

L.P. Lounibos · R.L. Escher · N. Nishimura · S.A. Juliano

Long-term dynamics of a predator used for biological control and decoupling from mosquito prey in a subtropical treehole ecosystem

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Abstract We identified, staged and counted the immature stages of mosquitoes from 1,826 censuses (with replacement) of the aquatic contents of ten treeholes surveyed every 2 weeks between 1978 and 1993. These time series were used to examine the population dynamics and effect on prey of the predatory mosquito *Toxorhynchites rutilus*. The mean annual frequency of occurrence of *T. rutilus* ranged from 0.02 to 0.67 among holes, and no fourth instars were recovered during a 30-month dry period. Oviposition and pupation by this species were recorded in all months, but most commonly in the spring. Overwintering larvae of the predator increased in weight during the prolonged fourth instar that preceded pupation in the spring. Time series analyses showed that the presence of a fourth instar *T. rutilus* significantly reduced the abundances of late-stage *Aedes triseriatus* mosquitoes. Pupal numbers of this prey species were more negatively affected by *T. rutilus* than were numbers of fourth instar *A. triseriatus*. Long-term declines in mean annual abundance of *A. triseriatus* prey during 16 years of observations on two holes were not correlated with increases in the mean annual frequencies of *T. rutilus*. Local extinctions of the aquatic stages of *A. triseriatus* within treeholes were common, but in most holes not significantly associated with the presence of *T. rutilus*, suggesting that predation does not routinely drive mosquito prey locally extinct in this ecosystem. The decoupling of *T. rutilus* and *A. triseriatus*, as revealed through these complete and long-term censuses, is contrasted with other reports of generalist predators causing extinctions of mosquito prey. Discrepancies

among reported outcomes probably result from differences in duration of sampling periods and statistical procedures along with real differences in the intensity of predation among systems and sites.

Key words *Aedes* · Extinction · Long-term censuses · Seasonality · Weights

Introduction

Traditional strategies for biological control have sought stable pest equilibria by means of specialist natural enemies, such as insect parasitoids (Hassell and May 1973; Hassell 1978). However, certain generalist predators may control insect populations even while causing instability and local pest extinctions (Murdoch et al. 1985). Local extinctions of prey by predators are more common in aquatic systems (Murdoch and Bence 1987) where nearly all efforts towards the biological control of mosquitoes are directed (Service 1983). Notonectid bugs and mosquitofish of the genus *Gambusia* are polypagous predators that have been shown to drive mosquito prey extinct in natural and semi-natural conditions (Blaustein et al. 1995; Murdoch et al. 1984; Bence 1988). Whether a generalist predator used for biological control promotes pest extinction or persistence may depend on the temporal and spatial scale of observations (e.g., Walde 1994).

Carnivorous mosquito larvae of the genus *Toxorhynchites* share traits in common with other predators such as polyphagy (Kazana et al. 1983; Lounibos et al. 1987), a relatively low intrinsic rate of increase (Trpis 1981), and asynchrony with prey populations (Trpis 1973; Lounibos 1979). Species of this genus have been introduced to control container-inhabiting mosquito pests and disease vectors in the city of New Orleans (Focks et al. 1982) and on islands in the Pacific (Paine 1934; Bonnet and Hu 1951; Petersen 1956; Rivière et al. 1979) and Caribbean (Gerberg and Visser 1978). Complete control of *Aedes aegypti* (L.) was reported for 6

L.P. Lounibos (✉) · R.L. Escher · N. Nishimura
University of Florida,
Florida Medical Entomology Laboratory,
200 9th St SE, Vero Beach, FL 32962, USA
Fax: 561-778-7205; e-mail: LPL@ICON.VERO.UFL.EDU

S.A. Juliano
Department of Biological Sciences, Ecology Group,
Illinois State University, Normal, IL 61790, USA

weeks on the island of St. Maarten after eggs of the African *Toxorhynchites brevipalpis* (Theobald) were dispersed into peridomestic breeding containers (Gerberg and Visser 1978). Ten weeks after release of the native North American *T. rutilus* Coq. in New Orleans, emergence of co-occurring *Aedes* and *Culex* pests was suppressed by 74% (Focks et al. 1982).

Based on a 9-month study in a natural hardwood forest in northern Florida, Bradshaw and Holzapfel (1983) reported that the presence of *T. rutilus* in treeholes was significantly associated with pupation failure and local extinction of the most susceptible prey species, *Aedes triseriatus* (Say). Although Bradshaw and Holzapfel's results seem to indicate that *T. rutilus* can, like *Notonecta* and *Gambusia*, drive mosquito prey locally extinct, this interpretation is open to question because they analyzed successive treehole censuses inappropriately, ignoring the fact that the data were a time series, and because they ignored heterogeneity among individual treeholes. Other field studies of natural populations of *T. rutilus* in discarded automobile tires have indicated that this predator may be capable of actual elimination of *A. aegypti* under certain circumstances (Bailey et al. 1983; Focks et al. 1980), but diapause by the predator may decrease its ability to control *Aedes* populations (Focks et al. 1980). Therefore, the question of whether *T. rutilus* drives *A. triseriatus* extinct under natural conditions warrants re-evaluation.

Stage-selective predation is common among many generalist predators that feed on mosquitoes (e.g., Scott and Murdoch 1983; Pastorok 1981). In treeholes, the net result of predation by *T. rutilus* is selective cropping of the older stages of mosquito prey (Bradshaw and Holzapfel 1983). Lounibos (1979) reported that fourth instar *T. brevipalpis* preferred pupal to fourth instar *Aedes* in the laboratory, but such selectivity has not been demonstrated in nature. Preferential elimination of the non-feeding pupal stage could be advantageous for control of container-inhabiting mosquitoes because it does not interfere with resource competition among larvae that naturally reduces pupation success (e.g., Gilpin and McClelland 1979).

The present study was undertaken to examine the effect of predation by *T. rutilus* on its principal prey species, *A. triseriatus*, in unmanipulated treeholes subjected to long-term censusing. In particular, we addressed three questions:

1. Does *T. rutilus* in a treehole reduce production of *A. triseriatus* and, if so, by how much?
2. Are long-term trends in *A. triseriatus* production associated with *T. rutilus* production?
3. Are local extinctions of *A. triseriatus* within individual treeholes associated with predation by *T. rutilus*?

Predator growth, development and dynamics are also described in order to call attention to habitat patchiness, weather and predator diapause that influence the linkage between predator and prey.

Methods

Study site and treeholes

Treeholes were located in a relatively undisturbed, 4-ha low hammock on the grounds of the University of Florida's Florida Medical Entomology Laboratory (27°35'N, 80°22'W). The dominant trees in this hammock, typical of subtropical peninsular Florida (Platt and Schwartz 1990), are the cabbage palm, *Sabal palmetto* (Walt.) Lodd. ex Schultes, and the southern live oak, *Quercus virginiana* Mill.

Weekly temperature means or maxima and minima, both ambient and in treeholes, from five representative years have been published previously (Lounibos 1983; Lounibos et al. 1992). Benthic treehole temperatures are typically stable from April through October, maxima ranging from 24 to 26°C and minima from 16 to 18°C during this period. The coldest months of the year are December through February when weekly minima within treeholes are commonly below 10°C and infrequently reach 0°C. The weekly range of temperature variation is much greater in the winter. Rainfall, obtained from daily records at a station 6.5 km northwest of the hammock, averaged 143.32 ± 7.30 (SE) cm per year during the study period.

Seven treeholes, one pan and six rot holes (*sensu* Kitching 1971) in separate *Q. virginiana* trees were found in 1978 to initiate fortnightly censuses (Table 1). Between 1980 and 1985, five of these holes were omitted (one temporarily) from censuses because of failures to hold water, and one new hole was added. Thus, only three holes were censused between 1985 and 1990, when hole 9 was added back to the program. Two new rot holes were added in 1992. For only two rot holes (numbers 2 and 6) were censuses uninterrupted throughout the 1978–1993 period.

Mosquito censuses and measurements

At 2-weekly intervals, all free-standing water was siphoned from each treehole with a plastic tube (internal diameter 12 mm), and the extract transported to the laboratory. After July 1979, each hole was rinsed with tap water, using a volume approximately equal to the original extract. The rinse water was siphoned into a separate bottle. Rinses increased the efficiency of recovery of mosquito immatures, estimated as approximately 90% of the actual numbers of larvae and pupae occupying a hole (Lounibos 1983).

In the laboratory the volume of the original extract was measured, and samples were passed through sieves to capture different-sized larvae. Larvae were rinsed in tap water and counted and identified in enamel trays under a strong light. In the first decade of study, *Corethrella appendiculata* Grabham, *T. rutilus*, *A. triseriatus* and *Orthopodomyia signifera* (Coq.) accounted for over 99.9% of all identifications, but from 1991 onward *Aedes albopictus* (Skuse), which was introduced into northern Florida in 1986 and subsequently spread southward (Peacock et al. 1988; O'Meara et al. 1993), occurred in some treehole samples. Early instars of the two *Aedes* and *O. signifera* were identified under a dissecting microscope (Breland 1959; Darsie 1986). All larvae were classified by instar, determined by comparative head capsule widths. Because it was the most abundant mosquito species in treeholes, and the most susceptible to predation (Chambers 1985), only numbers of *A. triseriatus* are considered in relationship to the presence or absence of *T. rutilus*. The second most abundant prey species in treeholes, *O. signifera* occurred in from zero to 26.6% of censuses of the six holes used for examinations of interactions between *T. rutilus* and *A. triseriatus*. The relative infrequency of *O. signifera* compared to *A. triseriatus* (Table 1), and the absence of competition between these two species where *O. signifera* densities are greater (Bradshaw and Holzapfel 1983) suggested that the latter did not affect directly the dynamics of interactions between *T. rutilus* and *A. triseriatus*.

Larval and pupal weights have been shown to be accurate predictors of developmental status in *Toxorhynchites* spp.

(Lounibos 1979; Russo 1986), and pupal weights are positively correlated with reproductive success in female *T. rutilus* (Frank et al. 1984). Therefore, fourth instar and pupal *T. rutilus* recovered in censuses were blotted dry and weighed on tared, waxed paper to the nearest 0.1 mg on an electrobalance. Pupal sexes were identified by shapes of gonopodia.

Within four hours after extraction, all larvae and pupae were returned to the original treehole fluid, together with particulate matter and debris retained in sieves, and replaced in their treehole of origin.

Recensusing of *T. rutilus* individuals

Counts of first through third instars and pupae were assumed to be independent for describing seasonal dynamics. Average stage durations in the laboratory at 21°C, of the first (4.4 days), second (5.8 days), and third (7.8 days) instars, and the pupal stage (8.4 days) of *T. rutilus* (Holzapfel and Bradshaw 1976) are each much less than the fortnightly interval between censuses, hence it is virtually impossible that the same individuals appeared in successive censuses. The infrequent recurrence of these stages of *T. rutilus* in successive censuses supports this assumption.

By contrast, the same individuals of fourth instar *T. rutilus*, the diapause stage in this species (Bradshaw and Holzapfel 1975, 1977; Trimble and Smith 1978, 1979) and the instar in which non-diapausing *Toxorhynchites* spp. can withstand long periods of starvation (e.g. Muspratt 1951), were frequently re-sampled in successive censuses. When individual weights and the absence of co-occurring *T. rutilus* of the same approximate size precluded misidentification of the same individual, wet weights on successive censuses were averaged or plotted, depending on the objectives of the analyses. When fourth instar *T. rutilus* co-occurred multiply in successive censuses, weights were averaged over censuses after arbitrary assignments of weights to individuals. The size similarity of co-occurring fourth instar *T. rutilus* (Lounibos 1985) minimized any errors in weight assignments.

Time series procedures

Prior to testing for effects of *T. rutilus* fourth instars on the abundance of *A. triseriatus*, relationships between life stages of *A. triseriatus* in successive censuses were evaluated by PROC AUTOREG of SAS (1988) as time series analyses in which the error terms are autoregressive processes. First and higher-order autocorrelations in the individual time series of *A. triseriatus* pupae and fourth instars were determined with PROC ARIMA of SAS (1988) through ten lags, and significant autocorrelations assessed as those greater than two times the standard error of the estimate. Both first and second-order autocorrelations were significant for some treeholes, so the lag for autoregressions was set equal to two. Autoregressive estimates for (1) numbers of *A. triseriatus* pupae versus total *A. triseriatus* larvae one census period previous and (2) numbers of *A. triseriatus* fourth instars versus total *A. triseriatus* of all younger stages one census period previous were calculated by the Yule-Walker method in PROC AUTOREG, also known as the two-step, full transform method (SAS 1988). Because of biases among estimators (Bence 1995), we also calculated autoregressions by the maximum likelihood and unconditional least squares methods in PROC AUTOREG. Although r^2 values differed slightly among methods, all three yielded similar conclusions concerning the significance of the structural portions of relationships and subsequent analyses of residuals. Autoregressions were performed only on time series with 90 or more successive observations on treeholes whose mean annual frequency of occurrence of *A. triseriatus* was > 0.50 (Table 1). *P* values from autoregressions are regarded as approximate because of deviations from normality in the *A. triseriatus* data sets.

Residual numbers of both pupae and fourth instars of *A. triseriatus* were obtained from the structural and time series portions of the regression models. These residuals are thus deviations above

or below the predicted abundance of each stage of *A. triseriatus* based on a model incorporating both serial dependence of numbers of that stage (autocorrelation components) and dependence of numbers of that stage on numbers of earlier stages in the previous census (structural component). We then tested whether the occurrence of *T. rutilus* fourth instars could account for these deviations from the autoregressive models. For these analyses, residual numbers of pupal or fourth instar *A. triseriatus* obtained for each treehole were analyzed by two-way, fixed effects ANOVA (PROC GLM, SAS 1985) with *T. rutilus* (\pm), treehole (1,2,3,6,9,11), and interaction as effects. In this analysis we assumed that the residuals within *T. rutilus* (\pm) groups within each treehole showed no trends with respect to the lagged variables from the autoregression analyses. Preliminary analyses supported this assumption. *Aedes* pupae and fourth instars were analyzed separately because *Toxorhynchites* appear to capture pupae preferentially (Lounibos 1979), hence the two stages may be differentially affected by the predator, and because of the greater relative importance of pupal survival in regulating prey reproductive rate. Other statistical analyses followed Sokal and Rohlf (1981), using non-parametric methods when assumptions of parametric tests were violated.

Extinction analyses

To examine whether extinctions of *A. triseriatus* within a treehole were associated with the presence of *T. rutilus*, we analyzed only censuses with water and only treeholes with records of more than forty wet censuses. The occurrence of *A. triseriatus* followed by its absence in the next census, if it had water, was regarded as an extinction. These extinctions could result in two ways. First, during the intercensus interval all *A. triseriatus* could pupate and emerge as adults, and if no new larvae hatch, the next census will record an extinction. This event may occur with or without a *T. rutilus* present. Second, all *A. triseriatus* immatures may be eaten by *T. rutilus*, and if no new larvae hatch, the next census will record an extinction. This event can occur only in the presence of *T. rutilus* larvae, and based on the size selectivity of *T. rutilus* predation (Bradshaw and Holzapfel 1983), only in the presence of late-instar predators. If most extinctions are of the first type, we expect extinctions and *T. rutilus* presence to occur together at random. If, however, most extinctions are of the latter type, we expect extinctions and *T. rutilus* presence to occur together more often than expected by chance. If a *T. rutilus* fourth instar or pupa was present at the time of extinction or in the previous census, the predator was considered associated with the extinction.

Determining whether extinctions are significantly associated with *T. rutilus* presence requires more than standard tests of association (e.g., a χ^2 test). The events recorded represent a time series and are clearly not a sequence of independent events, if for no other reason than that extinctions, by our definition, cannot occur in both of two successive censuses. To determine whether *T. rutilus* was associated with extinctions within each treehole significantly more than expected by chance, we used sampled randomization tests (Sokal and Rohlf 1981; Crowley 1992). We generated for each treehole one thousand randomized data sequences in which the number and temporal sequence of wet censuses, the frequency of *A. triseriatus* extinctions and their temporal sequence, and the number of occurrences (\pm) of *T. rutilus* fourth instars and pupae were fixed at the values observed in the data for the real treehole, but the temporal sequences of occurrences of *T. rutilus* were randomly assigned to the wet censuses. This procedure is equivalent to taking the observed series of wet censuses and *A. triseriatus* extinctions and then randomly placing the observed number of *T. rutilus* occurrences on those series 1,000 times. These 1,000 randomized data sets were used to provide the null distribution of numbers of *A. triseriatus* extinctions associated with the predator for each treehole. From the null distribution we determined the probabilities of equaling or exceeding the observed numbers of associations of *T. rutilus* with prey extinctions simply due to randomized placements of the predator with wet censuses. If, for an individual treehole, this probability is ≤ 0.05 , we take this to indi-

cate that extinctions of *A. triseriatus* occur in the presence of *T. rutilus* significantly more frequently than expected due to chance. Each individual test may have relatively low power so we used Fisher's method for combining probabilities from individual tests (Sokal and Rohlf 1981) to obtain a test of significance across all eight treeholes.

We conducted these randomization tests using two different sets of assumptions about the appropriate null hypothesis. First, we assumed that *T. rutilus* occurrence in a given wet census was a random, independent event, occurring with a probability (p) that was determined for each treehole such that the number of randomly placed occurrences of *T. rutilus* was equal to the observed number of occurrences. Independence in time implies that prior occurrence of *T. rutilus* had no effect on current probability of occurrence. At each step of the randomized sequence of occurrences, a random number (u) between 0 and 1 was generated, and if $u \leq p$, then the current random census was designated as *T. rutilus* positive. Although temporal independence is a reasonable initial assumption, inspection of the real data suggested that *T. rutilus* were aggregated in time, so if *T. rutilus* occurred in the previous census, the probability of *T. rutilus* occurring in the current census was greater. This led to our second null hypothesis, which assumed aggregation of *T. rutilus* in time. At each step of the randomization process, if the prior census had been assigned no *T. rutilus* occurrence, or had been dry, a random number (u) between 0 and 1 was generated, and if $u \leq p$, then the random census is designated as *T. rutilus* positive, just as in the randomization assuming temporal independence. If, however, the previous census had been assigned a *T. rutilus* occurrence, then two random numbers (u_1, u_2) between 0 and 1 were generated. If either $u_1 \leq p$ or $u_2 \leq p$ then the random census was designated as *T. rutilus* positive. This procedure resulted in patterns of *T. rutilus* occurrence that were temporally aggregated and that resembled the real data within most of our treeholes. Our purpose in testing the temporally aggregated null hypothesis was not to recreate every detail of *T. rutilus* occurrence in each real treehole. Instead, we were attempting to determine whether the general outcome of these tests was highly dependent on the assumption of independent occurrence of *T. rutilus*. Hence, we used the same basic procedure for all holes, varying only the value of p , in order to produce the correct number of *T. rutilus*-positive censuses.

To determine whether differences between our results and a previous study in north Florida on the same predator and prey species (Bradshaw and Holzapfel 1983) were attributable to biological or procedural (statistical) differences, we also analyzed our data by contingency tables, like those used by Bradshaw and Holzapfel (1983). We performed 2×2 association analyses using two alternative criteria for the presence of *T. rutilus*: (1) a predator of any life stage in the previous census (i.e. the criterion of Bradshaw and Holzapfel 1983) or (2) a fourth instar or pupa of the predator in the previous or current census (i.e., the criterion for our sampled randomization tests).

Table 1 Treehole capacities and mean annual frequencies of occurrence of mosquitoes (*Toxorhynchites rutilus*, *Aedes triseriatus*) during the 1978–1993 census periods

^a Highest amount of free-standing water recorded during censuses
^b Proportion of censuses with water that contained one or more *T. rutilus* or *A. triseriatus*
^c Treehole number nine did not hold water in the intervening 1983–1990 interval

Treehole no.	Maximum capacity ^a (l)	Census period	Total number of censuses	Mean annual freq. ^b <i>T. rutilus</i> (95% CL)	Mean annual freq. ^b <i>A. triseriatus</i> (95% CL)
1	2.0	IX/78 -XII/82	111	0.27(0.20)	0.99(0.02)
2	1.6	V/78 -XII/93	410	0.44(0.13)	0.64(0.13)
3	0.3	V/78 -IX/84	168	0.03(0.06)	0.72(0.13)
4	0.6	X/78 -IV/80	41	0.67(0.19)	0.25(0.43)
5	3.3	VIII/81 -III/89	198	0.53(0.16)	0.38(0.22)
6	1.1	V/78 -XII/93	410	0.63(0.15)	0.88(0.06)
9A ^c	0.1	IX/78 -X/83	132	0.02(0.03)	0.70(0.21)
9B ^c	0.5	VII/90 -XII/93	92	0.25(0.27)	0.86(0.14)
11	0.6	IX/78 -XI/85	187	0.11(0.09)	0.93(0.05)
12	0.1	VI/92 -XII/93	41	0.14(0.10)	0.71(0.31)
13	0.5	VIII/92 -XII/93	36	0.54(0.26)	0.34(0.26)

Results

Predator frequency

Mean annual frequencies of occurrence of *T. rutilus* in wet samples from ten treeholes ranged between 0.02 and 0.67 from 1,826 censuses during 1978–1993 (Table 1). The correlation across treeholes between mean annual frequencies of *T. rutilus* and *A. triseriatus* was not significant ($r = -0.45$ for arcsine-transformed proportions, $P = 0.16$, 9 *df*), and the transformed mean annual frequencies of neither species were correlated across treeholes with hole maximum capacities (*T. rutilus*: $r = 0.46$, $P = 0.15$; *A. triseriatus*: $r = -0.06$, $P = 0.87$).

In treeholes with the longest census records, numbers 2 and 6, the frequency of *T. rutilus* declined in 1980 and dropped to zero in 1981 (Fig. 1A), during the drought that reduced free-standing water below 50% of all censuses in 1981 (Fig. 1B). Between 1982 and 1984 the proportion of censuses containing water and *T. rutilus* both increased. A plateau in incidence of *T. rutilus*, especially in treehole 6, was maintained between 1984 and 1993, during which decade all but a few censuses of these holes contained some water (Fig. 1). Fourth instars of *T. rutilus* were absent for approximately 30 consecutive months from the six treeholes censused during the 1980–1982 drought period (Fig. 2).

Growth and development of *T. rutilus*

Because *T. rutilus* larvae hatch within 48–72 h after oviposition at 25°C (Trimble and Corbet 1975), the incidence of first and second instars was used as an index of recent oviposition. These early larval stages were recovered in all months of the year, most abundantly in the spring and least commonly in the winter (Fig. 3).

Pupae of *T. rutilus* were present in all months, most abundant in the spring and least common in the winter (Fig. 3). The frequency of pupae was greatest in April and showed a more distinct seasonal peak than that of the early instars.

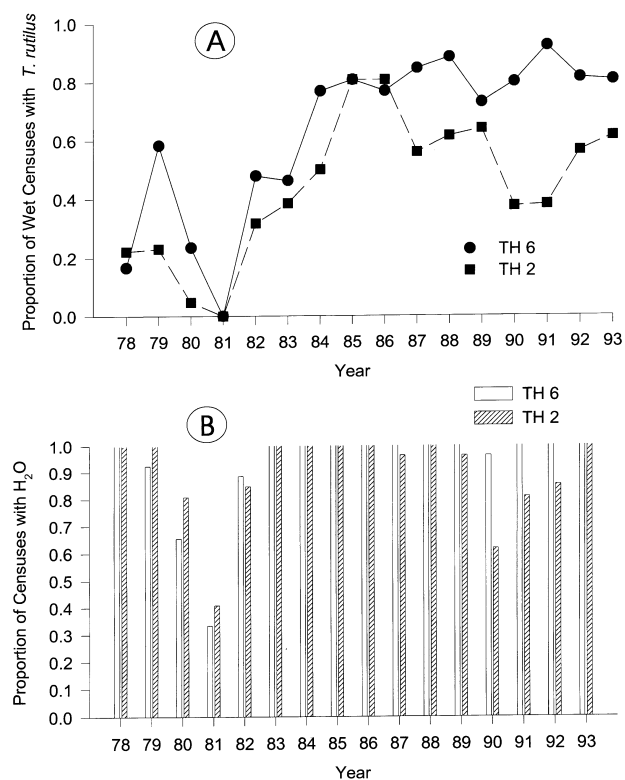


Fig. 1 **A** The annual frequencies of occurrence of one or more *Toxorhynchites rutilus* in water-holding samples of two treeholes censused fortnightly from 1978 through 1993. **B** The annual frequencies of censuses with some free-standing water in the same two treeholes. Holes were censused 26–27 times in all years except 1978, when 2-weekly censusing began in May

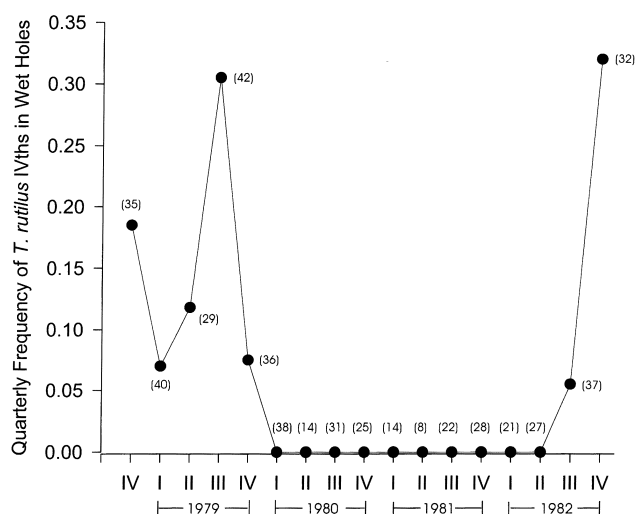


Fig. 2 The quarterly frequencies of occurrence of fourth instar *T. rutilus* (IVths) in six treeholes with water during a dry phase of the study period. Values near data points are numbers of treehole samples (out of a possible 36 or 42 total) that contained some free-standing water during that quarter

Almost identical numbers of instars I + II were recovered from holes 2 and 6, and no significant heterogeneity between holes was detected when frequencies of

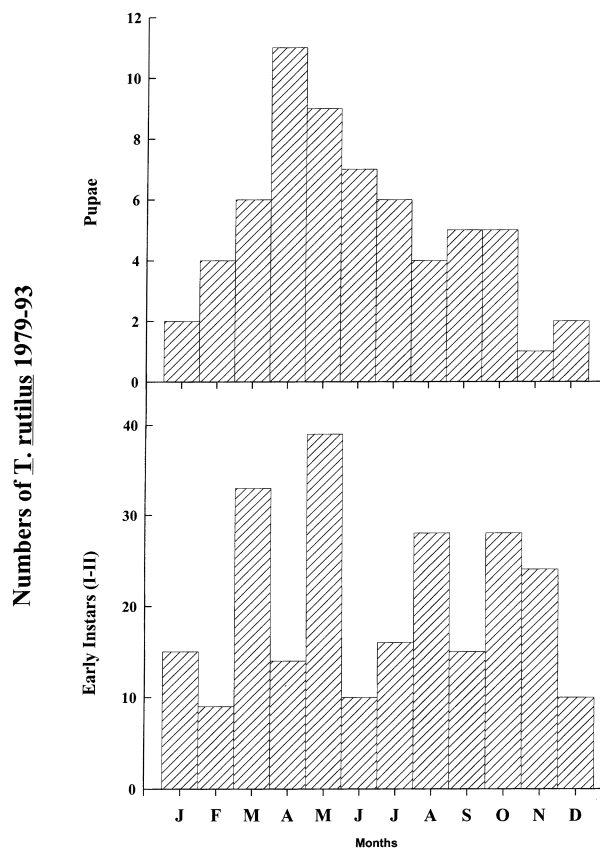


Fig. 3 The seasonal distribution of pupae and first plus second instars of *T. rutilus* recorded from all treehole censuses, 1979–1993

Table 2 Numbers of selected life stages of *T. rutilus* recovered in two treeholes censused fortnightly between 1978 and 1993

Treehole number	Stages		
	I + II	III	Pupae
2	96	51	15
6	95	40	24

Test of homogeneity (Sokal and Rohlf 1981) between holes: $G_{adj} = 3.4$, 2 *df*, $P > 0.10$

third instars and pupae were included (Table 2). A 2×2 test of independence indicated that survivorship from instars I + II to pupae did not differ between holes ($G_{adj} = 2.69$, ns, 1 *df*). However, when a contingency table test was applied to survival from the third instar to pupation, decreased survivorship in treehole 2 led to significant heterogeneity between holes ($G_{adj} = 8.49$, $P < 0.005$).

Treeholes 2 and 6, the only holes sampled continuously throughout the study period, produced 67% of all predator pupae which were weighed because of the positive relationship between female mass and fecundity (Frank et al. 1984). The pupal mass (mean \pm SE) from treehole 2 (36.6 ± 2.9 mg) was significantly less than that from treehole 6 (44.0 ± 1.3 mg) (Mann-Whitney

$U = 220, P = 0.022$), in which hole prey density was consistently higher. There was no significant difference in the masses of male (41.1 ± 1.7 mg, $n = 22$) and female (42.3 ± 1.9 mg, $n = 30$) pupae when all sexed specimens were considered ($t = 0.54, P = 0.60$). There was no significant variation among months in pupal masses from all holes ($F_{10,46} = 1.36, P = 0.23$).

Unequivocal recognition of the same individual fourth instar in successive censuses was possible only in the fall and winter months when fourth instars occurred singly in treeholes and lower temperatures retarded

development. For 11 fourth instars recognized individually in five or more successive censuses, reweighing usually revealed increases in mass, ranging from less than one to more than 25 mg between fortnightly intervals (Fig. 4). Of the 11 individuals tracked through the fall and winter 5 were recovered in the pupal stage in February and March.

Does predation by *T. rutilus* influence production of *A. triseriatus*?

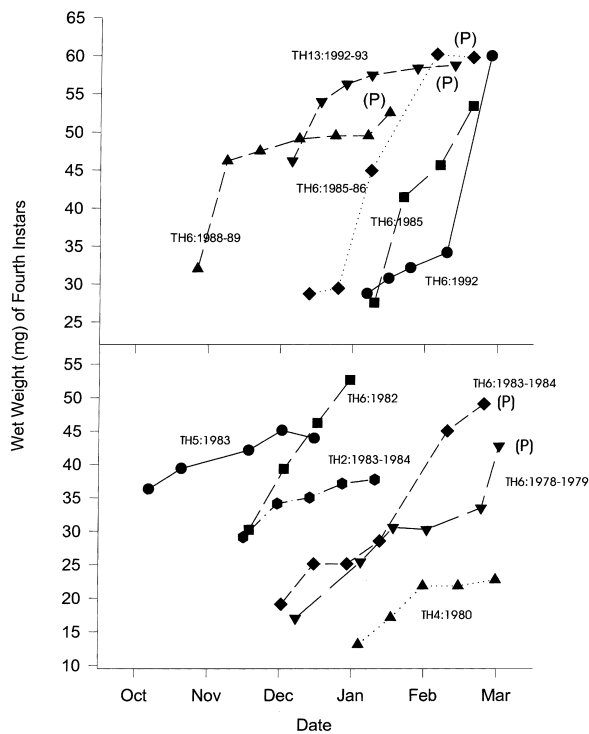


Fig. 4 Wet weights of individually recognized *T. rutilus* fourth instars in treeholes during fall and winter months of 1978–1984 (bottom) and 1985–1993 (top). (P) beside a final weight determination indicates pupation

Autocorrelations between late- and early-stage *A. triseriatus* were significant in all but one treehole and greater for larvae than for pupae in all but two holes (Table 3). A similar pattern was observed for the autoregressions of numbers of *A. triseriatus* pupae versus total numbers of *A. triseriatus* larvae of the previous census and for fourth instar *A. triseriatus* versus first through third instar *A. triseriatus* of the previous census, although only three of six pupal-larval autoregressions were significant. The overall coefficient of determination was substantially greater for the larval-larval autoregression, explaining 23% of the total variance, compared to only 3% for the pupal-larval autoregression (Table 3).

In two-way ANOVAs across all treeholes, both *T. rutilus* (\pm) and treeholes significantly affected residual numbers of pupal and fourth instar *A. triseriatus* (Table 4). For both variables, the interaction was not significant (Table 4) indicating that the effect of the predator was consistent across these six treeholes (Figs. 5 and 6). The overall effect of *T. rutilus* presence was to decrease the residual number of fourth instars (least squares mean \pm SE = -3.78 ± 2.21) and pupae (least squares mean = -0.78 ± 0.31) compared to those values observed in the absence of *T. rutilus* (fourth instar larvae = 0.70 ± 0.57 ; pupae = 0.27 ± 0.08), as we expected. However, only the least square means for residual numbers of pupae were significantly different from zero ($P = 0.013$ for + *T. rutilus* and $P = 0.001$

Table 3 Results from selected holes of autoregressions of later-stage (dependent variable) at $t + 1$ versus earlier stage (independent variable) at time t immature *A. triseriatus*

Hole no.	Pupae versus total larvae				Fourth versus younger instars		
	df	Order of sig. autocor ^a	Regr. ^b r^2	P^c	Order of sig. autocor ^a	Regr. ^b r^2	P^c
1	107	1	0.05	0.026	1	0.16	<0.001
2	408	2	0.19	<0.001	2	0.11	<0.001
3	127	1	0.02	0.154	2	0.19	<0.001
6	406	1	0.01	0.038	2	0.14	<0.001
9B	74	None	<0.01	0.542	None ^d	0.02	0.240
11	159	1	0.02	0.101	1	0.09	<0.001
Totals	1,300	2	0.03	<0.001	2	0.23	<0.001

^a Significant autocorrelations determined as $r > 2 \times SE$

^b Yule-Walker estimates for structural part of model after transforming for autocorrelation

^c P values assume normal distributions and, thus, are only approximate

^d Significant correlation at lag = 3 (but none at lags 1 or 2)

for *T. rutilus*). The ANOVA model for residual pupae explained approximately twice the variation as did the model for residual fourth instar larvae, though for both variables only a tiny proportion of the variation was explained (Table 4). The effect of *T. rutilus* was most clear cut in treeholes 2 and 6 (Figs. 5 and 6).

Are long-term trends in *A. triseriatus* related to predation?

The mean numbers of *A. triseriatus* per water-holding census declined in treeholes 2 and 6 during the course of the 16-year observation period (Fig. 7). Significant linear regressions described the decrease in mean numbers of *A. triseriatus* per year in both holes, and the addition of a quadratic term provided a significant improvement in the fit of treehole 6 data to a regression model. Be-

Table 4 Two-way analyses of variance of the effects of treeholes and presence/absence of *T. rutilus* fourth instars on the residual abundance of *A. triseriatus* pupae and fourth instars

Residual <i>Aedes</i> pupae				
Source	df	MS	F	P ^a
<i>T. rutilus</i> IV	1	159.37	32.02	<0.001
Treeholes	5	13.95	2.80	0.016
<i>T.r.</i> × holes	5	5.83	1.17	0.321
Error	1,292	4.98		
$(r^2 = 0.04)$				
Residual <i>Aedes</i> fourth instars				
<i>T. rutilus</i> IV	1	1937.12	7.77	0.005
Treeholes	5	604.27	2.42	0.039
<i>T.r.</i> × holes	5	212.51	0.85	0.513
Error	1,292	249.31		
$(r^2 = 0.02)$				

^a P values are approximate because underlying data sets from which residuals derived may be non-normal

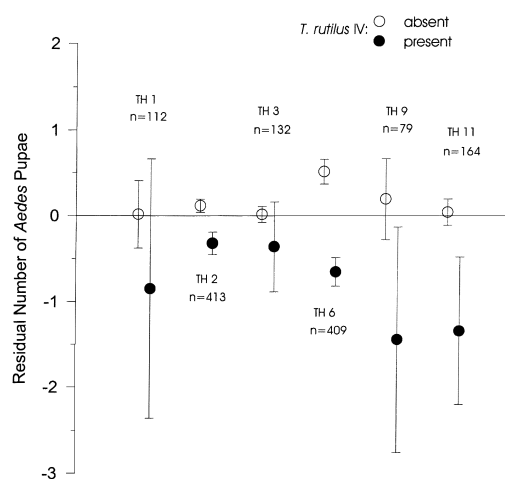


Fig. 5 Least squares means of residual numbers of *Aedes triseriatus* pupae (obtained from autoregressions of pupae versus lagged numbers of total larvae) versus the presence/absence of *T. rutilus* fourth instars. Error bars represent ± 1 SE

cause of possible serial correlations between years, the same data were analyzed by autoregression with the lag set to two. Autocorrelations were significant only for treehole 6, and autoregressions confirmed the significant decline over time in mean numbers of *A. triseriatus* for hole 6 ($r^2 = 0.53$, $P = 0.004$) and hole 2 ($r^2 = 0.35$, $P = 0.026$).

Because the decline in mean numbers of *A. triseriatus* in treeholes 2 and 6 appeared, at least superficially, to be related to increases in the annual frequency of occurrence of *T. rutilus* (Fig. 1A), we performed partial correlation analyses, including also the annual frequency of censuses without water (Fig. 1B). Using the method of

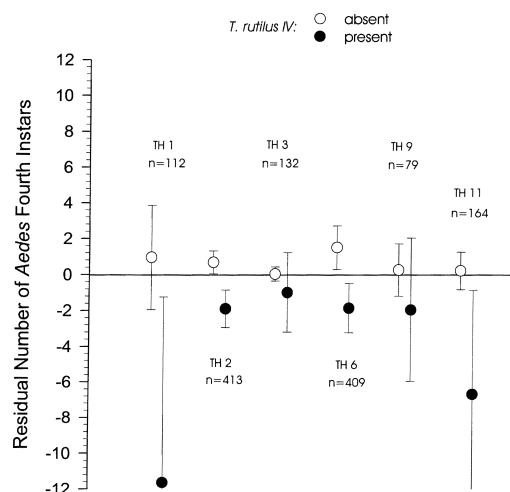


Fig. 6 Least squares means of residual numbers of *A. triseriatus* fourth instars (obtained from autoregressions of fourth instars versus lagged total numbers of younger instars) versus the presence/absence of *T. rutilus* fourth instars. Error bars represent ± 1 SE, and are truncated for two means to compress the figure

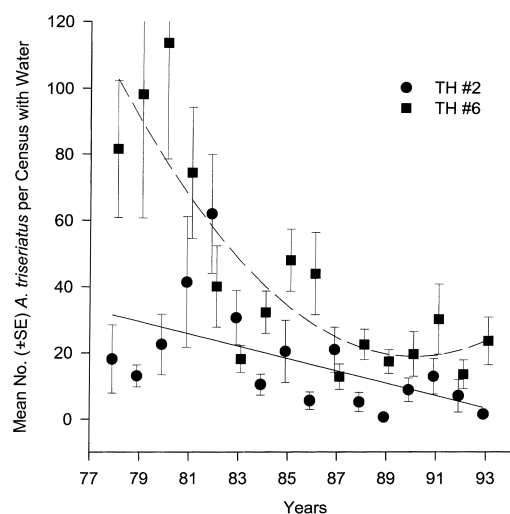


Fig. 7 Mean annual numbers of *A. triseriatus* in water-holding samples from treeholes 2 and 6 during the 16-year study period. Curves represent best-fitting regression models: treehole 2: $y = 141.6 - 1.49(\text{year})$, $F_{1,14} = 6.17$, $P = 0.026$, $r^2 = 0.31$; treehole 6: $y = 5373 - 119.1(\text{year}) + 0.66(\text{year}^2)$, $F_{1,13} = 19.09$, $P < 0.001$, $r^2 = 0.75$. Error bars represent ± 1 SE

partial correlations to hold one or the other variable fixed (Sokal and Rohlf 1981), neither the annual frequencies of occurrence of *T. rutilus* nor of dry censuses were significantly correlated with the mean annual abundances of *A. triseriatus* per water-holding census (Table 5).

Are extinctions of *A. triseriatus* associated with *T. rutilus*?

Extinctions of *A. triseriatus* were common, occurring in 8.7–18.0% of wet censuses of treeholes 2 through 9 (Table 6); in treeholes 1 and 11 the incidence of extinctions of *A. triseriatus* was below 4.0%. In most holes the occurrence of fourth instar or pupal *T. rutilus* outnumbered extinctions of *A. triseriatus* and in treehole 6 accounted for over half of all wet censuses (Table 6). Across all treeholes, less than half of all extinctions occurred in the presence of *T. rutilus* (Table 6). When we assumed *T. rutilus* occurred independently across time, randomization tests indicated that in all treeholes, number of extinctions of *A. triseriatus* that were associated with the presence of *T. rutilus* was not significantly greater than would be expected by chance.

Table 5 Partial correlation coefficients between mean annual numbers of *A. triseriatus* per wet census and two frequency variables for two treeholes surveyed for 16 years

Annual frequency	Mean numbers of <i>A. triseriatus</i>	
	Treehole 2	Treehole 6
<i>T. rutilus</i> per wet census	-0.28 ns	-0.11 ns
Dry censuses	-0.07 ns	0.41 ns

ns = non-significant, 14 *df*

^a Arcsine-transformed before calculation of correlations

Table 6 Sampled randomization tests for associations of extinctions of *A. triseriatus* with the presence of *T. rutilus* fourth instars or pupae in the same or previous census

Treehole no.	No. wet censuses	No. <i>A. triseriatus</i> extinctions	No. censuses w. <i>T. rutilus</i> ^a	No. <i>A.t.</i> extinctions with <i>T. rutilus</i>	Null hypothesis	
					<i>P</i> ^b : independent	<i>P</i> ^b : aggregated
1	73	2	10	2	0.070	0.057
2	364	46	119	26	0.458	0.195
3	128	23	4	0	1.000	1.000
4	41	5	16	1	1.000	0.997
5	259	27	88	17	0.314	0.343
6	379	33	201	27	0.387	0.020
9	162	22	12	3	0.656	0.649
11	135	5	7	1	0.447	0.402
Totals	1,541	163	457	77	$\chi^2 = 13.55$ $df = 16$ $P > 0.5^c$	$\chi^2 = 21.66$ $df = 16$ $P > 0.1^c$

^a Fourth instars or pupae

^b Based on null probability from 1,000 randomized distributions

^c Fisher's method for combining probabilities (Sokal and Rohlf 1981): $\chi^2 = 2\sum -\ln(P)$

Combined results for all eight treeholes indicated no significant association of extinction and *T. rutilus* across all treeholes (Table 6).

When we assumed that *T. rutilus* occurred aggregated in time, the results changed only slightly (Table 6). For seven of eight holes, the number of extinctions of *A. triseriatus* associated with *T. rutilus* was not greater than would be expected by chance. Only in treehole 6 were extinctions significantly associated with *T. rutilus* (Table 6). For all but treehole 6, actual *P* values from the tests were similar under both sets of assumptions (Table 6). For the tests assuming temporal aggregation, combined results for all eight treeholes again indicated no significant association of extinction and *T. rutilus* across all holes (Table 6).

Contingency table analyses of these same data indicated a significant negative association ($P < 0.05$) in two of seven treeholes between the presence of *A. triseriatus* and the presence of a *T. rutilus* fourth instar or pupa in the current or preceding census (Table 7). When we applied the criterion of Bradshaw and Holzapfel (1983) for *T. rutilus* presence, the association was also negative and significant ($P < 0.05$) in a single, albeit different, treehole (Table 7). The individual χ^2 values were significantly heterogeneous for both the criteria of Table 6 ($\chi^2 = 20.45$, $P < 0.005$, 6 *df*) and Bradshaw and Holzapfel (1983) ($\chi^2 = 14.95$, $P < 0.025$, 6 *df*), precluding the pooling of data to obtain composite χ^2 values for all treeholes combined (Zar 1996).

Discussion

Extinctions

The present study provides little evidence that *T. rutilus* is regularly associated in this ecosystem with extinctions of its principal prey, *A. triseriatus*. This conclusion was the same for nearly all treeholes whether we assumed

Table 7 Contingency table results for association of *A. triseriatus* presence with the presence of *T. rutilus*

Treehole no.	<i>T. rutilus</i> presence as in:	χ^2 ^a	
		Table 6	Bradshaw and Holzapfel (1983)
1		0.53(-)	0.46(+)
2		6.55*(-)	3.70(-)
3		1.15(+)	1.15(+)
4		nd ^b	nd
5		5.28*(-)	2.82(-)
6		3.19(-)	6.28*(-)
9		<0.01(+)	0.41(+)
11		0.14(+)	0.03(+)

* $P < 0.05$ for 1 *df*^a χ^2 corrected for continuity. Positive or negative direction of association indicated in parentheses.^b nd not done (sample size too small)

that *T. rutilus* occurred independently across censuses, or occurred aggregated in time. This similarity of conclusions under two different sets of assumptions suggests that the lack of an association is not a product of the particular assumptions built into our randomization tests (as is often the case with ecological null models – Colwell and Winkler 1984; Crowley 1992). The length of our data set supports the conclusion that such an association is really absent in these treeholes, not just undetectable due to power, sampling, or other factors. This result contrasts with reports that other generalist predators in aquatic ecosystems drive mosquito prey locally extinct (Murdoch et al. 1985). The original research that attributed mosquito extinctions to predation by *Notonecta* and *Gambusia* was based on short-term (season-long) observations of experimental plots stocked with predators (Murdoch et al. 1984; Bence 1988), in contrast to our long-term analyses of natural, unmanipulated communities. Criteria for local extinction in the current study are more rigorous than in the cited, experimental works because of the greater accuracy of our treehole censuses. An estimated 90% of all mosquito larvae and pupae are counted in extractions of the aquatic contents of treeholes (Lounibos 1983), whereas residual mosquitoes in cattle tanks or rice paddies could be easily missed by inadequate visual observation or incomplete sampling by dipping, and results scored as extinctions. However, in clear, temporary pools of the Negev Desert, Israel, the evidence is strong that *Notonecta maculata* cause local extinctions of the mosquito *Culiseta longiareolata* (Blaustein et al. 1995).

The current results contrast with the reported relationship between the presence of *T. rutilus* and local extinctions of *A. triseriatus* in north Florida treeholes (Bradshaw and Holzapfel 1983), although the two studies agree that pupation success of this prey species was reduced by the predator. Even when we apply the contingency table method of Bradshaw and Holzapfel (1983) to our data we find no significant association

between the presence of *T. rutilus* and *A. triseriatus* prey in most of these treeholes (Table 7). Because only one significant association was detected by sampled randomization tests, and then only under the assumption that *T. rutilus* presence in a hole is not independent, these contrasting results suggest that the serial dependency of successive censuses may lead to an inappropriate interpretation of associations from the contingency table analyses. The levels of significance for the few negative associations that we detected were much less ($P < 0.05$) than that reported by Bradshaw and Holzapfel (1983) from their pooled data from numerous holes in northern Florida ($P < 0.001$), suggesting that predation intensity may have been higher in the latter study. Supporting this interpretation, the overall extinction rate of *A. triseriatus* and frequency of occurrence of *T. rutilus* in northern Florida were approximately double the values observed in our data from southern Florida (W.E. Bradshaw, personal communication). If our results of significant heterogeneity of extinctions among holes fit a general pattern for *T. rutilus*, then predation intensity of this species may be very patchy within woodlands.

Causes of predator-prey decoupling

The limited evidence for an association between *A. triseriatus* extinctions and presence of *T. rutilus* in our study may be in part the result of inclusion of the full range of seasonal data on this predator-prey system. Bradshaw and Holzapfel (1983, 1988) did not examine predation by *T. rutilus* in the winter when this species is in fourth instar diapause in north Florida (Bradshaw and Holzapfel 1984). Reduced prey consumption is characteristic of diapausing *T. rutilus* (McCrary 1965), so the predator would be less likely associated with extinctions if winter months were included. In tires on an island in the Gulf of Mexico, while *T. rutilus* larvae were in diapause, the number positive for *A. aegypti* increased from 13% to 43% (Focks et al. 1980). In the milder climate of southern Florida, *T. rutilus* are not confined to this developmental stage during winter (Fig. 3), but hibernal temperatures are commonly low enough (Lounibos 1983; Lounibos et al. 1992) to retard development and lead to a concentration of individuals in, and prolongation of, the final larval instar (Fig. 4). Relatively low temperatures reduce prey consumption even in non-diapausing *T. rutilus* (Trimble and Smith 1978), which may further limit the potential of this predator to cause local extinctions in the winter.

Differential sensitivity to drought is one factor that may reduce predator impact and decouple populations of *T. rutilus* and *A. triseriatus*, as revealed from long data series that include dry periods. Fourth instars of the predator were completely absent during one 30-month dry interval (Fig. 2), and such droughts are commoner in peninsular Florida than at higher latitudes (Bradshaw and Holzapfel 1984). In the patchy habitats of southern

Florida, recovery of populations of *T. rutilus* reduced by drought may be slow owing to low adult dispersal between discontinuous hammocks, as was observed for *T. brevipalpis* in small relict forests on the Kenya coast (Lounibos 1979). Because of the absence of a drought-resistant life history stage, *T. rutilus* preferentially colonize treeholes that resist drying out (Bradshaw and Holzapfel 1988). By contrast, *A. triseriatus* possess a drought-resistant egg stage and do not choose holes according to their probability of desiccation (Bradshaw and Holzapfel 1988). After rainfall fills previously dry holes, pupal productivity of *A. triseriatus* is stimulated by an enriched detritus and the absence of *T. rutilus* (Lounibos 1985).

Fourth instar *T. rutilus* may also reduce search and attack rates at low prey densities which may enable the last few *A. triseriatus* larvae to linger in treeholes and to avoid extinction. Inspection of our data shows the frequent co-occurrence of one or two, usually early instar *A. triseriatus* with a fourth instar *T. rutilus*. These delayed-hatch *A. triseriatus* achieve relatively high fitness in the absence of predation (Livdahl and Koenekoop 1985). Attack rates by *T. rutilus* fourth instars on small prey are known to increase in the presence of fourth instar *A. triseriatus* (L.P. Lounibos, unpublished work).

The hibernal accumulation of fourth instar *T. rutilus* culminates in spring pupation (Fig. 3). The pupation peak of the predator in April coincides with that of *A. triseriatus* (Lounibos 1985), which may escape predation during the relatively lengthy non-feeding pupal stage of *T. rutilus*. Thus, over the entire annual cycle many apparent extinctions of *A. triseriatus* will occur by the completion of preadult development in the absence of *T. rutilus* and, in other cases, *A. triseriatus* immatures will co-exist with *T. rutilus*, particularly at lower temperatures.

Spatial patchiness also leads to a reduced predator impact (Folt and Schulze 1993). Treeholes are highly heterogeneous in characteristics that determine their inquiline fauna (Kitching 1971), and *A. triseriatus* and *T. rutilus* respond to different oviposition cues (Bradshaw and Holzapfel 1988). Thus, it is not surprising that frequencies of the two species should be inversely correlated among holes, although the negative correlation in this study was non-significant.

Do treeholes harbor metapopulations?

The patchiness of treehole habitats raises the question whether this predator-prey system may exhibit metapopulation dynamics characteristic of partially subdivided populations. An answer awaits information on immigration and dispersal of *A. triseriatus* and *T. rutilus* among holes, which is best obtained experimentally (e.g. Walde 1994). Colonization by *Aedes* is enhanced by the capacity of dried eggs to remain dormant on inner treehole walls, awaiting rainfall to fill the cavity and to stimulate hatch. Taylor (1991) contends that container ecosystems with populations of predatory and prey

mosquitoes are not true metapopulations and instead are affected by the spatial processes addressed in classical, within-population predator-prey theory. Facilitated by the dispersive attributes of the dormant aedine egg, the *Toxorhynchites-Aedes* system probably behaves more like a single, patchy population in which dispersal between patches is sufficiently high to maintain a single, extinction-resistant population (Harrison 1991).

Cannibalism, prey selectivity and biological control

The relative infrequency of early instars of *T. rutilus* (Fig. 3) is probably a consequence of cannibalism, known to differentially impact the younger stages (Corbet 1985). Cannibalism is common among *Toxorhynchites* in nature (e.g. Trpis 1973; Lounibos 1979; Bradshaw and Holzapfel 1983) and more likely when alternative prey are scarce (Corbet 1985).

From the standpoint of biological control, the issue of predator-induced extinctions of prey is unimportant if the target pest is reduced to innocuous levels. However, in many aquatic ecosystems, larval predators do not provide satisfactory control of mosquitoes (e.g., Service 1983; Blaustein 1992). The relatively small amount of variation of residual *Aedes* attributable to predation by *T. rutilus* in this natural ecosystem (Table 4) suggests that satisfactory biological control of mosquitoes may only be achieved by inundative and sequential releases of *Toxorhynchites* that augment natural numbers (Gerberg 1985). However, the current study did confirm that *T. rutilus* reduces pupal *Aedes* more effectively than fourth instars in nature (Fig. 5 vs. 6; Table 4). In principle, such selectivity on the final preadult stage should enhance the efficiency of *Toxorhynchites* for the biological control of mosquitoes because the predators remove those prey individuals with the greatest reproductive value.

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