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## The relationship between vulnerability to predation and behavior of larval treehole mosquitoes: geographic and ontogenetic differences

Steven A. Juliano and Lauren Reminger

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We tested predictions of the hypothesis that behavioral differences are the proximate cause of interpopulation differences in vulnerability to predation in *Aedes triseriatus*. The predictions were: 1) *A. triseriatus* from sites where the predator *Toxorhynchites rutilus* is abundant vs rare differ in types of activities and positions occupied, or the degree to which they change behavior in the presence of *T. rutilus*; 2) Some activities or positions entail greater risks of predation than others; 3) High-risk behaviors are more common among larvae from sites where *T. rutilus* is rare vs sites where *T. rutilus* is common. Behavior was quantified by direct observation of individual larvae with *T. rutilus* present or absent. Riskiness of behaviors was quantified by recording the frequency with which different behaviors led to capture. Populations from Normal, IL (*T. rutilus* very rare) and Vero Beach, FL (*T. rutilus* common) differ in patterns of activity and position occupied, confirming prediction 1). Changes in behavior in the presence of *T. rutilus* did not differ between populations. Thrashing was the most- and resting the least-risky activity; the bottom was the most- and the surface the least-risky position, confirming prediction 2). Larvae from Normal (low-predation population) spent more time thrashing and at the bottom than did larvae from Vero Beach (high-predation population), confirming prediction 3). For both populations, earlier instars spent more time thrashing and less time at the surface than did later instars. Activity and position were correlated: larvae at the surface rarely thrashed and frequently filtered. Fixed interpopulation behavioral differences seem to be the proximate causes of differences in vulnerability to predation. Changes in behavior in response to the presence of *T. rutilus* were minor and did not account for the interpopulation difference in vulnerability to predation.

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A variety of behaviors are believed to have evolved to reduce vulnerability to predation (reviewed by Endler 1986, Sih 1987). Most studies of the function and evolution of antipredator behaviors rely on comparisons among different species (e.g., Woodward 1983, Sih 1986, Henrikson 1988, Lawler 1989, McPeck 1990), rather than the relationship between predation pressure and intraspecific differences in behavior. Because of this, we know relatively little about the intraspecific variation in antipredator behavior that serves as the raw

material for natural selection by predation. The few existing studies of intra- or inter-population variation in antipredator behavior have revealed the functions of antipredator behavior and the variation available for selection (Arnold and Bennett 1984, Fawcett 1984, Kats, et al. 1988, Dowdey and Brodie 1989), suggesting that further studies of intraspecific variation in antipredator behavior will increase our understanding of the evolution of antipredator behavior.

Behavioral avoidance of predation may be fixed or

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flexible (Sih 1987). Prey may show a fixed or constitutive pattern of low-risk behaviors, thereby reducing vulnerability to predation. In contrast, many organisms show flexible behavior patterns, adopting low-risk behaviors only when predators are perceived (Sih 1986, 1987, Lawler 1989, McPeck 1990). Intraspecific variation in vulnerability to predation could result from differences in either fixed or flexible behavioral avoidance of predation.

Treehole mosquitoes are a useful study system for investigations of intraspecific variation in antipredator behavior. Larvae of *Aedes triseriatus* (Say) develop only in water filled treeholes or similar containers in forests in eastern North America (Bradshaw and Holzapfel 1985). In the southern part of the range of *A. triseriatus*, predatory larvae of another treehole mosquito, *Toxorhynchites rutilus* (Coq.), are common and prey heavily on *A. triseriatus* and on other insects (Bradshaw and Holzapfel 1983, 1985, Lounibos 1983, Chambers 1985). Abundance of *T. rutilus* declines to the north, and this predator is absent in the extreme northern portion of *A. triseriatus*'s range (Livdahl 1979, Bradshaw and Holzapfel 1985). Two separate laboratory studies have shown that *A. triseriatus* larvae from northern populations where *T. rutilus* is rare or absent are more vulnerable to predation by *T. rutilus* than are larvae from southern populations where *T. rutilus* is abundant (Livdahl 1979, Juliano 1989). In conjunction with these studies differences between northern and southern populations of *A. triseriatus* in survival, development time, size at maturity, and rate of population increase have been documented (Livdahl 1984, Juliano 1989).

These studies on *A. triseriatus* document consistent geographic differences in population performance under controlled conditions, with northern (low-predation) populations consistently different from southern (high-predation) populations. However, both the proximate mechanisms behind these differences, and the ultimate evolutionary causes for these differences remain unknown. Livdahl (1979) suggested that the difference in predation rate was due to a greater handling time for high-predation strains, however reanalysis of Livdahl's data showed that handling times did not differ and that a difference in attack rates was a more likely explanation (Juliano and Williams 1985). Juliano (1989) suggested that behavioral differences between northern and southern populations could be the proximate mechanism for observed differences in vulnerability to predation and population performance. *T. rutilus* is an ambush predator, and anecdotal evidence suggests that position in the water and type of activity are two aspects of prey behavior that influence vulnerability to *T. rutilus* (Crans and Slaff 1977, Russo 1986). Differences in behavior of treehole mosquitoes seem to explain interspecific differences in vulnerability to predation (Chambers 1985). Amount of movement is positively associated with vulnerability to predation in several mosquito species, and some mosquito species modify

position and type of activity in response to the presence of predators (Rubio et al. 1980, Sih 1986).

If behavioral differences are the proximate mechanism producing differences in vulnerability to predation, natural selection produced by predation by *T. rutilus* may be the ultimate cause. Heavy predation on southern strains may favor genotypes that reduce contact with the predator by reducing movement and avoiding positions where predation is likely. However, even if behavioral differences are the proximate mechanism producing differential vulnerability, it is possible that such differences are the result of other ultimate causes. The first step in determining whether natural selection by predation is the cause of intraspecific variation in vulnerability to predation is to determine the proximate mechanism producing the differences in vulnerability to predation.

In this paper, we test the following predictions based on the hypothesis that differences in behavior are the proximate mechanism producing intraspecific variation in vulnerability to predation in *A. triseriatus*:

- 1) Populations of *A. triseriatus* with different exposure to *T. rutilus* (already shown to differ in vulnerability to predation) show either: a) fixed differences in pattern of behavior, specifically, type of activity and position occupied, or b) differences in the degree to which they change behavior in the presence of *T. rutilus*.
- 2) Some types of activities or some positions entail greater risks of predation than do others.
- 3) The specific interpopulation differences in behavior (fixed or flexible) are that the low-predation population shows greater frequencies of high-risk behaviors than does the high-predation population.

## Methods

### Collection and maintenance of mosquitoes

The two populations of *Aedes triseriatus* involved in this study originated from Normal, Illinois, where *T. rutilus* is very rare, and Florida Medical Entomology Laboratory (FMEL), Vero Beach, Florida, where *T. rutilus* is common. At the Normal site, no *T. rutilus* were observed in 2 of 3 years of regular collecting from tree holes. In one year, 8 larvae were collected in late summer. In contrast, in Vero Beach, *T. rutilus* are present and abundant every year, and larvae are present year round (Lounibos 1983). Juliano (1989) demonstrated that these two populations differed in vulnerability to predation, with the Normal population more vulnerable. These populations were studied from 1987 to 1990. In 1987, behavior of the two populations was quantified. The Normal population originated from larvae collected from a large treehole in September 1986. The FMEL population originated from larvae collected

from tires in November 1987. For both populations, individuals used in 1987 were products of 5–10 laboratory generations. In 1989 and 1990 risks associated with different behaviors and capacity to modify behavior in the presence of *T. rutilus* were quantified. For both populations experimental animals were F<sub>1</sub> progeny of larvae collected in the same forest stands that furnished founders of populations used in 1987. Thus, the laboratory populations used in different years represented independent collections from the same natural populations. Rearing methods for *A. triseriatus* were those described by Munstermann and Wasmuth (1985) and Juliano (1989). Adults were maintained as free-mating colonies in 0.6 m<sup>3</sup> cages, and blood-fed on anaesthetized laboratory mice. *T. rutilus* larvae used in experiments were from Ashland, Virginia, and were either field-collected or were products of a laboratory colony propagated using a forced mating technique similar to that described by Munstermann and Wasmuth (1985).

### Behavior of *A. triseriatus* larvae

Instantaneous scan samples (Martin and Bateson 1986) were used to quantify behavior of larvae from each population. Five larvae from each population were placed individually into 50 ml microbeakers filled with 30 ml tap water. After five minutes' acclimation, scan samples were conducted every minute for 30 minutes. For each larva, activity and position within the container were recorded. This whole process was repeated at least 3 times for each instar, for a total of 130 larvae observed.

Four major activities could be identified reliably: 1) Resting. Larvae were not feeding and were not moving through the water. Our resting category included the "underwater/still", "rise", and "autogroom" behaviors described by Walker and Merritt (1991). 2) Browsing. Larvae moved along the surfaces of the container, working their mouthparts against the surface, presumably collecting microbes (which should have been minimal). Our browsing category included the "brush wall" and "float/substrate brush" behaviors described by Walker and Merritt (1991). 3) Filtering. Larvae in open water floated through the water propelled by movements of the mouthparts. No movement of the body was observed. Our filtering category included the "float/suspension feed", "float/interfacial feed", and "mouth swim" behaviors described by Walker and Merritt (1991). 4) Thrashing. Larvae moved through the water propelled by vigorous lateral movements of the whole body. Our thrashing category included the "dive", "startle dive", and "wiggle swim" behaviors described by Walker and Merritt (1991).

Four positions within the container could be reliably identified: 1) Surface. The spiracular siphon of larvae in contact with the water's surface; 2) Wall. Larvae within 1 mm of the container wall; 3) Bottom. Larvae within 1

mm of the container bottom; 4) Middle. Larvae away from the surface, wall, and bottom. Larvae in contact with the surface and within 1 mm of the wall were recorded as at the surface.

For an individual, the frequency with which each activity occurred and each position was occupied is estimated by the proportion of observations of that individual in that activity or position (Martin and Bateson 1986). These proportions (arcsine transformed) were analyzed using multivariate analysis of variance (MANOVA) with population (FMEL, Normal), instar (1 to 4), and interaction as model effects, and proportions of the four activities (or the four positions) as the multivariate observations. We are primarily interested in the population effect. MANOVA was followed by univariate analyses of variance (ANOVA). Significant differences are based on Bonferroni multiple comparisons ( $\alpha=0.05$ ).

Analysis of proportions of activities and positions does not indicate the relationship between activity and position, which was analyzed using canonical correlation (Lindeman et al. 1980). This is a multivariate technique designed to test for a relationship between two sets of variables. For each set of variables, the first canonical variate is the linear combination of the variables that has the maximum correlation with the first canonical variate from the other set. The second canonical variate is the linear combination of the variables (independent of the first canonical variate) having the maximal correlation with the second canonical variate of the other set of variables. The null hypotheses that the true correlations of canonical variates are 0 were tested using approximate *F*-tests (SAS Institute Inc. 1987, Procedure CANCORR).

When determining the relationship between activity and position, we removed population- and instar-specific differences in activity and position. Clearly, if populations differ in both activity and position, a correlation of activity and position will result from this between-group difference, and may not indicate a correlation between activity and position within groups. To remove population and instar effects, we performed canonical correlation analysis on residuals from univariate ANOVA's (described above) on activity and position. This is equivalent to testing for correlations among activities and positions within groups. Significant canonical correlations indicate that activity and position are related, regardless of the population or instar involved.

### Flexibility of behavior in response to *T. rutilus*

We recorded the activities and positions of *A. triseriatus* larvae in the presence and absence of *T. rutilus*. Trials were done in plastic microbeakers 60% full with tap water. For young larvae (1st or 2nd instars) we used 10 ml beakers. For old larvae (3rd or 4th instars) we used

Table 1. ANOVA's and MANOVA for activity of *Aedes triseriatus* larvae from two populations, across 4 instars.

| Activity Source | Df  | Thrashing<br><i>F</i><br>( <i>P</i> ) | Filtering<br><i>F</i><br>( <i>P</i> ) | Browsing<br><i>F</i><br>( <i>P</i> ) | Resting<br><i>F</i><br>( <i>P</i> ) | MANOVA<br>Lambda<br>( <i>P</i> ) |
|-----------------|-----|---------------------------------------|---------------------------------------|--------------------------------------|-------------------------------------|----------------------------------|
| Population      | 1   | 13.31<br>(0.0004)                     | 2.15<br>(0.1448)                      | 3.78<br>(0.0543)                     | 0.46<br>(0.4985)                    | 0.898<br>(0.0115)                |
| Instar          | 3   | 36.85<br>(0.0001)                     | 3.80<br>(0.0120)                      | 69.80<br>(0.0001)                    | 4.39<br>(0.0057)                    | 0.297<br>(0.0001)                |
| Interaction     | 3   | 0.15<br>(0.9313)                      | 1.16<br>(0.3277)                      | 1.05<br>(0.3713)                     | 0.63<br>(0.5957)                    | 0.866<br>(0.1349)                |
| Error           | 122 |                                       |                                       |                                      |                                     |                                  |

50 ml beakers. Two replicates with and two without *T. rutilus* were done at one time. The entire process was replicated at least 10 times per instar for a total of 366 larvae observed. Old larvae were exposed to 3rd or 4th instar *T. rutilus* and young larvae were exposed to 1st or 2nd instar *T. rutilus*. An open-ended plastic tube, placed vertically into each beaker, was used to isolate *T.*

*rutilus* from *A. triseriatus* for a 5 min acclimation period. Half the beakers had a single *T. rutilus* larva (starved 24 h) added to the tube. Then a single *A. triseriatus* was added to each beaker. After acclimation, the tubes were slowly withdrawn, releasing *T. rutilus* larvae. Position and activity of *A. triseriatus* larvae were again recorded every minute for 30 minutes. Upon capture, we recorded the position and activity of the captured larvae immediately prior to capture, then we recorded position and activity for the other larva exposed to *T. rutilus* but not captured. Recording of position and activity of remaining larvae continued for 30 min or until the second larva was captured. Simultaneous observations were always done on larvae from the same population and age class.

As in the previous experiment, we analyzed arcsine transformed proportions of different activities or positions using MANOVA with Instar (1 to 4), Population (FMEL, Normal), Predator (+, -), and all interactions as effects. Only proportions based on at least 10 observations (i.e., 10 min) were used in this analysis to reduce errors inherent in proportions based on very low sample sizes. As before, MANOVA was followed by univariate ANOVA and Bonferroni multiple comparisons ( $\alpha=0.05$ ). If larvae modify their behavior in response to the presence of *T. rutilus*, we predict a significant main effect for Predator, or a significant interaction involving Predator. A significant Predator-Population interaction would indicate a greater change in behavior in response to *T. rutilus* in one of the populations.

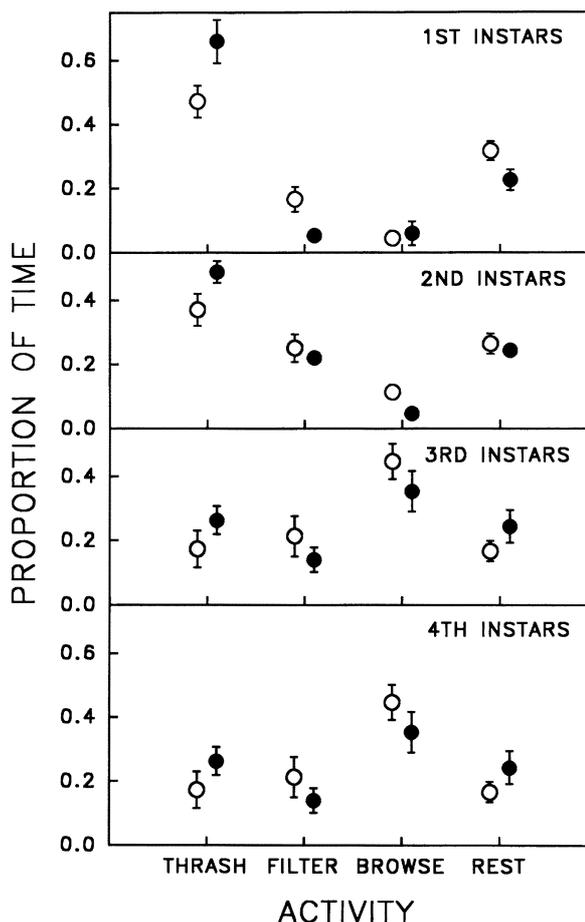


Fig. 1. Estimated proportions (mean±SE) of time spent in different activities for larvae of *Aedes triseriatus* from Normal (filled symbols) and FMEL (open symbols) across four instars.

### Riskiness of different behaviors

We tested whether some behaviors entail a greater risk of predation by comparing positions and activities observed immediately before capture with those observed for larvae exposed to *T. rutilus*, but not captured. Because *T. rutilus* is an ambush predator, prey are not pursued, hence prey behavior immediately before capture does not represent fleeing. Observations were obtained from the previous experiment. For pairs of larvae observed in the presence of *T. rutilus*, we recorded

Table 2. ANOVA's and MANOVA for position within the container for larvae of *Aedes triseriatus* from two populations, across four instars.

| Position    |     | Bottom           | Middle            | Wall             | Surface           | MANOVA            |
|-------------|-----|------------------|-------------------|------------------|-------------------|-------------------|
| Source      | Df  | F<br>(P)         | F<br>(P)          | F<br>(P)         | F<br>(P)          | Lambda<br>(P)     |
| Population  | 1   | 7.23<br>(0.0082) | 0.68<br>(0.4115)  | 0.04<br>(0.8455) | 8.04<br>(0.0054)  | 0.903<br>(0.0154) |
| Instar      | 3   | 7.25<br>(0.0002) | 14.88<br>(0.0001) | 0.55<br>(0.6468) | 18.70<br>(0.0001) | 0.446<br>(0.0001) |
| Interaction | 3   | 2.13<br>(0.1000) | 0.07<br>(0.9734)  | 0.90<br>(0.4431) | 2.20<br>(0.0917)  | 0.784<br>(0.0034) |
| Error       | 122 |                  |                   |                  |                   |                   |

position and activity immediately preceding capture for the first larva captured (capture group), and then recorded position and activity for the other, uncaptured larva (no capture group).

If all behaviors of *A. triseriatus* are equally risky, the frequencies of different behaviors in capture and no capture groups should not differ. If some activities or

positions entail greater risk of predation, those activities or positions should be significantly over-represented in the capture group relative to the no capture group. We tested for differences in these frequencies using maximum likelihood analysis of multiway contingency tables, with effects of group (capture, no capture), age (young, old), and population (FMEL, Normal) as independent variables, and frequencies of the four positions or activities as dependent categorical variables (SAS Institute Inc. 1987, procedure CATMOD).

## Results

### Behavior of *A. triseriatus* larvae

#### Activity

MANOVA indicated that the proportions of the four activities differed significantly between populations and among instars, but interaction of population and instar was not significant (Table 1). These results indicate that pattern of activity differs among populations and instars, but that the pattern of difference between populations is consistent across instars.

Examination of mean frequencies of the four activities reveals that the two populations differ primarily in the frequency of thrashing (Fig. 1). The Normal population spent more time thrashing than did the FMEL population (Fig. 1) and the difference was significant in ANOVA (Table 1). The FMEL population usually spent more time resting, browsing, and filtering (Fig. 1), but ANOVA indicated that these differences were not significant (Table 1). Thus, the main behavioral difference between the two populations appears to be the greater frequency of thrashing in the Normal population and corresponding lower frequencies of other activities (Fig. 1).

The four instars differed in proportions of all four activities (Table 1). Proportion of time browsing was significantly greater in 3rd and 4th instars than in 1st and 2nd instars (Fig. 1, Table 1). Proportion of time thrashing was significantly less in 3rd and 4th instars than in 1st and 2nd instars (Fig. 1, Table 1). Although effects of instar on resting and filtering were significant

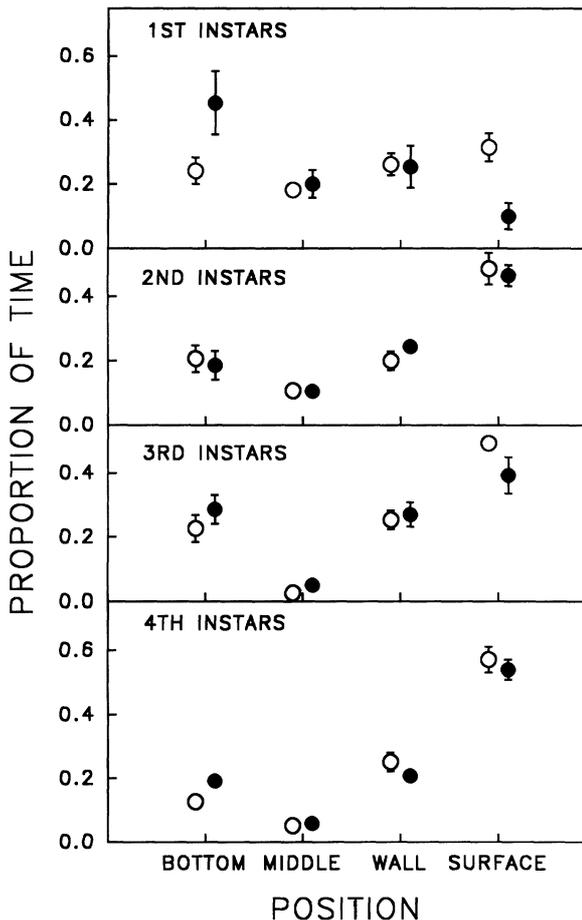


Fig. 2. Estimated proportions (mean±SE) of time spent in different positions for larvae of *Aedes triseriatus* from Normal (filled symbols) and FMEL (open symbols) across four instars.

Table 3. Coefficients for original variables for the first two canonical variates, and correlations of original variables with the first two canonical variates. Original variables are residuals from analyses of variance involving population and instar.

| Residual of        | First canonical variate |             | Second canonical variate |             |
|--------------------|-------------------------|-------------|--------------------------|-------------|
|                    | Raw coefficient         | Correlation | Raw coefficient          | Correlation |
| <b>A. Activity</b> |                         |             |                          |             |
| Thrashing          | -0.079                  | -0.840      | 0.157                    | 0.213       |
| Filtering          | 0.033                   | 0.782       | 0.133                    | 0.381       |
| Browsing           | -0.030                  | -0.030      | 0.131                    | -0.295      |
| Resting            | -0.016                  | 0.261       | 0.162                    | 0.341       |
| <b>B. Position</b> |                         |             |                          |             |
| Bottom             | -0.036                  | -0.825      | 0.216                    | 0.390       |
| Middle             | 0.015                   | 0.056       | 0.163                    | 0.361       |
| Wall               | -0.016                  | -0.574      | 0.122                    | -0.428      |
| Surface            | 0.058                   | 0.952       | 0.226                    | 0.063       |

(Table 1), multiple comparisons revealed no significant differences among means. The strongest pattern evident in the mean proportions is that early instars spend a great deal of time thrashing and relatively little time browsing, whereas later instars spend more time browsing and much less time thrashing (Fig. 1).

#### Position

Effects of Population, Instar, and Interaction on positions occupied were all significant in MANOVA (Table 2). This result indicates that the multivariate difference between populations depended on the instar examined.

Examination of univariate population-by-instar means shows that the main differences involved proportion of time at the surface vs at the bottom. Time at the bottom was significantly greater for 1st instars vs 4th instars and was always greater for Normal vs FMEL (Fig. 2). Both main effects were significant (Table 2), but interaction was not significant in ANOVA of time at the bottom (Table 2). Both main effects were significant for time at the surface, but Interaction was again not significant (Table 2). Univariate means (Fig. 2) indicate that time at the surface was significantly greater for 3rd and 4th instars vs 1st instars, and that individuals from FMEL spent a greater proportion of time at the surface than did individuals from Normal (Fig. 2). Time spent in the middle of the water column differed only among instars (Table 2), and was significantly less in 3rd and 4th instars vs 1st instars (Fig. 2). Time spent at the container walls did not differ among instars or between populations (Fig. 2). The significant multivariate interaction effect was not a clear product of a significant interaction in any one variable (Table 2), however it appeared that differences between populations were most pronounced in the 1st instar (Fig. 2). These results indicate that individuals from FMEL and Normal differ primarily in the proportions of time spent at the surface vs at the bottom, with the FMEL population spending more time at the surface. Time at the surface also increased greatly with instar.

#### Correlation of activity and position

After the effects of population and instar were removed, position and activity showed significant canonical correlations, indicating that what larvae did depended on where they were. Of four canonical correlations determined, the first ( $F_{16,373}=8.38$ ,  $P<0.0001$ ) and second ( $F_{9,00}=2.54$ ,  $P<0.0089$ ) were statistically significant, and accounted for 86.0% and 9.9%, respectively, of the total variance in the data set. The third and fourth canonical correlations were not significant ( $P>0.10$ ) and each accounted for  $\leq 4\%$  of the variance.

The first canonical correlation was 0.730. The first activity canonical variate was positively correlated with time filtering and negatively correlated with time thrashing. The first position canonical variate was positively correlated with time spent at the surface and negatively correlated with time at the wall and bottom (Table 3). Thus, larvae at the surface filtered frequently, but rarely thrashed. Conversely, larvae below the surface (at the wall or the bottom) thrashed frequently, but rarely filtered.

The second canonical correlation was 0.341. The second activity canonical variate was positively correlated with time resting, thrashing, and filtering, and negatively correlated with time browsing (Table 3). The second position canonical variate was positively correlated with time at the bottom and in the middle, and negatively correlated with time at the wall (Table 3). The second canonical correlation indicates that after the relationship between time at the surface and frequency of thrashing and filtering is taken into account (first canonical correlation), larvae at the wall tend to be browsing, whereas larvae away from the wall tend to be resting, thrashing, or filtering.

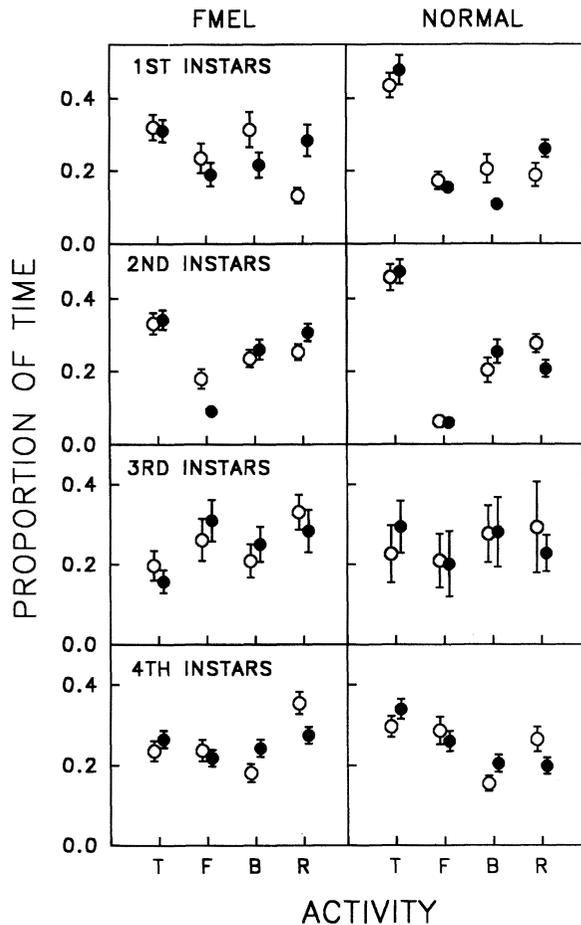


Fig. 3. Estimated proportions (mean±SE) of time spent in different activities for larvae of *Aedes triseriatus* from Normal and FMEL across four instars. Filled symbols – observed in the absence of *T. rutilus*, open symbols – observed in the presence of *T. rutilus*.

## Flexibility of behavior in response to *T. rutilus*

### Activity

MANOVA revealed significant effects of Population, Instar, Population-Instar interaction, and Instar-Predator interaction on activity pattern (Table 4). The main effect for Predator was not significant (Table 4), however the significant Instar-Predator interaction indicates that how activity patterns were affected by the presence of *T. rutilus* was dependent on which instar was observed. ANOVA's for browsing and resting had significant Instar-Predator interactions (Table 4). First instars browsed significantly more and rested significantly less in the presence of *T. rutilus* than they did in the absence of *T. rutilus* (Fig. 3). In contrast, fourth instars browsed significantly less and rested significantly more in the presence of *T. rutilus* than they did in the absence of *T. rutilus* (Fig. 3). Thus, the presence of *T. rutilus* had some effect on activity pattern. The absence of any significant interaction involving population and predator indicates that the presence of *T. rutilus* affected activity patterns of the two populations in the same way (Table 4, Fig. 3).

The results of this analysis also confirmed some of the major differences between populations and among instars. ANOVA's revealed significant population effects for thrashing, filtering, and resting (Table 4). As in the previous experiment, thrashing was significantly more frequent in the Normal population than in the FMEL population. In this experiment filtering and resting were slightly but significantly more frequent in the FMEL population than in the Normal population (Table 4, Fig. 3). This same trend was present, but not significant, in the previous experiment (see Fig. 1). The instar effect was significant for all four variables (Table 4). Interpretation of the instar effect for filtering, browsing, and resting is complicated by significant two way interac-

Table 4. ANOVA's and MANOVA for activity of *Aedes triseriatus* larvae in the presence vs absence of the predator *Toxohynchites rutilus*.

| Activity                 |     | Thrashing         | Filtering         | Browsing         | Resting          | MANOVA            |
|--------------------------|-----|-------------------|-------------------|------------------|------------------|-------------------|
| Source                   | Df  | F<br>(P)          | F<br>(P)          | F<br>(P)         | F<br>(P)         | Lambda<br>(P)     |
| Population               | 1   | 23.80<br>(0.0001) | 5.35<br>(0.0213)  | 1.86<br>(0.1793) | 4.11<br>(0.0434) | 0.924<br>(0.0001) |
| Instar                   | 3   | 18.72<br>(0.0001) | 27.42<br>(0.0001) | 2.99<br>(0.0311) | 3.38<br>(0.0186) | 0.735<br>(0.0001) |
| Predator                 | 1   | 0.82<br>(0.3656)  | 3.26<br>(0.0717)  | 0.08<br>(0.7775) | 0.07<br>(0.7980) | 0.983<br>(0.2008) |
| Population<br>× Instar   | 3   | 0.87<br>(0.4580)  | 5.22<br>(0.0016)  | 2.31<br>(0.0761) | 2.40<br>(0.0676) | 0.911<br>(0.0012) |
| Population<br>× Predator | 1   | 1.25<br>(0.2652)  | 0.00<br>(0.9803)  | 0.02<br>(0.8981) | 1.92<br>(0.1670) | 0.991<br>(0.5509) |
| Instar<br>× Predator     | 3   | 0.21<br>(0.8895)  | 0.99<br>(0.3962)  | 4.44<br>(0.0044) | 8.73<br>(0.0001) | 0.882<br>(0.0001) |
| 3-way<br>Interaction     | 3   | 0.23<br>(0.8758)  | 0.71<br>(0.5469)  | 0.31<br>(0.8196) | 1.14<br>(0.3334) | 0.985<br>(0.9449) |
| Error                    | 350 |                   |                   |                  |                  |                   |

Table 5. ANOVA's and MANOVA for position within the container for larvae of *Aedes triseriatus* in the presence vs absence of the predator *Toxorhynchites rutilus*.

| Position Source       | Df  | Bottom F (P)   | Middle F (P)  | Wall F (P)     | Surface F (P)  | MANOVA Lambda (P) |
|-----------------------|-----|----------------|---------------|----------------|----------------|-------------------|
| Population            | 1   | 3.75 (0.0535)  | 8.61 (0.0036) | 30.13 (0.0001) | 1.03 (0.3110)  | 0.884 (0.0001)    |
| Instar                | 3   | 10.93 (0.0001) | 3.06 (0.0284) | 17.50 (0.0001) | 25.06 (0.0001) | 0.750 (0.0001)    |
| Predator              | 1   | 0.83 (0.3638)  | 0.04 (0.8400) | 1.81 (0.1799)  | 0.02 (0.8999)  | 0.991 (0.5403)    |
| Population × Instar   | 3   | 1.53 (0.2072)  | 1.14 (0.3314) | 2.88 (0.0358)  | 2.48 (0.0607)  | 0.929 (0.0114)    |
| Population × Predator | 1   | 0.32 (0.5733)  | 0.14 (0.7105) | 0.03 (0.8690)  | 0.01 (0.9273)  | 0.998 (0.9586)    |
| Instar × Predator     | 3   | 2.35 (0.0723)  | 1.71 (0.1636) | 4.66 (0.0033)  | 2.45 (0.0634)  | 0.913 (0.0015)    |
| 3-way Interaction     | 3   | 1.01 (0.3866)  | 1.95 (0.1215) | 0.09 (0.9637)  | 0.44 (0.7211)  | 0.961 (0.3110)    |
| Error                 | 350 |                |               |                |                |                   |

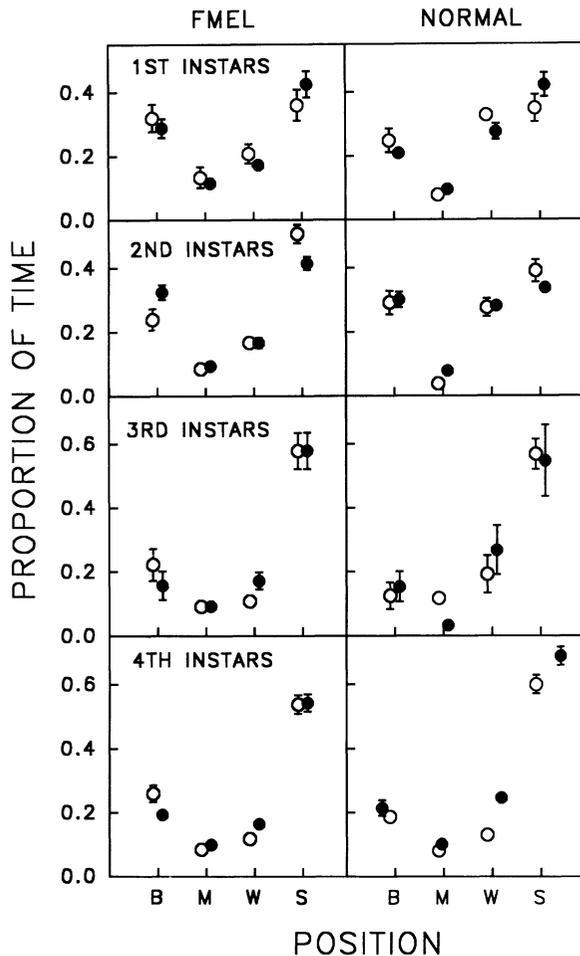


Fig. 4. Estimated proportions (mean±SE) of time spent in different positions for larvae of *Aedes triseriatus* from Normal and FMEL across four instars. Filled symbols – observed in the absence of *T. rutilus*, open symbols – observed in the presence of *T. rutilus*.

tions (Table 4), however, for thrashing, the nature of the instar effect is clear: thrashing is significantly less frequent in 3rd and 4th instars than in 1st and 2nd instars (Fig. 3). This confirms the pattern observed in the previous experiment.

#### Position

As was true for activity, MANOVA revealed significant effects of Population, Instar, Population-Instar interaction, and Instar-Predator interaction on positions occupied (Table 5). The main effect for Predator was not significant (Table 5), however the significant Instar-Predator interaction indicates that how positions occupied were affected by the presence of *T. rutilus* was dependent on which instar was observed. ANOVA's yielded a significant Instar-Predator interaction only for time at the wall (Table 5). Fourth instars were at the wall significantly less often in the presence of *T. rutilus* than they were in the absence of *T. rutilus* (Fig. 4). Thus, the presence of *T. rutilus* appeared to have some effect on positions occupied. The absence of any significant interaction involving population and predator indicates that the presence of *T. rutilus* affected positions occupied for the two populations in the same way (Table 5, Fig. 4).

The results of this analysis also confirmed the major differences among instars, but did not confirm the previously observed difference between populations in positions occupied. ANOVA's revealed significant population effects for time at the wall and in the middle (Table 5). Individuals from Normal were at the wall significantly more often and in the middle significantly less often than were individuals from FMEL (Fig. 4). This differs from the interpopulation differences observed in the previous experiment, which involved time at the surface vs bottom. The instar effect was significant for all four variables (Table 5) but interpretation of

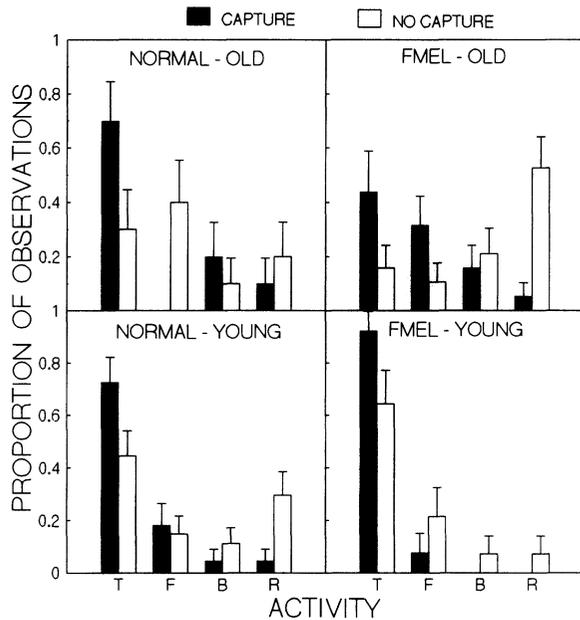


Fig. 5. Proportions ( $\pm$ SE) of different activities of *A. triseriatus* larvae for all combinations of population (FMEL vs Normal) and age (old vs young), observed in the presence of *T. rutilus*. Solid bars – capture group, open bars – no capture group.

this main effect for time at the wall is complicated by significant two way interactions (Table 5). However, for time at the bottom and at the surface, the nature of the instar effect is clear: 3rd and 4th instars spend significantly less time at the bottom and significantly more time at the surface than do 1st and 2nd instars (Fig. 4). This confirms the pattern observed in the previous experiment.

### Riskiness of different behaviors

#### Activity

Frequencies of different activities differed significantly between capture and no capture groups ( $\chi^2=16.28$ , Df=3,  $P=0.0010$ ). Frequencies of activities also differed between old and young larvae ( $\chi^2=11.50$ , Df=3,  $P=0.0093$ ), but not between FMEL and Normal populations ( $\chi^2=0.20$ , Df=3,  $P=0.9771$ ). The likelihood ratio ( $\chi^2=18.41$ , Df=12,  $P=0.1038$ ) was not significant, indicating that the main effects model adequately fit the data.

Thrashing was clearly over-represented for all Population-Age combinations in the capture group, and resting was over-represented in the no capture group (Fig. 5). The two feeding activities, filtering and browsing, did not differ consistently between groups (Fig. 5). The significant Age effect was due to a greater frequency of thrashing among young larvae (Fig. 5), a pattern also evident in the previous experiments (see Fig. 1).

### Position

Observations of larvae in the middle were rare in this experiment, and were completely absent for the Normal population (Fig. 6). To eliminate these zero frequencies we pooled wall and middle categories for analysis. Frequencies with which different positions were occupied differed between capture and no capture groups ( $\chi^2=7.09$ , Df=2,  $P=0.0289$ ). Frequencies of different positions did not differ between populations ( $\chi^2=5.53$ , Df=2,  $P=0.0631$ ), and between ages ( $\chi^2=1.55$ , Df=2,  $P=0.4616$ ). The likelihood ratio was not significant ( $\chi^2=1.40$ , Df=8,  $P=0.9942$ ), indicating that the main effects model adequately fit the data.

Fig. 6 shows frequencies with which each position was occupied for each Population-Age-Group combination. The significant Group effect resulted from observations at the bottom being consistently over-represented in the capture group, and observations at the surface being consistently over-represented in the no capture group across all four Population-Age combinations (Fig. 6). There was no clear pattern for pooled observations at the wall and in the middle, however, observations at the wall were over-represented in the no capture group in 3 of the 4 Population-Age combinations (Fig. 6).

### Discussion

Prediction 1), that these populations differ in behavior, was clearly confirmed by the fixed difference in behav-

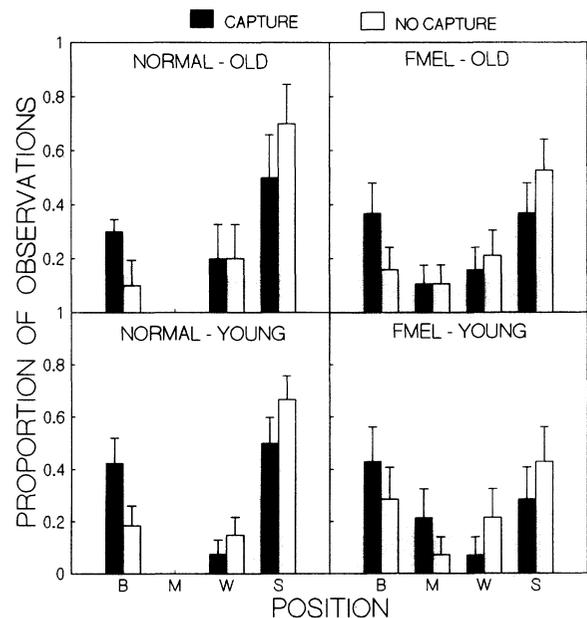


Fig. 6. Proportions ( $\pm$ SE) of different positions of *A. triseriatus* larvae for all combinations of population (FMEL vs Normal) and age (old vs young), observed in the presence of *T. rutilus*. Solid bars – capture group, open bars – no capture group.

ior between the FMEL and Normal populations. Although we found some evidence for a flexible behavioral response to the presence of *T. rutilus*, the degree to which this flexible response is expressed in the two populations does not differ.

Prediction 2), that risk of predation differs among behaviors, was confirmed for both activity and position. Specifically, thrashing was consistently the most- and resting the least-risky activities, and the bottom was the most- and the surface the least-risky positions.

Finally, prediction 3), that the more vulnerable population shows a higher frequency of riskier behaviors, was confirmed. The Normal population showed a significantly greater frequency of the riskiest activity (thrashing) than did the FMEL population. In one experiment the Normal population also showed a greater frequency of the riskiest position (bottom) than did the FMEL population, although in the second experiment this difference was not significant.

These results support the hypothesis that differences in behavior patterns between FMEL and Normal populations of *A. triseriatus* are the proximate cause of the differences in vulnerability to predation documented by Juliano (1989). Specifically, it appears that interpopulation differences in fixed behavior patterns, as opposed to flexible responses, are responsible for differences in vulnerability to predation by *T. rutilus*.

We expected that *A. triseriatus* would adopt low-risk behaviors when *T. rutilus* was present, however, the observed flexible behavioral response to the presence of *T. rutilus* was not consistent with this prediction. First instar larvae actually spent less time in a low-risk activity (resting) when *T. rutilus* was present. Fourth instars did spend significantly more time in a low-risk activity (resting) when *T. rutilus* was present, and reduced the frequency of browsing. They also spent significantly less time at the wall when *T. rutilus* was present, and this position was not consistently related to risk of predation. Overall, the relationship of behavioral changes and *T. rutilus* presence provides little support for the hypothesis that *A. triseriatus* modify behavior so as to reduce risk of predation by *T. rutilus*. It is especially clear that any inter-population differences in vulnerability are not due to differential flexible responses, as the two populations responded to *T. rutilus* in similar ways.

Low frequency of movement and microhabitat selection are commonly associated with low risk of predation in aquatic organisms (e.g., Woodward 1983, Fawcett 1984, Sih 1986, 1987, Kats, et al. 1988, Lawler 1989, McPeck 1990). In many instances interspecific differences in frequency of movement are correlated with co-occurrence with predators and are inferred to be products of natural selection via predation (Woodward 1983, Kats, et al. 1988, McPeck 1990). Although prey often alter their frequency of movement in response to the presence of predators (Sih 1986, 1986, Lawler 1989, McPeck 1990), and this may explain some interspecific differences in vulnerability to predation, fixed inter-

specific differences in behavior may be better predictors of vulnerability than differential flexible responses (e.g., Woodward 1983, Lawler 1989). Our experimental results fit the latter pattern: fixed intraspecific differences in behavior seem to determine vulnerability to predation.

The behavioral differences between these strains of *A. triseriatus* are correlated with predation by *T. rutilus*, which may be the ultimate evolutionary cause of the behavioral differences. The southern (high-predation) strain spends less time in the most vigorous activity than does the northern (low-predation) strain, and one interpretation of this observation is that predation selects for the "slow life-style" (Sih 1987). If this is true, it suggests that low-predation strain has been selected for a "faster life-style" since the recession of Pleistocene glaciers, when *A. triseriatus*'s range probably expanded north with the deciduous forest (Livdahl 1979). This seems to imply some cost to the slow life-style in the absence of significant predation (Sih 1987).

As pointed out by Livdahl (1984) and Juliano (1989), there are many other differences between southern and northern regions, and these environmental differences, either alone or in conjunction with predation, may select for different patterns of behavior. For example, in the north, the warm season is short, and in the extreme north, *A. triseriatus* is univoltine, with diapausing eggs deposited by the first generation of the year (Sims 1985). Further south, *A. triseriatus* becomes multivoltine with late autumn egg diapause, then multivoltine with continuous development and egg diapause only at extremely low temperatures in southern Florida (Sims 1985). The constraint of a short warm season may favor greater movement and feeding in northern populations (Juliano 1989). However, only one activity, thrashing, was more frequent in the Normal population, whereas the two feeding activities were actually less frequent. Thus, we find only partial support for the warm-season hypothesis.

Because position and activity are so closely correlated, it is difficult to decide whether activity or position is a more important determinant of risk of predation. Thrashing seems to be the riskiest activity, and because *A. triseriatus* rarely thrashes at the surface, the surface may appear to be associated with lower risk of predation. Despite this complication, it seems clear that the overall behavior pattern of the low-predation Normal population is likely to lead to a greater risk of predation than the behavior pattern of the high-predation FMEL population.

There are unanswered questions about the connection between behavior of *A. triseriatus* and predation by *T. rutilus*. We have documented a difference in behavior between single populations. Further behavioral tests with other populations would show whether or not these differences are consistently correlated with *T. rutilus* predation. Although we observed some short-term changes in behavior in response to the presence of *T.*

*rutilus*, the changes are rather subtle and do not seem to correlate well with differences in vulnerability to predation. It remains possible, however, that over a longer term (e.g., several days or weeks) the continued presence of *T. rutilus* may induce more marked behavioral changes that may contribute to interpopulation differences in vulnerability to predation.

There are pronounced ontogenetic changes in the behavior of *A. triseriatus* larvae. Later instars thrash less and occupy the bottom more frequently than do earlier instars. Because later instars engage in these risky activities less frequently, one interpretation of these ontogenetic changes is that an individual's expected future reproductive success (reproductive value) increases as it approaches metamorphosis (and associated maturity and reproduction), and this increase creates a greater potential for behavioral differences to affect fitness (Sih 1987). Selection for low-risk behaviors may thus be more effective on late instar larvae than on early instar larvae. There are, however, numerous alternative explanations for the ontogenetic changes in behavior that do not involve risk of predation. For example, energy costs of aquatic locomotion are often size dependent (Nachtigall 1985). If the cost of thrashing as a form of locomotion increases disproportionately as the size of the larva increases, this mode of locomotion may be used less frequently by older, larger larvae. Because the ontogenetic changes in behavior occurred in populations with and without histories of predation, this suggests that factors other than predation at least contribute to these ontogenetic changes.

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