INFLUENCE OF TADPOLE SHRIMP, *TRIOPS LONGICAUDATUS* (NOTOSTRACA: TRIOPSIDAE), STOCKING RATE ON *CULEX TARSALIS* DEVELOPMENT IN EXPERIMENTAL FIELD MICROCOSMS

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ABSTRACT. The effectiveness of 5 tadpole shrimp (TPS) stocking rates to reduce cohorts of *Culex tarsalis* was studied in field microcosms (0.81-m^2). Larval/pupal abundance in microcosms containing > 5 TPS/m^2 was significantly ($P < 0.05$) lower than that of controls lacking tadpole shrimp due to predation. Adult mosquito abundances captured in emergence units above the microcosms stocked with tadpole shrimp were significantly ($P < 0.05$) lower than that of controls at rates ≥ 10 TPS/m^2. Tadpole shrimp growth during 17 days of this study was inversely proportional to their stocking rate, and a linear relationship between size and stocking rate was plotted. Tadpole shrimp stocking rates also influenced rate of mosquito development, causing significantly ($P < 0.05$) shorter periods for 50% emergence where shrimp were present when compared with that of controls. Adult male *Cx. tarsalis* emerged significantly earlier than females in microcosms stocked at 5 TPS/m^2, while no significant ($P > 0.05$) differences were detected between the sexes at the remaining predator stocking rates.

INTRODUCTION

Tadpole shrimp (Notostraca: Triopsidae spp.) are inhabitants of temporary waters worldwide (Longhurst 1955). In experimental ponds in the Coachella Valley, California, *Triops longicaudatus* (LeConte) was the first predaceous arthropod to appear in tow net samples 5 days after flooding (Walton et al. 1990b). In contrast to most aquatic insect predators, tadpole shrimp produce desiccation-resistant eggs which lie dormant in the soil for months or years and hatch when hydrated; an ecological adaptation to living in temporary bodies of water. A review of the natural history of *Triops* can be found in Hempel-Zawitkowska (1967).

Early field observations by Mail (1934) and Maffi (1962a, 1962b) suggested tadpole shrimp to be predators of mosquito larvae in temporary waters. Further studies in the laboratory indicated that tadpole shrimp feed upon mosquito larvae (Mail 1934, Scott and Grigarick 1979, Tietze 1987, 1989). The impact of tadpole shrimp on *Culex* populations was studied in vegetated and unvegetated field plots, and under both conditions tadpole shrimp yielded > 95% control. We found that both tadpole shrimp induced mosquito oviposition deterrence and predation responsible for reducing mosquito larval populations (Tietze and Mulla 1990).

We are currently evaluating tadpole shrimp as biological control agents for mosquitoes. If they are to be used as operational biological control agents of mosquitoes, the basic question, "What predator density is needed for effective mosquito control?" must be addressed. This study investigates the effect of tadpole shrimp stocking rates on the degree of mosquito control in field microcosms.

MATERIALS AND METHODS

We used fiberglass microcosms (0.81-m^2) located at the Aquatic and Vector Control Research Facility in Riverside, California, for our study. The microcosms were equipped with float-valve systems to maintain a water depth of 30 cm and volume of 0.243 m^3. Approximately 2 kg of dry soil void of tadpole shrimp eggs were added to each microcosm prior to flooding. Each microcosm was enclosed by a pyramid-shaped emergence unit 67 cm in height, covered with nylon window screen and tapered from 0.75 m^2 at the base to 0.015 m^2 at the apex. A pint jar (1.1-liter) was secured at the apex of each emergence unit for collecting emerged adult mosquitoes. A plastic funnel was inserted into the neck of each pint jar, which effectively trapped the adult mosquitoes in the jars. Black cloth collars were placed around the exterior portion of the apex and top 20 cm of the emergence units to enhance the positively phototropic response of emerged mosquitoes and their entry into the collection jar. The emergence units excluded oviposition by wild gravid mosquitoes and kept other mosquito predators from entering the system. Water temperature was recorded using a maximum-minimum thermometer (Taylor Instruments, Arden, NC) placed into a shaded portion of a microcosm.

Microcosms were flooded on July 16, 1989, and treated with cypermethrin (1.21 g/ha) on July 17 to kill hatching tadpole shrimp, about 24 h before stocking, because these units were stocked with tadpole shrimp before and their eggs were present in most of the units. Cypermethrin applied at this rate has no adverse impact on mosquito larvae (Walton et al. 1990a). Mosquito populations were created by adding 5

locally field-collected *Culex tarsalis* Coq. egg rafts per microcosm on July 18. Mosquito larval food consisting of a mixture of finely ground rodent laboratory chow (Ralston Purina Co., Denver, CO) and yeast (3:1) was added to each microcosm on days 2, 4, 6 and 8 post-stocking. About 1.5 g of larval food was added to each unit and evenly dispersed across the entire water surface. Mosquito abundance was measured daily (except on days 12 and 14 post-stocking) by taking three, 400-ml dips per microcosm with replacement. Dips were taken from the corners of the microcosms. On each sampling day, larval instar and stage of development of mosquitoes in each microcosm was noted, and adult mosquitoes present in the emergence units were counted, sexed and identified.

Five tadpole shrimp (TPS) stocking rates (0, 5, 10, 20 and 40 TPS/m^2) were established in microcosms by adding 0, 4, 8, 16 and 32 TPS/microcosm, respectively. Tubs were randomly assigned stocking rates, and each rate was replicated 3 times. Tadpole shrimp used in this study were collected from a previously flooded pond located at the same facility. At the time of addition and end of the study (17 days post-stocking), tadpole shrimp density and size of tadpole shrimp per microcosm were recorded.

Larval and adult abundances were compared among tadpole shrimp stocking rates using an one-way analysis of variance procedure and Duncan's multiple range test (SAS 1985). The null hypothesis tested was: there is no significant difference in larval or adult mosquito abundance between tadpole shrimp stocking rates. Male and female adult mosquitoes were thus analyzed separately.

The relationship between tadpole shrimp stocking rate and their growth after 17 days post-stocking was analyzed using an one-way analysis of variance procedure (SAS 1985) to test for significant differences in tadpole shrimp size between tadpole shrimp stocking rates. The stocking rate-growth relationship was plotted.

The time for 50% adult emergence was determined for each replicate of each predator stocking rate. This was done by plotting cumulative numbers of male and female mosquitoes on a per microcosm basis and thus determining the day corresponding to 50% of the total emergence. Differences in time for 50% emergence among stocking rates were analyzed by multivariate analysis of variance (SAS 1985). Difference in emergence time was also compared between mosquito sexes using paired *t*-tests. Due to low numbers of adults emerging at the tadpole shrimp stocking rate of 20, this rate was excluded from the above 2 tests.

RESULTS

Significant differences ($P < 0.05$) in larval/pupal and adult mosquito abundances were detected among the 5 different tadpole shrimp stocking rates (Fig. 1). Larval/pupal mosquito abundance was significantly ($P < 0.05$) lower in microcosms stocked with tadpole shrimp compared with controls, particularly for the highest 2 stocking rates (Fig. 1). Five and 10 TPS/m^2 yielded intermediate larval/pupal abundances, but were still significantly ($P < 0.05$) different from that of controls. Similar trends were evident when comparing adult abundance among the stocking rates (Fig. 1). Male and female adult *Cx. tarsalis* abundances were significantly
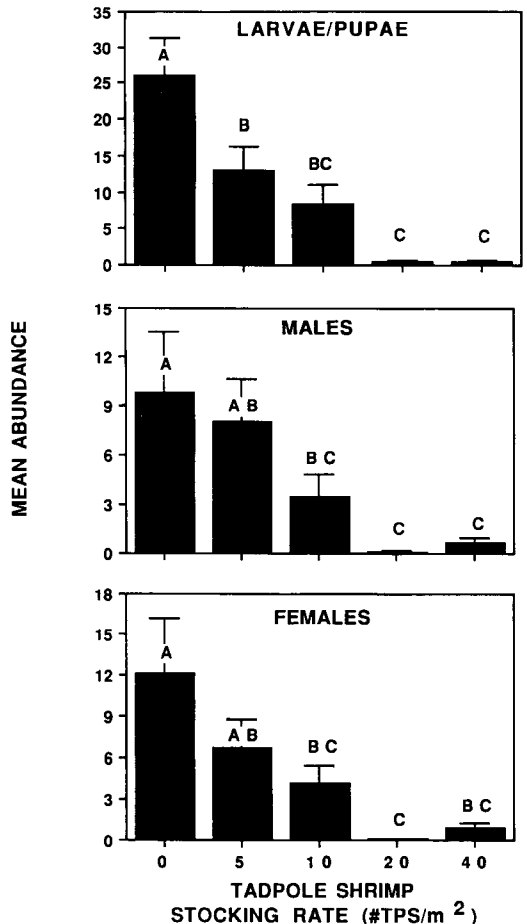

Fig. 1. Mean abundance per microcosm of larval/pupal and adult male and female *Culex tarsalis* stocked at 5 tadpole shrimp densities. Unshared letters indicate significant ($P < 0.05$) differences determined using Duncan's multiple range test. Bars represent standard errors.

($P < 0.05$) lower than that of controls at stocking rates of greater than or equal to 10 TPS/m^2. The lowest mosquito abundances were detected at 20 and 40 TPS/m^2, where the former rate produced slightly lower abundances, especially for adult female mosquitoes (Fig. 1).

Tadpole shrimp averaged 3.0 mm in carapace length when stocked in the microcosms, but at 17 days post-stocking their size was found to be significantly ($P < 0.001$) different among the 4 stocking rates. Tadpole shrimp carapace length was inversely proportional to stocking rate, and a linear relationship was plotted (Fig. 2). Tadpole shrimp density 17 days post-stocking ranged from 47 to 60% of the original stocking density.

Time for 50% emergence of male and female *Cx. tarsalis* was significantly ($P < 0.05$) shorter in microcosms stocked with tadpole shrimp when compared with that of controls (Fig.3). No significant ($P > 0.05$) difference in emergence rate was detected among the stocked treatments. Males emerged significantly ($P < 0.05$; *t*-test) earlier than females in microcosms stocked at 5 TPS/m^2, but no difference in emergence rates between adult mosquito sexes was detected in the remaining treatments. Water temperature in the microcosms ranged from 21 to 33.5°C during the course of the study.

DISCUSSION

Tadpole shrimp-stocked field microcosms produced significantly lower immature and adult mosquito abundances than that of controls when stocked at densities greater than or equal to 10 TPS/m^2. Twenty and 40 TPS/m^2 caused the
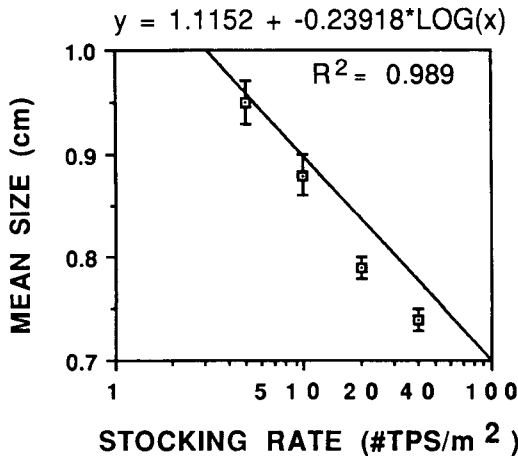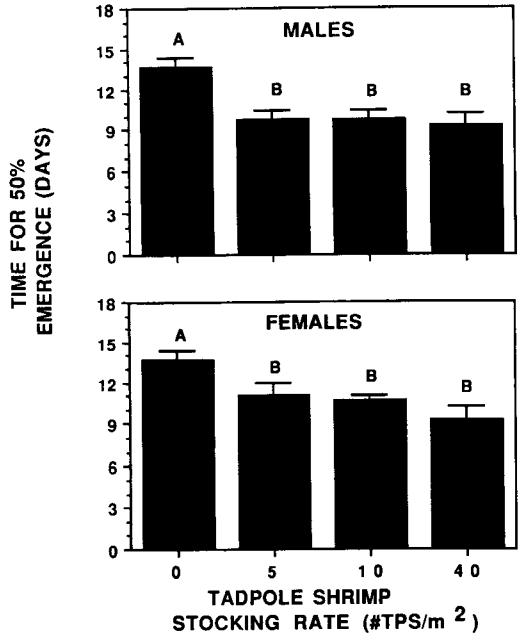

Fig. 3. Mean time for 50% emergence of adult male and female *Culex tarsalis* in field microcosms stocked at 5 tadpole shrimp densities. Unshared letters indicate significant ($P < 0.05$) differences determined using Duncan's multiple range test. Bars represent standard errors.

lowest *Cx. tarsalis* abundances, while 5 and 10 TPS/m^2 produced intermediate results. While larval/pupal abundances in microcosms stocked with 5 TPS/m^2 were significantly ($P < 0.05$) lower than that of controls, no significant ($P > 0.05$) differences were detected between this stocking rate and controls regarding adult male and female mosquitoes. This discrepancy can be attributed to increased variation in abundance of male and female mosquitoes emerging in controls due to lower numbers of adult mosquitoes recovered from one control replicate. The general absence of most mosquito natural enemies from the microcosms assured us that mosquito decline was primarily due to tadpole shrimp predation.

While successful control of *Cx. tarsalis* was achieved in fiberglass microcosms using certain shrimp stocking rates, it would be incorrect to assume the same stocking rates to be equally effective in any aquatic habitat. Fiberglass microcosms represent an highly simplified habitat; extrapolation of results of this study to various other field habitats could be misleading. Other studies in progress have indicated that larval mosquito populations are reduced less by tadpole shrimp in experimental field ponds contain-

$$y = 1.1152 + -0.23918*LOG(x)$$

$$R^2 = 0.989$$

Fig. 2. Regression of mean tadpole shrimp carapace length ($\pm$SE) and tadpole shrimp stocking rate in field microcosms.

ing dense emergent vegetation than in those lacking emergent vegetation. Thus higher tadpole shrimp stocking rates may be necessary to achieve mosquito reduction in densely vegetated aquatic habitats.

Tadpole shrimp stocking rate influenced both their own growth and that of the mosquitoes. Density-dependent processes caused tadpole shrimp to grow less when stocked at higher densities. The nature of this relationship remains unstudied, but may be due to nutritional deficiency. Stocking with tadpole shrimp also caused a significant ($P < 0.05$) decrease in the time needed for 50% mosquito emergence when compared to controls lacking shrimp. Why the later emergence of mosquitoes in the controls? At high densities, larval mosquitoes are scramble-type competitors (Nicholson 1954) which filter-feed upon organic particles (Mogi 1981). Increasing larval density means a decreasing abundance of food on a per larva basis thus causing slower development, as has been documented for larval *Aedes aegypti* (Linn.) and *Ae. vexans* Meigen (Brust 1968). Since larval densities were reduced by tadpole shrimp predation, intraspecific competition for food was probably also reduced. In addition, tadpole shrimp activity on the microcosm benthos increased the availability of suspended organic matter (unpublished data), which may form an important food source to the developing mosquito larvae. Selective predation upon smaller instar larvae, as observed for *Cx. quinquefasciatus* Say in the laboratory (Tietze and Mulla 1989), may have also skewed the larval profile toward larger instars and subsequently earlier emergence.

Longer larval and pupal periods of *Cx. quinquefasciatus* have been attributed to chemical "overcrowding factors" (Ikeshoji and Mulla 1970). Such overcrowding factors were probably not a primary cause for slowed development in this study, due to relatively low maximal larval densities calculated to be about 4 larvae/liter (assuming an average of 175 larvae hatch per egg raft) compared with a single overcrowding unit derived from 5,000 larvae/liter (Ikeshoji and Mulla 1970).

Tadpole shrimp mortality during the stocking period was characteristic of natural mortality trends observed in larger experimental ponds. In a constant volume of water, TPS density is highest during the first 2 or 3 weeks post-flooding and then declines (Tietze and Mulla 1990). Tadpole shrimp eggs hatch soon after inundation and develop as a single synchronous cohort during the flooding period. Since subsequent hatching does not occur or smaller tadpole shrimp are consumed by mature tadpole shrimp

(Hempel-Zawitkowska 1967), tadpole shrimp densities decline during the flooding period.

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