars in experimental habitats for the multiple cohort egg treatments, as well as the newly hatched multiple cohort treatment, for each density level. Cohorts added as covered eggs (□), uncovered eggs (▨), or newly hatched larvae (■). The newly hatched treatment group provides a reference point for the other two groups, as the numbers that could be expected if hatching were immediate. n = initial density.

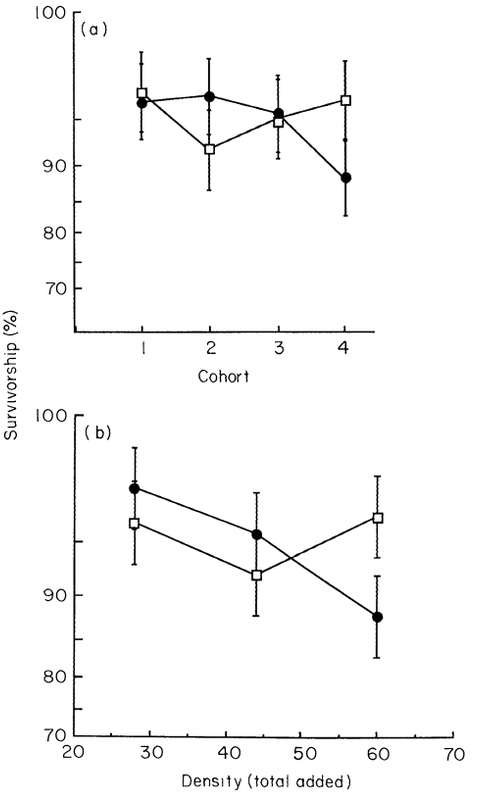

Fig. 8. Survival of newly hatched (□) or 2-day-old (●) larvae during the first 2 days after introduction to experimental habitats. Results were analysed with a three-factor analysis of variance using cohort, density and treatment, in which only the density × cohort ($F_{6,96} = 2{\cdot}664$, $P < 0{\cdot}02$) and density × treatment ($F_{2,96} = 3{\cdot}7$, $P < 0{\cdot}03$) interaction effects were significant. (a) Survival of each cohort of the two larval addition groups. (b) Influence of the treatment × density interaction on 2-day survivorship. Tukey's least significant difference is shown for visual comparison between cell means. For both (a) and (b), the vertical scale converts survivorship into the more appropriate angular transformation for proportionate data.

mortality rates than did the similar treatment in which newly hatched larvae were added. We suspect that by increasing the synchrony within cohorts in this case we destined the larvae to compete directly for food particles of similar size (Merritt 1987). Populations composed of cohorts added as newly hatched larvae were not as synchronized as those added as 2-day-old larvae, but were more so than egg-derived cohorts, and also suffered from competitive interactions in an unexpected way. The number of females emerging from these populations at high density was substantially reduced, indicating that sexual asymmetries may exist in the response by larvae of different ages to density. Other researchers have detected sexual dimorphism in response to density in other insects (Frank, Curtis & Rickard 1985; Wall & Begon 1986; Simmons 1987). Overall, larval cohorts suffered more from the negative effects of density than did egg cohort groups. This result is striking given that experimental studies of density-dependent effects on mosquito populations are traditionally conducted by manipulation of larval groups, usually in single cohorts (e.g. Istock, Wasserman & Zimmer 1975; Fish & Carpenter 1982; Hard, Bradshaw & Malarkey 1989), which are never introduced as eggs.

Studies on diverse organisms have revealed cohort interactions among juveniles that yield significant effects on various measures of success. Asynchronously developing odonates demonstrate the potential negative effects of density and inter-instar interactions, including interference competition and cannibalism (Crowley *et al.* 1987a; Wissinger 1988; Gribbin & Thompson 1990). For example, small damselfly larvae suffered a decrease in developmental rate and in size at moult in the presence of large larvae, which experienced no reciprocal

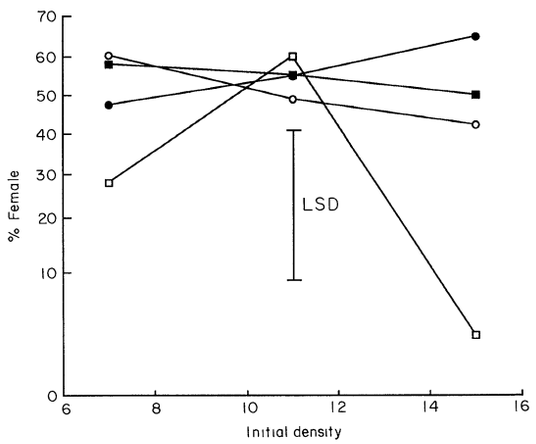

Fig. 9. Percentage of females for all populations for each treatment and density combination. Cohorts added as newly hatched larvae (□), 2 days old larvae (○), uncovered eggs (●) or covered eggs (■). We analysed the results with a three-factor analysis of variance using cohort, density and treatment, in which the following effects were significant: treatment ($F_{3,84} = 6{\cdot}21$, $P < 0{\cdot}001$), cohort ($F_{1,84} = 19{\cdot}34$, $P < 0{\cdot}001$), treatment × cohort ($F_{3,84} = 2{\cdot}76$, $P < 0{\cdot}05$), and treatment × density ($F_{6,84} = 3{\cdot}59$, $P < 0{\cdot}005$). The vertical scale converts percentage female into the more appropriate angular transformation for proportionate data.

impairment (Gribbin & Thompson 1990). Averill & Prokopy (1987) found that tephritid fruitfly larvae with a 2-day head start gained advantage over other larvae experimentally added to the fruit 2 days later. Similarly, our results show an advantage of earlier over later interacting cohorts.

Even though insect miners in fruits and leaves differ in many ways from treehole mosquitoes, their lifestyles are similar in that they cannot migrate out of their larval habitat even when overcrowded or in other ways stressed. Thus, females may influence potential larval interactions by their choice of oviposition sites. Female tephritids subvert potentially competitive interactions by depositing an oviposition pheromone that inhibits future oviposition into the fruit. By rejecting inhabited leaves, adult female leaf miners, *Scolioneura betulei* Klug. (Hymenoptera), also avoid multiple infestations of their larval habitats where interference of small larvae by large occurs (Tuomi, Niemalä & Mannila 1981). Adult behaviour of both these herbivores may have evolved in response to larval competitive interactions. In contrast to the behaviour of avoidance of occupied habitats, mosquito females tend to lay clumps of eggs into treeholes, which appear to be relatively limited in availability. Given our findings of strong density-dependent effects in the larval population, the discovery of oviposition attractants arising from mosquito-produced compounds becomes even more provocative (Bentley *et al.* 1976; 1981; Maire 1984; Dawson *et al.* 1989). We suspect that competitive larval interactions within treeholes may have triggered the evolution of unequal hatching responses within clutches of eggs (Livdahl & Koenekoop 1985; Koenekoop 1985) rather than evolution of rejection of occupied, but limited, larval habitats by ovipositing females.

The augmented r' values attained by cohorts initiated by eggs hatching into larval habitats corroborates our hypothesis that selection may have acted to promote the evolution of life-history traits that limit competitive interactions. Stimulated by previous studies on egg hatch inhibition in response to density conducted in natural treeholes (Livdahl & Edgerly 1987), we designed our experiment to test for its significance to overall productivity, and not for the existence of hatch inhibition itself. Although our experimental densities and our two egg treatments did not differ in egg hatch rates, we expect that the phenomenon of larva-induced hatch inhibition would have appeared had we chosen a lower density level for comparison.

Separate components of success (development time, survivorship, size and per capita growth rate) are often assessed by investigators of density-dependent population regulation in insects. Our results are consistent with many of these studies in that with increasing density, development rate is slowed, average size of adult declines, mortality increases, and per capita growth rate becomes depressed. Our results also add details towards understanding the interaction of density with cohort structure, an interaction that influences productivity in ways not previously reported. Many investigators have found that survivorship declines with increasing density for a variety of insects, e.g. damselflies (Crowley *et al.* 1987b), bark beetles (Beaver 1974), crickets (Simmons 1987), *Armigeres theobaldi* Barraud (Culicidae) (Mogi & Yamamura 1988), *A. triseriatus* (Fish & Carpenter 1982; Livdahl 1982). In contrast, the western treehole mosquito, *A. sierrensis* (Ludlow) (Hawley 1985) and one of three bark beetles studied by Beaver (1974), *Scolytus multistriatus* (Marsh.), exhibited no relationship between density and larval mortality. Increasing density can also trigger responses in growth that may circumvent the increased risk of mortality. Sea urchins (*Diadema antillarum* Philippi), for example, adjust body size and gonad volume up or down in response to density such that higher density populations have proportionately smaller individuals (Levitan 1989). Some insects respond to increased density by altering their development, perhaps by speeding their development rate because of habitat modifications via group effects, e.g. *Sarcophaga bullata* Parker (Baxter & Morrison 1983) or alternatively, by entering diapause, e.g. dragonflies (Van Buskirk 1987), pitcher plant mosquito (Istock, Wasserman & Zimmer 1975). The cricket, *Gryllus bimaculatus* De Geer, responds to increased density by sacrificing adult body mass rather than development time (Simmons 1987).

A brief review of empirical studies of density dependence reveals numerous complexities. For example, competitive interactions increase with density not only because of the constraints of food limitation (e.g. Lamberti, Feminella & Resh 1987; So & Dudgeon 1989; Wissinger 1988), but also because of interactions between food and density (*A. triseriatus*, Hard, Bradshaw & Malarkey 1989). Competitive interactions due to increased contact between individuals include cannibalism (Johnson *et al.* 1985; Van Buskirk 1987; Baur 1988; Wissinger 1988; Gribbin & Thompson 1990), behavioural interference (Wissinger 1988), or build-up of growth-inhibiting factors (e.g. Dye 1984; Petranka & Sih 1986). In the pitcher plant mosquito, reduced food availability increased the percentage of larvae that entered diapause, but an increase in density alone significantly affected per capita growth rate (Istock, Wasserman & Zimmer 1975). Increasing density in land snail populations caused a decrease in adult size, development rate and egg clutch sizes, even in the presence of excess food (Baur 1988).

In our study, increases in density resulted in especially severe declines in r' for each successive cohort in the multiple cohort populations, whereas in single cohort populations, r' was maintained at consistently low levels for all three density levels. In addition, while obvious at low density, evidenced by the distinct peaks in female emergence, cohort structure was eroded at medium and high densities. The third and fourth cohorts suffered severe mortality and prolonged development times.

This latter point brings up an interesting, and potentially confusing, by-product of density–cohort interactions. When computed as population averages, development rates as a function of treatment appear to be consistently depressed in the single cohort group (Fig. 10), as would be predicted from all other analyses of success. However, multiple cohort populations in which cohorts were added as covered eggs also appear to exhibit elongated developmental periods at medium and high density (Fig. 10). This result is inconsistent with the premise that rapid development rate contributes to a comparatively high r', a feature attained by egg treatment groups at all densities (Fig. 1). These apparent inconsistencies are resolved when one realizes that later cohorts derived from covered eggs produced more adults during the final weeks of the experiment than did the other treatments which experienced high mortality rates in these cohorts (Fig. 5). Hence, because of the lack of a modifier to account for higher survivorship of late cohorts, average development time for the covered egg treatment fails as a comparative measure of success. This ambiguity underscores the need to gather information about various aspects of success into a single population statistic, such as r' (Livdahl 1982; Hard, Bradshaw & Malarkey 1989; Juliano 1989; So & Dudgeon 1989).

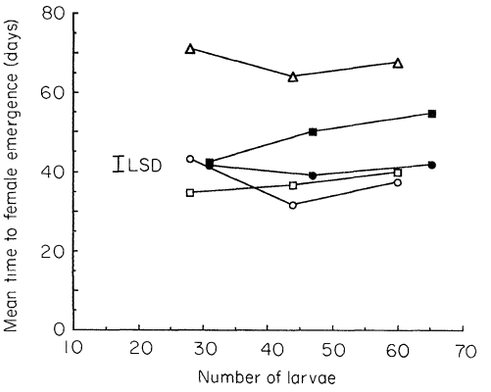

Fig. 10. Mean emergence time for females for each treatment–density combination: single cohort (△), newly hatched larvae (□), 2 days old larvae (○), uncovered eggs (●), and covered eggs (■). Tukey's least significant difference (LSD) is provided for visual comparison of cell means.

Our results also underscore the importance of allowing individuals to complete development before measuring success. Instar-specific mortality occurred differentially among our treatments, including higher mortality of pupae in the single cohort populations. If we had used pupal weights as a predictor of fecundity, as did Hard, Bradshaw & Malarkey (1989) for *A. triseriatus*, we would have overestimated the relative success of the single cohort populations.

A puzzling result emerged from our results of egg hatch inhibition and its relationship to egg–larva contact. By isolating the eggs beneath a mesh cover in one egg treatment, we may have created a refuge for hatching larvae, as well as a source of uncontested food. This confounding effect became apparent during censuses when we discovered first instars within the vials. The period spent by these larvae within the vials may explain the consistently higher success of these populations over the other egg treatment groups, in which larvae hatched directly into the larval habitat. If this refugium explanation is valid, these results again emphasize the subtle, yet potent, effect of larva–larva interactions, which may occur in very brief episodes relative to the length of larval development.

In contrast to observations on odonates, where cannibalism may play a significant role in population regulation (Merritt & Johnson 1984; Van Buskirk 1989), our results suggest that this form of interference competition is not important for *A. triseriatus*. Provisioning the jars with beech leaves and treehole fluid with detritus particles may have provided enough food during the first 4 weeks when susceptible first instars were introduced or enough hiding places to prevent cannibalistic interactions. These methods alone may explain the difference between our lack of evidence for cannibalism and the presence of such in the simple laboratory habitat previously utilized

by Koenekoop & Livdahl (1986). As with the egg treatment analysis, we obtained a surprising result in the multiple cohort groups: cohorts started as 2-day-old larvae suffered unexpectedly high mortality in the later cohorts and at higher densities, again emphasizing the importance of competition in experimentally synchronized groups.

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