AN EVALUATION OF *GAMBUSIA AFFINIS* AND *BACILLUS THURINGIENSIS* VAR. *ISRAELENSIS* AS MOSQUITO CONTROL AGENTS IN CALIFORNIA WILD RICE FIELDS

VICKI L. KRAMER,[1] RICHARD GARCIA[2] AND ARTHUR E. COLWELL[3]

ABSTRACT. The mosquito control potential of the mosquitofish and *Bacillus thuringiensis* var. *israelensis* (*Bti*) were evaluated in experimental wild rice fields in Lake County, California. Fields were assigned one of six treatments: control, 1.1 kg/ha *G. affinis*, 3.4 kg/ha *G. affinis*, *Bti* only (6 kg/ha Vectobac™ granules), 1.1 kg/ha *G. affinis* plus *Bti* and 3.4 kg/ha *G. affinis* plus *Bti*. *Gambusia affinis*, at both release rates, significantly reduced the mosquito population at densities exceeding 100 fish/minnow trap. Treatments with *Bti* significantly reduced larval populations; however, the populations in the fields without fish rebounded to pretreatment levels within two weeks. In fields stocked with *G. affinis* and treated with *Bti*, populations remained low after *Bti* treatment. Nontarget populations of arthropods were significantly lower in fields stocked with *G. affinis* than in fields without fish on one or more sampling dates.

INTRODUCTION

Wild rice (*Zizania palustris* Linn.) is grown in Lake County, California from May through October, providing ca. 300 hectares of breeding habitat for *Culex tarsalis* Coquillett, *Anopheles freeborni* Aitken and *An. franciscanus* McCracken.[4] Wild rice acreage is increasing in California and totaled more than 6,400 hectares in 1986 (M. D. Andres, unpublished data).

The mosquitofish, *Gambusia affinis* (Baird and Girard), has been shown by several researchers (Hoy and Reed 1970, Craven and Steelman 1968) to be an effective mosquito control agent in white rice (*Oryza sativa* Linn.) fields. Consequently, mosquito abatement districts (MADs) in the Central Valley of California make seasonal releases of the fish into their rice fields. The use of *G. affinis* instead of chemicals can reduce mosquito control costs in white rice fields substantially (Lichtenberg and Getz 1985). Wild and white rice plants, although cultivated in a similar manner, have several differences, such as plant height and structure and length of the growing season, that could affect the mosquito control effectiveness of *G. affinis* (Kramer et al. 1987).

Gambusia affinis was evaluated in experimental wild rice fields in Lake County in 1986 at release rates of 0.6 and 1.7 kg/ha (0.5 and 1.5 lbs/acre), and no significant differences were found among the mosquito populations in fields with or without fish (Kramer et al. 1987). The authors postulated several reasons for this lack of control, such as the short (90 day) growing season, the wild rice plant structure, and the omnivorous feeding nature of *G. affinis*. They concluded that higher release rates of *G. affinis* may be necessary for significant mosquito control in wild rice fields.

This study therefore evaluated *G. affinis* at a higher release rate of 3.4 kg/ha (3 lbs/acre). This rate is 15 times greater than the usual release rate (0.2 lbs/acre) in California white rice fields (Combs 1986) and, although feasible for Lake County, is unrealistic for many mosquito abatement districts. Thus *G. affinis* was also evaluated at a more practical rate of 1.1 kg/ha (1 lb/acre). Although significant mosquito control was not achieved at 1.7 kg/ha during the 1986 season, the 1.1 kg/ha rate was selected for 1987 because many variables in a rice field system can change from one season to the next, potentially affecting the control capabilities of *G. affinis*. Also, the Lake County experimental wild rice fields were first year fields in 1986 and this may have affected mosquito production (Collins and Washino 1980). The impact of *G. affinis* on aquatic insect and zooplankton populations was also evaluated in this study.

Bacillus thuringiensis Berliner var. *israelensis* de Barjac (*Bti*) has been used effectively to control mosquito larvae in a wide range of habitats (Lacey and Undeen 1986), including white rice fields, but had not previously been tested in wild rice. Studies (ibid) have shown that *Bti* is a highly selective control agent and that natural enemies of mosquito larvae are conserved. To evaluate whether natural enemies, including introduced fish, can maintain mosquito populations at a low level following an initial reduction by *Bti*, tests were conducted using the pathogen alone and in combination with *G. affinis*.

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[4] Tompkins, D. 1987. Lake County Agricultural Crop Report. Depart. Food and Agriculture, Lakeport. 5 pp.

MATERIALS AND METHODS

The eighteen 0.1 hectare (0.25 acre) Lake County rice plots used to evaluate *G. affinis* in 1986 (Kramer et al. 1987) were used for the 1987 study. The fields were flooded and seeded on June 9 using a seed broadcaster attached to an all-terrain vehicle. The wild rice plants matured in about 90 days, and the fields were drained for harvest on September 16.

Maximum/minimum thermometers were placed in two of the plots, and the water temperatures were recorded weekly throughout the season. Plant height and water depth were also monitored weekly. A water sample from the center of each field was collected on September 13 and analyzed for nitrate and phosphate content, alkalinity, hardness, conductivity and pH.

There were three replicates of each of the following six treatments: control, 1.1 kg/ha (low fish rate) *G. affinis*, 3.4 kg/ha (high fish rate) *G. affinis*, *Bti* only, 1.1 kg/ha *G. affinis* plus *Bti* and 3.4 kg/ha *G. affinis* plus *Bti*. All *Bti* treatments were 6 kg/ha of granular Vectobac™ (200 ITU/mg). Prior to flooding, treatments were assigned to the fields using a randomized block design (Fig. 1).

Gambusia affinis were seined from the Lake County MAD rearing ponds, weighed and released into the 12 treatment plots on June 23, two weeks post-flooding. Fish survival is believed to be optimized by this two week post-flooding release schedule (Farley and Younce 1977a). Fish released included adults and fry of both sexes.

Monitoring stations were flagged at 2 meter intervals around the perimeter of each field. Each flag was assigned a number so that stations could be randomly selected as monitoring sites for the fish, mosquito and other aquatic insect populations.

Every two weeks, four minnow traps (3.2 mm mesh) per field were set overnight (ca. 24 hr.) to sample *G. affinis* and other aquatic organisms. The trap stations, one per each side of the field, were randomly selected on each trapping date. On the final monitoring date (September 8), four traps were also set in the interior of each field (a total of eight traps per field). All organisms were counted at the study site and returned to their trapping location. On August 11, ten trapped fish from each field (120 total) were frozen for later gut content analysis.

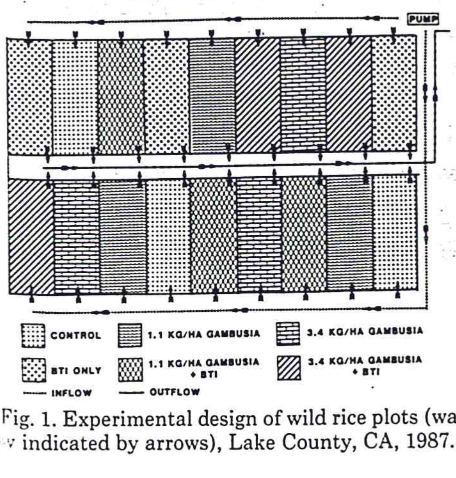

Fig. 1. Experimental design of wild rice plots (water flow indicated by arrows), Lake County, CA, 1987.

The immature mosquito populations were monitored weekly by taking 40 dips around the perimeter and 20 dips through the interior of each field. The perimeter density was sampled by randomly selecting eight (two per field side) of the flagged stations each week. Five dips (400 ml each) were taken at each station in a semicircular pattern, within 2 meters from the levee. The interior samples were taken along a transect beginning at a randomly selected station on one side of the field and ending at a randomly selected station on the opposite levee. A single dip was taken at 2 m intervals along the transect. All dip samples were concentrated, placed in containers with rice field water, and brought to the laboratory to be immediately counted and identified. Insects other than mosquitoes were also identified and recorded. A New Jersey light trap (Mulhern 1942) was operated adjacent to the wild rice fields to monitor adult mosquito populations in the area. The light trap sample was collected and counted weekly.

The zooplankton populations were sampled using a 202 micron net to concentrate the interior transect samples taken on August 3 and September 13. Thus 8 liters of water per field (20 dips) were filtered on each date. After the mosquito larvae and other aquatic organisms were identified, the zooplankton were stained with rose bengal to aid identification; they were then preserved with 5% formalin solution. Two 5 ml subsamples were drawn from each concentrated 200 ml plot sample, and the zooplankton were identified and counted at 30× magnification.

The *Bti* was applied at 6 kg/ha with a backpack blower to the nine *Bti* fields on August 20 and again to the three *Bti* only fields on September 8. Treatments were made when mosquito population densities were relatively high and near peak numbers, as based on expected population trends. Treated and control fields were sampled 1, 3 and 5 days post-treatment after the first application, and 2 days post-treatment after the second application.

One-way analysis of variance and Tukey's test (for pairwise comparisons, $P = 0.05$) were used to detect differences in the mosquito, aquatic insect and zooplankton populations among the treatments. The mosquito population data were analyzed in two groups: 1) Control, 1.1 kg/ha *G. affinis* and 3.4 kg/ha *G. affinis* (to evaluate the impact of *G. affinis*) and 2) Control, *Bti* only, 1.1 kg/ha *G. affinis* plus *Bti* and 3.4 kg/ha *G. affinis* plus *Bti* (to evaluate the impact of *Bti* alone and in combination with *G. affinis*). Since the effect of *Bti* on rice field nontarget organisms appears to be negligible (except for mortality among some chironomids and dixids) (Garcia et al. 1980, Garcia 1986, Miura et al. 1980), the six treatment groups were combined into three groups—1) control plus *Bti* only, 2) all 1.1 kg/ha *G. affinis* fields and 3) all 3.4 kg/ha *G. affinis* fields—to analyze the aquatic insect and zooplankton populations.

RESULTS AND DISCUSSION

The seasonal average minimum water temperature was 17°C and the average maximum 30°C. Water quality was similar among the treatments. The nitrate concentration on September 13 averaged 0.34 ppm (range 0.31–0.40), phosphate 0.76 ppm (0.47–0.86), alkalinity 207 ppm (200–220), hardness 153 ppm (110–190), conductivity 392 micromhos/cm (380–410) and pH 7.6 (7.5–7.9). Water depth averaged 17 cm and maximum plant height was 2.6 m.

The *G. affinis* population increased steadily throughout the growing season (Fig. 2). The average trap count at the end of the season was 182 fish/trap in the low rate fish fields and 230 fish/trap at the high release rate. The interior trap counts were similar to the perimeter catches. These counts were well above the 1986 peak trap counts of 20 and 76 fish/trap in the 0.6 and 1.7 kg/ha fields, respectively (Kramer et al. 1987). The much larger *G. affinis* population in 1987 than 1986 may have been due to a more abundant prey population, a larger proportion of pregnant females released into the fields and an earlier (by 10 days) fish release date.

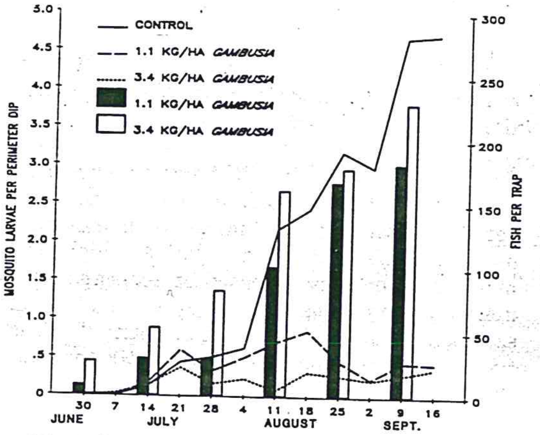

Fig. 2. *Gambusia affinis* population in wild rice fields (right axis—bars) and larval mosquito populations in *G. affinis* treated and control wild rice fields (left axis—lines), Lake County, CA, 1987.

Although the 3.4 kg/ha release rate is three times greater than the 1.1 kg/ha rate, fish trap counts throughout the season seldom approached a three-fold difference (Fig. 2). Thus, the stocking of *G. affinis* at increasingly higher rates does not appear to yield proportionally greater fish populations. This result may be related to factors that determine the carrying capacity of the rice field habitat. As Norland and Bowland (1976) stated, fish stocking rates do not necessarily determine ultimate population density, and food supply is likely the limiting factor to final population numbers. In their study, two white rice fields stocked at the same rate (1.1 kg/ha mature female *G. affinis*) had vastly different final (September) trap counts (30 vs. 130 fish/trap). Another field stocked at 3.4 kg/ha of mature females yielded a final catch of 150 fish/trap, substantially less than the Lake County wild rice catch at the same release rate. Their fish populations (in the 1.1, 2.2 and 3.4 kg/ha fields) leveled off after ca. 4 weeks suggesting the carrying capacity of the fields had been reached.

Based on the regression equation developed by Stewart and Miura (1985) to estimate absolute densities of *G. affinis* in white rice fields, the 1987 Lake County trap counts of 182 and 230 in the low and high fish fields respectively are equivalent to densities of ca. 586,000 and 746,000 fish/ha (ca. 244 and 311 kg/ha since about 2,400 fish, including fry, males and mature females, captured from the wild rice fields at the end of the season, equaled one kilogram). The growth curves of Stewart and Miura (1985) in white rice estimate a maximum carrying capacity of ca. 120,000 fish/ha. Our studies indicate that wild rice fields may support higher population densities of *G. affinis* than white rice fields; however, additional studies (e.g., mark and recapture) would have to be applied to verify this hypothesis. Studies in the Central Valley have indicated that the nutrient content of wild rice field water is greater than that of white rice water (Kramer and Garcia 1988).

Immature mosquito populations in *G. affinis* treated fields were significantly ($P < 0.01$) lower than in control fields from August 11 until harvest (Fig. 2). The late instar (3rd and 4th) populations were separately evaluated and also found to be significantly lower. The *G. affinis* trap counts on August 11 were 101 and 161/trap in the low and high fish fields, respectively. The data imply that a count of more than 100 fish/trap will effectively control mosquitoes.

The perimeter mosquito counts averaged (all fields combined throughout the entire season)

0.31 larvae/dip higher than the interior dip counts. However, differences were not significant.

The larval *Cx. tarsalis* population was much lower in 1987 than in 1986, when it reached a maximum of 1.6 larvae/dip (Kramer et al. 1987). In 1987, *Cx. tarsalis* reached a maximum of 0.7 larvae/dip (16% of the total larval count) in the control plots in early September. The population never exceeded 0.2 larvae/dip (7%) in the fish-treated fields or 0.08 (6%) in the *Bti* only fields.

Anopheles franciscanus larvae comprised ca. 5% of the total anopheline count (based on 4th instar identification); whereas, in 1986, 40% of the anophelines were *An. franciscanus.* Thus *Anopheles freeborni* clearly dominated in 1987 with a peak of 4.7 larvae/dip in the control fields, which exceeded the peak larval count for all species combined (3.6/dip) in 1986.

Culex tarsalis was the most abundant species in the adult light trap collections (Fig. 3). The peak *Cx. tarsalis* population was 444 females per trap night in mid-July. *Anopheles freeborni* and *An. franciscanus* peaked at 110 and 70 females per trap night, respectively, in early September. Nearby breeding sources, including sloughs, ditches and adjacent commercial wild rice fields (planted one month after the experimental fields), probably contributed substantially to the light trap collections. Differential species attraction to the light trap may also have influenced the proportion of species collected.

Just prior to *Bti* treatment on August 20, larval mosquito densities in the low fish plus *Bti* fields were substantially greater than in the low fish only fields (2.6 versus 0.9 larvae/dip). This was probably due to habitat and sampling variation as fish populations were similar (97 versus 104 fish/trap on August 12 in the low fish plus *Bti* and low fish only fields, respectively). Larval populations were relatively similar between these two groups on all previous sampling dates.

Applications of *Bti* were applied to all *Bti* fields on August 20 (Fig. 4). The mosquito larvae were reduced by 72%, 70% and 38% in the *Bti* only, low fish plus *Bti* and high fish plus *Bti* fields, respectively, one day post-treatment. The percent mortality in the interior of the fields was slightly less than the perimeter reduction. These reduction rates are low compared to reduction rates achieved in other Lake County wild rice fields (>95% mortality) where the plants were of similar height and the application procedure and dosage rate were the same but the field size many times larger (Garcia and Colwell 1987, personal communication). The relatively high rate of water flow through the comparatively smaller experimental rice plots might have diluted the *Bti* and lessened its effectiveness.

All post-treatment larval densities were significantly ($P < 0.01$) less than densities in the control fields (pretreatment population numbers were not significantly different). Mosquito densities in the *G. affinis* plus *Bti* fields remained significantly lower than in the controls for the duration of the season. On September 1 and 8, the number of larvae in the *Bti* only fields did not differ significantly from the control density, and by September 8, the larval number in these plots was significantly greater than in the high *G. affinis* plus *Bti* fields. Apparently, the mosquito populations in the *Bti* only fields rebounded while the populations in the *G. affinis* fields were kept at a low level by the fish. Stewart et al. (1983), working with white rice fields, obtained the greatest mosquito control in a field treated with *Bti* and stocked with *G. affinis* (0.2 kg/ha).

The *Bti* only fields were treated a second time on September 8, and water flow through the

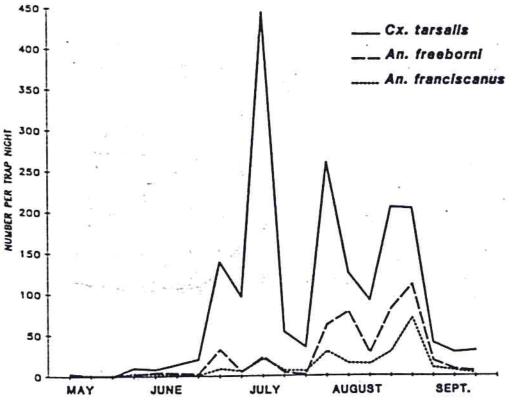

Fig. 3. Light trap counts of *Culex tarsalis*, *Anopheles freeborni* and *An. franciscanus* females adjacent to wild rice fields, Lake County, CA, 1987.

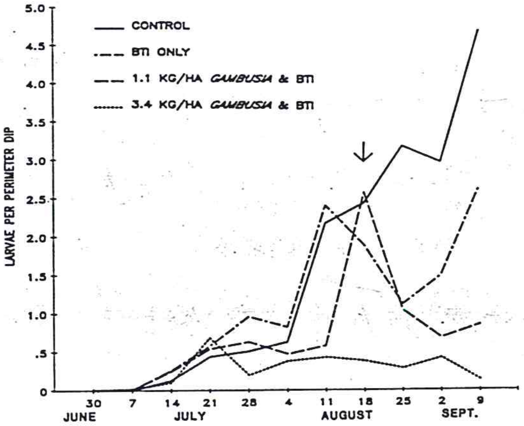

Fig. 4. Larval mosquito densities in *Bti* treated and control wild rice fields, Lake County, CA, 1987. *Bti* applied on August 20 (arrow).

fields was stopped for 24 hours following the treatment. The mortality rate of 85% exceeded that of the first treatment. The two day post-treatment population was significantly ($P <$ 0.01) smaller than the control population. The fields were drained shortly after this monitoring date.

Odonata, Corixidae, Belostomatidae, Notonectidae, Hydrophilidae and Dytiscidae populations were monitored with minnow traps (Fig. 5) and by dipping (Fig. 6). The Ephemeroptera were sampled only by dipping as few were captured by the minnow traps, which only retain organisms greater than 4 mm in width. Five the six insect groups monitored by the minno traps and four of the seven groups monitored dipping had population densities significant lower in the fish-treated fields than in the control fields on one or more sampling dates (Fig 5 and 6). Species collected in 1987 were simila to those in the 1986 wild rice fields and a reported by Kramer et al. (1987).

Dragonfly, primarily *Anax junius* (Drury) and *Pantala hymenaea* (Say), and damselfly, primar-

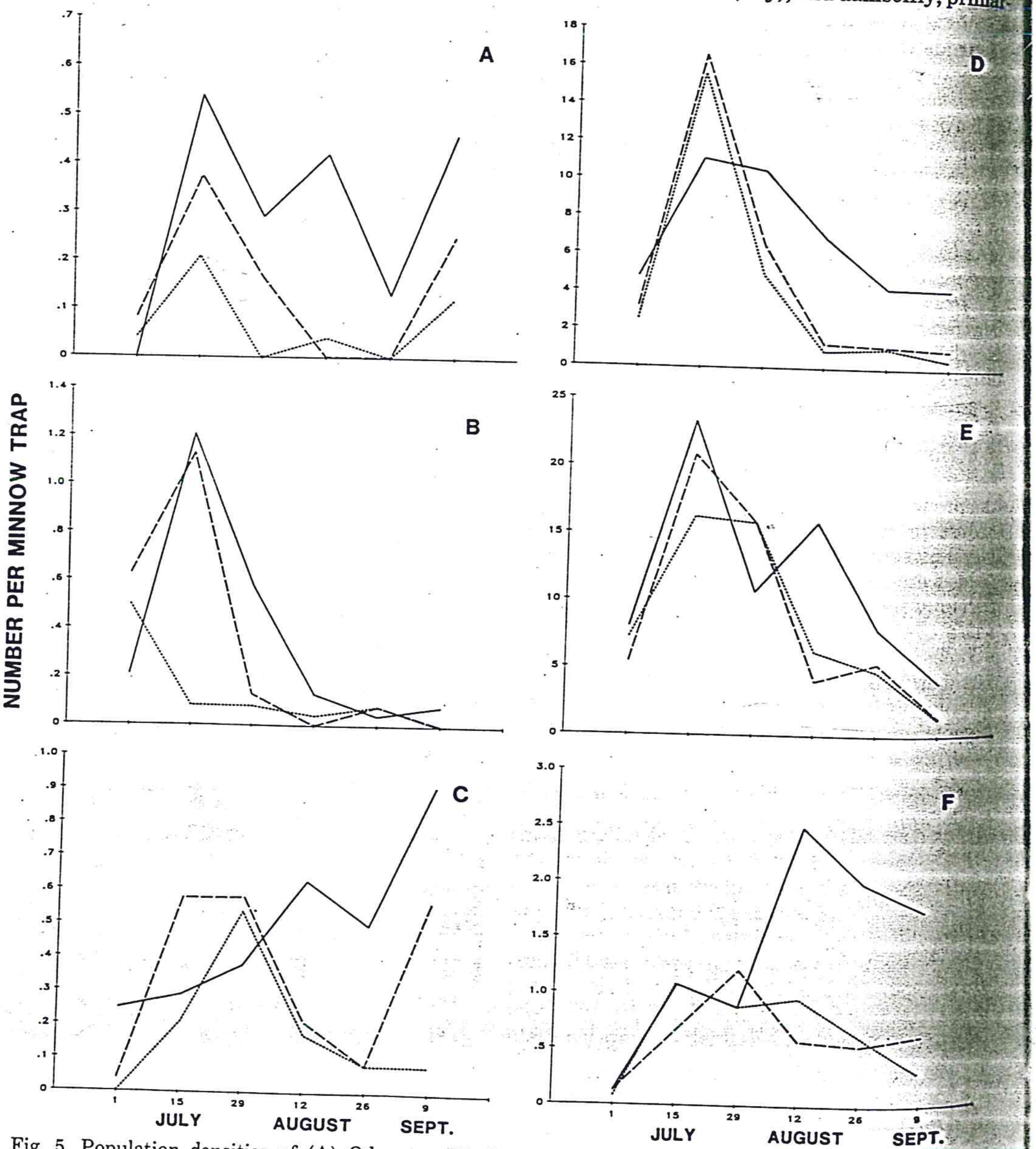

Fig. 5. Population densities of (A) Odonata, (B) Corixidae, (C) Belostomatidae, (D) Notonectidae, (E) Hydrophilidae and (F) Dytiscidae (number per minnow trap) in wild rice fields with and without *Gambusia affinis*, Lake County, 1987 (no *G. affinis* ——, 1.1 kg/ha *G. affinis* – – –, 3.4 kg/ha *G. affinis* . . . .).

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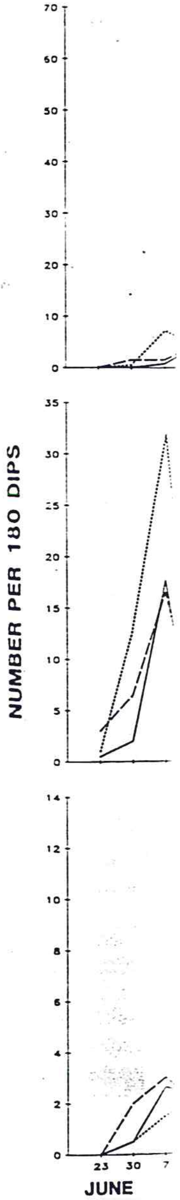

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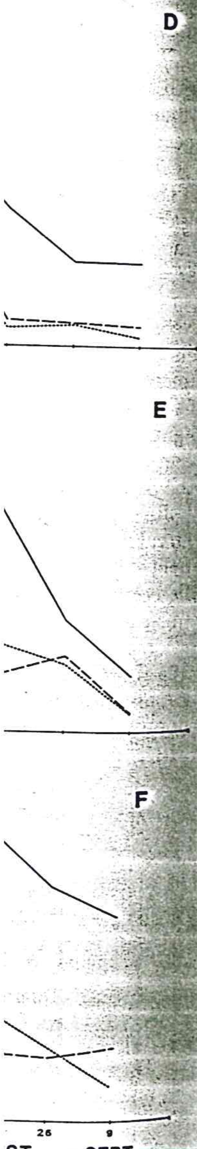

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ily *Enallagma carunculatum* Morse, numbers in the minnow traps were low and there were no significant differences among the treatment groups. Dip counts of Odonata naiads were significantly ($P < 0.01$) greater in the control fields than in the *G. affinis*-treated fields at the end of the season (September 8 and 15). The mayfly population, primarily *Callibaetis* sp. (Ephemeroptera), peaked in early July and no differences were detected among populations in fields with or without fish.

Numbers of the water boatmen, *Corisella decolor* Uhler and *Hesperocorixa laevigata* (Uhler) (Corixidae), were significantly higher in minnow traps in the control fields than in the fields with the high rate of *G. affinis* on July 15 ($P < 0.05$) and greater than all fish-treated fields on July 29 ($P < 0.01$). However, maximum population density was only ca. 2 corixids/minnow trap. No significant differences were found among populations monitored by dipping. A giant water bug, *Belostoma flumineum* Say (Belostomatidae),

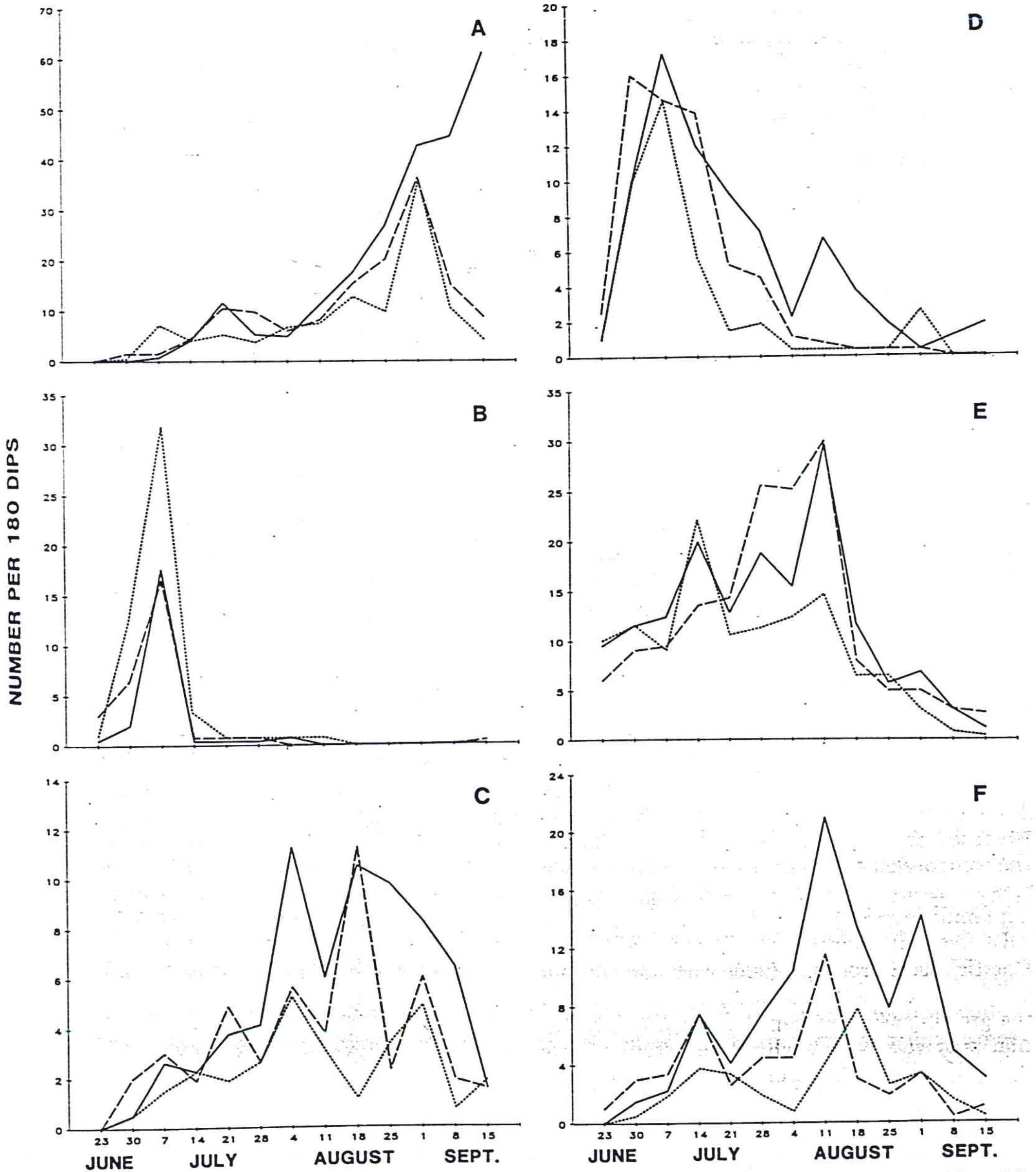

Fig. 6. Population densities of (A) Odonata, (B) Corixidae, (C) Belostomatidae, (D) Notonectidae, (E) Hydrophilidae and (F) Dytiscidae (number per 180 dips) in wild rice fields with and without *Gambusia affinis*, Lake County, 1987 (no *G. affinis* ——, 1.1 kg/ha *G. affinis* – – –, 3.4 kg/ha *G. affinis* · · · ·).

was significantly more abundant in the control fields than in the fish-treated fields toward the end of the growing season (August 26 and September 9) as monitored by minnow traps ($P < 0.01$) and by dipping ($P < 0.05$).

Backswimmer populations, primarily *Notonecta unifasciata* Guerin and *N. undulata* Say (Notonectidae), peaked in mid-July and minnow trap counts were significantly ($P < 0.01$) greater in the control fields than in the *G. affinis* treated fields from August 12 to September 9. The notonectids monitored by dipping showed significant differences between the control and fish-treated fields earlier in the season. The control field density was significantly greater than that in the high *G. affinis* fields on July 21 and 28 ($P < 0.05$) and than in all fish-treated fields on August 11 ($P < 0.01$). The bias of the minnow traps for the larger (later instar) notonectids and of the dipping for the smaller (early instar) notonectids may explain why the two sampling regimes detected population differences on different dates.

Adult beetles were monitored primarily with minnow traps while beetle larvae were sampled via dip counts. Water scavenger beetles, primarily *Tropisternus lateralis* (Fabricius) and *Hydrophilus triangularis* Say (Hydrophilidae), were abundant throughout the season, reaching a maximum of 23/trap in the controls in mid-July. The hydrophilid density was significantly ($P < 0.01$) greater in the control fields than in the *G. affinis* treated fields on the final trapping date, September 9. No significant differences were detected among populations monitored by dipping. Predaceous diving water beetles, primarily *Thermonectes bassilaris insignis* (McWilliams), *Rhantus gutticollis* (Say) and *Laccophilus* sp. (Dytiscidae), were less abundant than hydrophilids. There were significantly more dytiscid beetles trapped in the control fields than in all fish-treated fields on August 12 ($P < 0.01$) and significantly more than in the low *G. affinis* fields on August 26 ($P < 0.05$). Dytiscids collected by dip sampling were more abundant in the control fields than in either or both the fish-treated fields on August 4 and August 18 to September 8.

In general, significant differences among aquatic insect groups in fields with and without *G. affinis* were detected during the later part of the growing season when the *G. affinis* population was relatively high. Other studies have shown that populations of notonectids and damselflies (Farley and Younce 1977b, Miura et al. 1984), dragonflies (Farley and Younce 1977b), and mayflies and chironomids (Miura et al. 1984) were significantly lower in *G. affinis*-treated fields. Aquatic beetle, corixid, and belostomatid populations were not significantly lower in *G. affinis* fields (Farley and Younce 1977, Miura et al. 1984). In Lake County wild rice fields in 1986, when the *G. affinis* population was much lower than in 1987, there were no significant differences among insect populations in fields with or without fish (Kramer et al. 1987). The impact of *G. affinis* on aquatic insects probably varies depending on fish numbers, the availability of refugia and alternative prey densities as well as other factors.

Two cladocerans (*Ceriodaphnia* sp. and *Chydorus* sp.), a copepod (*Cyclops* sp.), ostracods and chironomids were commonly found in the zooplankton samples (Fig. 7). Total body lengths of *Ceriodaphnia* ranged from 0.27 to 0.74 mm and of *Chydorus* from 0.17 to 0.39 mm (40 individuals of each measured). In August, *Ceriodaphnia* were significantly ($P < 0.01$) more abundant in the control fields than in all *G. affinis* treated fields. No significant differences were detected among the copepod, ostracod or chironomid populations in fields with or without fish.

All zooplankton populations increased in September, and the *Ceriodaphnia, Cyclops* and ostracod populations were significantly ($P < 0.01$,

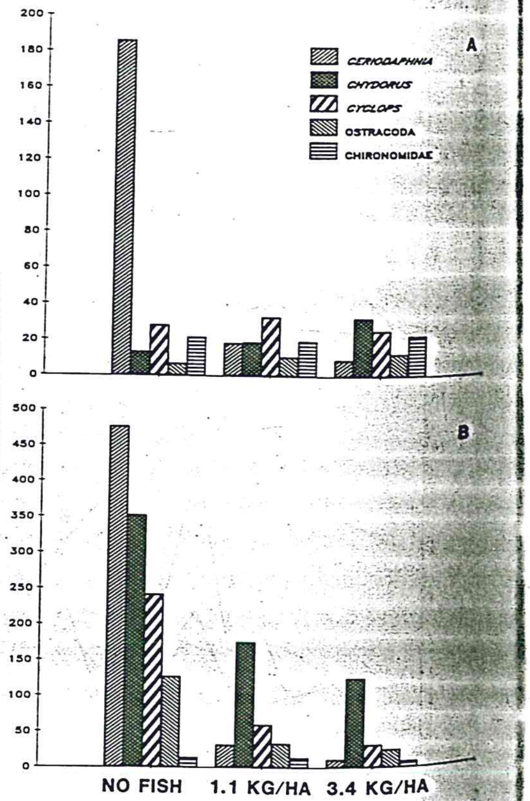

Fig. 7. Zooplankton populations on (A) August 3 and (B) September 13 in wild rice fields, Lake County, 1987.

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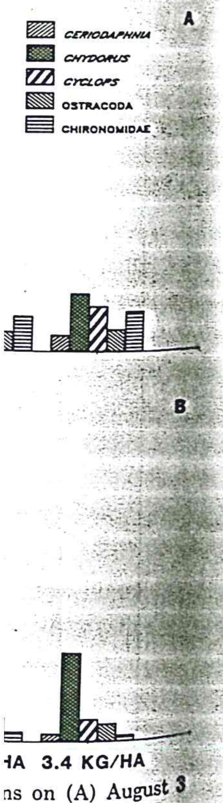

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01, and 0.05, respectively) lower in the *G. affinis* versus control fields. *Chydorus* and chironomid populations were not significantly lower in the fish-treated fields. In contrast to these data, Miura et al. (1984) found that *G. affinis* did not reduce populations of copepods or ostracods. Bay and Anderson (1966) found that *G. affinis* did not reduce populations of chironomids even at a fish density of more than 250 lbs/acre; whereas, Miura et al. (1984) found that the fish significantly reduced chironomid larvae. Different sampling regimes (benthic vs. free-swimming) or the presence of different chironomid species may account for the varying results.

Apparently *G. affinis* prefer to feed on the larger *Ceriodaphnia* than the smaller *Chydorus*. *Gambusia affinis* has been shown to essentially eliminate *Ceriodaphnia* in experimental ponds (Hurlbert and Mulla 1981). Bence and Murdoch (1983) found that *G. affinis* reduced the abundance and mean size of zooplankton in white rice fields (in fields without fish, zooplankton were <1.6 mm and with fish, <0.8 mm).

Since *Bti* potentially reduces populations of some chironomids, the data were analyzed to detect differences among chironomid populations in fields treated with *Bti* versus untreated fields. No significant differences were found (average of 12 and 12.7 chironomids/liter in the untreated and *Bti* treated fields, respectively). However, only free swimming larvae were sampled (not epiphytic or benthic larvae).

Of the 120 *G. affinis* dissected for gut analyses, 42% contained zooplankton only (fish size range 16–50 mm standard length (S.L.), $\bar{x} = 27$ mm), 28% had zooplankton and insects or snails (17–50 mm S.L., $\bar{x} = 29$), 11% had insects only (19–52 mm S.L., $\bar{x} = 31$) and 39% had empty guts (16–50 mm S.L., $\bar{x} = 31$). Based on these data, there does not appear to be a correlation between fish size and general prey preference.

Nine female fish (7.5% of those dissected), ranging in size from 25 to 47 mm S.L., contained 58 mosquito larvae (all anophelines: 6 first instar, 27 second, 12 third, 12 fourth and 1 pupa). One fish (female, 44 mm S.L.) had consumed 38 larvae (2nd–4th instars) and a second fish (female, 25 mm S.L.) contained 10 larvae (1st–3rd instars). The presence of large numbers of mosquito larvae in only 2 of the 120 fish examined suggests that some individuals form a search preference and consistently seek the same prey item. The remaining seven *G. affinis* had 1–3 larvae in their guts. The fish containing mosquito larvae were only found in half of the fields sampled. These fields had a higher average mosquito population (0.68/dip vs. 0.27/dip) and lower *G. affinis* population (112/trap vs. 150/trap) than the fields where no larvae were detected in the fish guts. Although the mosquito population was not significantly reduced by *G. affinis* in 1986 (Kramer et al. 1987), the percentage of fish (9%) containing mosquito larvae was similar to the 1987 finding. Therefore, the percentage of fish containing mosquitoes does not provide an index of the control effectiveness of *G. affinis*. The percent of fish guts containing larvae seems to be, in part, a function of the relative abundance of mosquito larvae and fish. Other factors, such as availability of alternative prey and prey accessibility, undoubtedly influence the number of mosquito larvae consumed.

Gambusia affinis had an extensive array of alternative prey available in the wild rice fields. Cladocerans (primarily *Chydorus* and some *Ceriodaphnia*) were the most abundant organisms in the fish guts. Copepods, ostracods and rotifers were also found. Forty chironomid larvae were found in 20 (16.7%) of the fish, and there were 20 hydrophilid larvae in 8 (6.7%) of the fish dissected. Other insects found in the guts of *G. affinis* included the following: 2 Anisoptera, 1 Zygoptera, 1 Cercopidae, 1 Corixidae, 2 Homoptera, 5 Thysanoptera, 1 Cecidomyidae, 1 Stratiomyidae, 4 other Diptera larvae and 1 Diptera adult. Eighty-six snails (*Physa*) were found in 9 (7.5%) fish (one fish had consumed 38 snails and a second, 20). One fish contained 3 *G. affinis* fry. Five fish (4.2%) had tapeworms (*Bothriocephalus*) in their gut. Algae was also found in the guts of many fish.

Eight of the 39 fish containing insects or snails had more than 7 organisms of the same type (mosquitoes, chironomids, hydrophilids or snails) in their guts. These numbers are more than would be expected by random encounter and support the notion of a prey search preference. The omnivorous feeding behavior of *G. affinis* is evident by these gut dissections and has been reported by many researchers (Hess and Tarzwell 1942, Washino and Hokama 1967, Miura et al. 1979, Farley 1980).

In conclusion, *G. affinis* significantly reduced the mosquito population in Lake County wild rice fields at fish densities exceeding 100/trap. In 1986, when mosquito populations were not significantly different in fields with and without fish, densities of *G. affinis* did not reach this level (Kramer et al. 1987). Since one release rate in 1987 (1.1 kg/ha) was less than one 1986 release rate (1.7 kg/ha), the assumption that *G. affinis* will be an effective control agent of mosquitoes at a given release rate is not always reliable. These studies indicate that the *G. affinis* population must be monitored post-release to assess its control potential.

Granular *Bti* effectively reduced mosquito populations in wild rice. When *G. affinis* were present, the larval populations did not rebound after a *Bti* treatment. Thus effective mosquito control can be achieved in wild rice when fields are stocked at 1.1 kg/ha of *G. affinis*, monitored and treated with *Bti* when the mosquito population increases beyond an acceptable level.

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